Acute Leukaemia

PH-P001

FACTORS AFFECTING INITIAL CYCLOSPORINE A LEVEL AND ITS CORRELATION WITH CLINICAL OUTCOME IN ACUTE LEUKEMIA PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

A. Gupta[1,], S. Punatar[1], J. Gawande[1], B. Bagal[1], L. Mathew[1], S. Kannan[2], N. Khattry[1]*
[1]Medical Oncology, Bone Marrow Transplant Unit, ACTREC, Tata Memorial Centre, [2]Biostatistics, ACTREC, Tata Memorial Centre, Mumbai, India

Introduction: Initial trough Cyclosporine A (CsA) blood level can influence incidence of GVHD and relapse in patients with acute leukemia. We sought to evaluate the impact of initial CsA level (CSA-1) on the incidence of acute and chronic GVHD, relapse and survival and also explore factors that may affect CSA-1.
Materials (or patients) and Methods: All patients who underwent allogeneic stem cell transplant (ASCT) for acute leukemia from January 2008 to March 2013 were included in this retrospective study. GVHD prophylaxis used was CsA (starting dose 1.5 mg/kg twice daily) from day -1 in combination with either methotrexate (MTX) or mycophenolate mofetil (MMF). CSA-1 was measured on day 4 or day 5 of starting CsA. Dose of CsA was modified depending on CSA-1 to achieve therapeutic level of 150-200 ng/ml. For analysis, patients were divided into three groups based on modification of CsA dose - Group A (dose escalated), Group B (dose de-escalated) and Group C (dose unchanged). Comparisons were done between 3 groups for baseline characteristics, incidence of acute and chronic GVHD, incidence of relapse, transplant related mortality (TRM), relapse free survival (RFS) and overall survival (OS). Comparison between 3 groups was done by using chi-square test and survival outcomes by Kaplan Meier method. Multivariate analysis to determine factors predicting CsA dose escalation or de-escalation was done using logistic regression.
Results: Seventy-four patients underwent 77 transplants; AML-52, ALL-23, biphenotypic-2; 42 in CR1, 20 in CR2, 15 in relapsed/refractory state; 65 from matched related, 10 from matched unrelated and 2 from haploidentical donors. Source of stem cells was peripheral blood in 70, bone marrow in 5 and cord blood in 2 transplants. The median age was 30 years (range 6-51). Conditioning regimen was full intensity (FI) i.e. TBI-Cy or Bu-Cy in 42 and reduced intensity (RI) i.e. fludarabine based in 35 transplants. Total body irradiation (TBI) was used in 32 patients. GVHD prophylaxis was CsA+MTX in 53 and CsA+MMF in 24 patients. There were 27 patients in group A, 13 in group B and 37 in group C. On univariate analysis, use of FI regimen, cyclophosphamide and TBI; and Body Mass Index (BMI) <22 kg/m^2 were associated with lower CSA-1 while use of fludarabine, RI regimen and BMI>22 kg/m^2 were associated with higher CSA-1. On multivariate analysis, fludarabine use and BMI>22 kg/m^2 predicted for higher CSA-1 requiring CsA dose de-escalation (*P*=0.038 and 0.034 respectively). Incidence of all grade and grade II-IV acute GVHD was 48% and 11% in group A, 38% and 31% in group B, and 43% and 19% in group C respectively (*P*= NS). Incidence of chronic GVHD was 52% in group A, 38% in group B and 57% in group C (*P*=NS). Incidence of relapse was 41% in group A, 23% in group B and 32% in group C (*P*= NS). TRM was 7% in group A, 38% in group B and 11% in group C (*P*= NS). OS and RFS at 4 years was 33% and 29% in group A, 43% and 46% in group B, and 53% and 45% in group C respectively (*P*=NS).
Discussion: BMI <22 kg/m^2 is associated with lower CSA-1 requiring CsA dose escalation. Use of fludarabine based conditioning and BMI>22 kg/m^2 is associated with higher CSA-1 requiring CsA dose de-escalation. Transplant outcomes including rates of acute and chronic GVHD, TRM, relapse incidence and overall survival are not significantly affected by initial CsA level.
Disclosure of Interest: None Declared.

PH-P002

SECONDARY AML IN CHILDREN SUFFERING FROM FANCONI ANEMIA IN GERMANY: A 20 YEARS EXPERIENCE

R. Beier[1,], S. Hoseini[1], B. Maecker-Kolhoff[1], R. Schneppenheim[2], W. Ebell[3], H. Schmid[1], P. Bader[4], K. Ehlert[5], K.-W. Sykora[1], U. Creutzig[1], C. Kratz[1], M. M. Chao[1], D. Reinhardt[1], M. Sauer[1]*
[1]Pediatric Haematology and Oncology, Hannover Medical School, Hannover, [2]Pediatric Haematology and Oncology, University Hospital Hamburg-Eppendorf, Hamburg, [3]Pediatric Haematology and Oncology, Charite Berlin, Berlin, [4]Pediatric Haematology and Oncology, University Hospital Frankfurt, Frankfurt, [5]Pediatric Haematology and Oncology, University Hospital Münster, Münster, Germany

Introduction: Allogeneic stem cell transplantation (HCT) is the only curative approach for patients with Fanconi anemia (FA) and associated myeloid malignancy (AML). The data about the impact of chemotherapy and HCT are rare.
Materials (or patients) and Methods: Between January 1993 and May 2013, 15 patients were identified in the AML-BFM database (Germany) with FA and AML. AML was diagnosed at a median age of 11.6 yrs [0.9-19.6]. In 10 patients,FA preceded the occurrence of AML by a median of 32.3 months [0-161]. One patient was further excluded, because she received HCT before AML.
Results: Six of 14 patients underwent HCT achieving a 5 year OS of 47%. The median time to transplant after diagnosis of AML was 2 months [0.3-4]. One patient died after relapse, one due to severe graft versus host disease (GvHD), another of unknown cause. Of the three surviving patients++, two had chronic GvHD. They had received a radiation free preparative regimen (mainly busulphan based) including antibodies for intensive GVHD prophylaxis. Two surviving patients received low dose cytoreductive therapy (thioguanine and cytarabine) to bridge time to HCT. None of them achieved complete remission (CR) before HCT. Eight of 14 patients did not undergo HCT with median survival of 40 days, despite in some patients intensive AML driven chemotherapy.
Discussion: Three of 14 patients survived and 2/3 surviving patients received low dose cytoreductive therapy for disease control before HCT. Intensive chemotherapy is associated with treatment related mortality by infections. Achieving CR pre transplant might not be necessary for survival.
Disclosure of Interest: None Declared.

PH-P003

THIOTEPA-BASED CONDITIONING PRIOR TO ALLOGENEIC STEM CELL TRANSPLANTATION (HSCT) FOR ACUTE MYELOID LEUKEMIA (AML) – A SURVEY FROM THE ALWP OF THE EBMT

S. Eder[1,], M. Labopin[1], J. Finke[2], D. Bunjes[3], A. Olivieri[4], S. Santarone[5], A. Rambaldi[6], L. Kanz[7], G. Messina[8], A. Nagler[9], M. Mohty[10] and Acute Leukemia Working Party*

[1]EBMT Office Paris, Hôpital Saint-Antoine, Paris, France, [2]Department of Medicine - Hematology, Oncology, University of Freiburg, Freiburg, [3]Klinik fuer Innere Medzin III, Universitätsklinikum Ulm, Ulm, Germany, [4]Department of Hematology, Azienda Ospedali Riuniti di Ancona, Ancona-Torrete, [5]Department of Hematology, Ospedale Civile, Pescara, [6]USC Ematologia, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy, [7]Abteilung II, Universität Tübingen, Tübingen, Germany, [8]Centro Unico Regionale Trapianti, Reggio Calabria, Italy, [9]Chaim Sheba Medical Center, Tel Hashomer, Israel, [10]Service d'Hématologie et Thérapie Cellulaire, Hôpital Saint Antoine, Paris, France

Introduction: Thiotepa is an alkylating compound with an anti-neoplastic activity. In the past, its use in the field of hematology became more and more frequently. Thus, we decided to investigate the outcome after conditioning with Thiotepa in patients with AML.

Materials (or patients) and Methods: We could identify 1908 adults with AML receiving Thiotepa in the EBMTs data base ProMISe and sent a questionnaire to these centres; the response rate was 22%.

Our survey was based on first allogeneic (optionally after autologous) HSCT until 2012, thus 310 patients were eligible.

Results: Median age was 46.5 years, the median interval from diagnosis to HSCT was 222 days. The median year of HSCT was 2008.

53.5% of the patients were male, 46.5% female. Donor sex was male in 55.7%. Female donor to male recipient was the case in 23.9%. Secondary AML was the indication for HSCT in 13% and 14% of all patients had a previous autologous HSCT.

Disease status at time of HSCT was CR1 in 155 patients (50%), CR2+ in 73 (23,5%) and 82 patients (26.5%) were in an advanced status.

Patients in CR1 had in 31.6% an HLA-matched sibling, in 16.1% an unrelated, in 31% an haplo-identical donor and cord-blood was the stem cell source in 21.3%.

Patients in CR2+ had in 21.9% an HLA-matched sibling, in 20.5% an unrelated, in 37% an haplo-identical donor and cord-blood was the stem cell source in 20.5%.

Patients transplanted in advanced status had in 20.7% an HLA-matched sibling, in 26.8% an unrelated, in 41.5% an haplo-identical donor and cord-blood was the stem cell source in 11%.

Conditioning regimen was myeloablative in 71.4% in patients in CR1, 72.2% in CR2+ and 65% in those with advanced status.

Total Body Irradiation (TBI) was used in 29% and Anti-Thymocyte Globuline (ATG) was administered in 58.5% of all cases. *In-vitro* T-cell depletion was performed in 35% (63% of all HSCT with an haplo-identical donor).

Cytogenetic risk group was "Good" in 3.9% in patients in CR1, 17.8% in CR2+ and 8.5% in patients with advanced status.

An "Intermediate" risk had 73.5% of the patients in CR1, 63% in CR2+ and 67.1% of those with advanced status.

Cytogenetic risk group was "Poor" in 14.8% in patients in CR1, 6.8% in CR2+ and 18.3% in patients with advanced status.

The median follow-up was 37 months (6-196).

Engraftment of PMN > 500 at day 60 was 92+/-3 % and platelets were above 20 G/l at 6 months after HSCT in 89+/-2 %.

In those 82 patients who had an an HLA-matched sibling as donor, acute GvHD grade 0-1 occurred in 71.6%, grade 2-4 in 28.4%.

In 62 patients with an unrelated donor 68,9% developed grade 0-1 and 31.1% grade 2–4 acute GvHD. 109 patients had a haplo-identical donor and the incidence of grade 0 – 1 and grade 2–4 acute GvHD were 79.2% and 20.8% respectively.

57 patients were transplanted with cord-blood: 70.4% had grade 0-1 and 29.6% grade 2-4 acute GvHD.

The 3 years outcome was as follows:

	CR1	CR2+	advanced
Chronic GvHD	43 +/-6	23+/-5	19+/-4
Relapse incidence	20+/-3	31+/-5	41+/-5
NRM	38+/-4	50+/-6	45+/-6
LFS	41+/-4	20+/-10	14+/-4
OS	46+/-4	28+/-5	14+/-4

Discussion: We could show, that the use of Thiotepa is comparable to other conditioning regimens, especially with a lower incidence of chronic GvHD in CR2+ and advanced disease patients.

Disclosure of Interest: S. Eder: None Declared, M. Labopin: None Declared, J. Finke: None Declared, D. Bunjes: None Declared, A. Olivieri: None Declared, S. Santarone: None Declared, A. Rambaldi: None Declared, L. Kanz: None Declared, G. Messina: None Declared, A. Nagler: None Declared, M. Mohty Conflict with: Riemser.

PH-P004

T REPLETED HAPLOIDENTICAL MISMATCH ALLOGENEIC VERSUS AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH ACUTE LEUKEMIA IN COMPLETE REMISSION: A PAIR-MATCHED ANALYSIS FROM THE ALWP

N. Claude Gorin[1,2,], M. Labopin[1,2], F. Ciceri[3], S. Piemontese[4], W. Arcese[5], P. Di Bartolomeo[6], D. Blaise[7], G. Meloni[8], H. Huang[9], W. Depei[10], J. Chen[11], A. Bacigalupo[12], A. Olivieri[13], M. Mohty[14,15] and Acute Leukemia working party of EBMT*

[1]Hematology and Cell Therapy, Hopital Saint ANTOINE PARIS, [2]EBMT Paris Office, University UPMC, Paris, France, [3]Haematology and BMT, San Raffaele Scientific Institute, Milan, Italy, [4]EBMT Paris Study Office / CEREST-TC, HOPITAL SAINT ANTOINE PARIS, Paris, France, [5]Stem Cell Transplant Unit, Tor Vergata¨ University of Rome, Rome, [6]Department of Hematology, Ospedale Civile, Pescara, Italy, [7]Transplantation et therapie cellulaire, Institut Paoli Calmettes, Marseille, France, [8]Dip. Biotecnologie Cellulari ed Ematologia, Univ.`La Sapienza`, Rome, Italy, [9]Bone Marrow Transplantation Center, First Affiliated Hosp., Zhejiang University, Hangzhou, [10]Stem cell transplantation, [11]Stem Cell Transplant Unit, First Affiliated Hospital of Soochow University, Suzhou, China, [12]Department of Haematology II, Ospedale San Martino, Genova, [13]Department of Hematology, Azienda Ospedali Riuniti di Ancona, Ancona, Italy, [14]Department of Haematology, [15]EBMT Paris Office, HOPITAL SAINT ANTOINE PARIS, Paris, France

Introduction: In the absence of a fully matched donor, adult patients with Acute Leukemia (AML and ALL) in CR are usually offered an allogeneic transplant from an alternative donor, but there has been thus far no demonstration that outcome when using an alternative donor is superior to outcome following an autologous stem cell transplantation (ASCT). We reported last year on a matched pair analysis comparing 113 AML patients transplanted in CR1 with an haplo-mismatch family donor to 226 autologous stem cell transplantations done in the period from January 2000 to December 2010 (Blood 2013, 3093a). Improvements in both transplant approaches may have changed the outcome, justifying a new analysis.

Materials (or patients) and Methods: In the present study, we considered the period from January 2007 to December 2011 and we collected information on patients with transplanted in first (CR1) and second CR (CR2). Also, we considered only T repleted haplo mismatch transplants. We used patient age, diagnosis, status at transplant and interval from diagnosis to transplant (< > 6 months for CR1; < > 18 months for CR2) and cytogenetics as matching factors. The pair match analysis was done on 130 haplo versus 235 ASCT.

Results: The median follow up was 26 months (range, 2-66) for haplo and 20 months (1-72) for ASCT. Patients allografted were transplanted more recently (median year of transplant : 2010 vs 2009, p<10e-4), and they received less frequently total body irradiation in the pretransplant regimen (17% vs 29 %; *P*= 0.01). 7% of the patients in the group haplo received a second allograft, while

2 year outcome		OS	LFS	RI	NRM
CR1 patients	haplo (101)	52+/-6	42+/-5	32+/-5	26+/-4
	auto (191)	63+/-4	42+/-4	54+/-4	3+/-2
	p	0,05	0,81	<0,0001	<0,0001
CR2 patients	haplo (29)	43+/-10	34+/-10	37+/-10	29+/-9
	auto (44)	58+/-9	36+/-9	54+/-10	10+/-5
	p	0.37	0.83	0.17	0.08
Intermediate cytogenetics	Haplo	60+/-7	47+/-7	32+/-7	36+/-13
	Auto	57+/-6	41+/-6	54+/-6	3+/-3
	p	0.95	0.24	0.002	0.01

in the ASCT group 21% of the patients received a subsequent allogeneic transplant and 5% a second autograft. The outcome at two years is presented in the table below:
Following an haplo mismatch transplantation, the percentage of acute GVHD grade III/IV+ was 12% and the incidence of chronic GVHD and extensive GVHD were respectively: 37+/– 3 and 14+/–3%. By Cox regression analysis introducing age, year of transplantation, diagnosis, status at transplant, TBI in conditioning and the nature of the transplant, Haplo was associated with a lower OS (P =0.003, HR= 1.88 95% CI: 1.24 -2.86), a higher non relapse mortality ($P< 10^{-4}$; HR = 6.84, 95% CI: 2.97 – 15.78) and a lower Relapse incidence (P=0.004, HR 0.51, 95% CI 0.32-0.81).
Discussion: These results suggest that the outcome following ASCT is presently not inferior to the one following haplo MM transplantation. We feel this study supports a randomized controlled trial comparing the two transplant modalities in a specific patient population with Acute Leukemia to be defined.
Disclosure of Interest: None Declared.

PH-P005

PRE-TRANSPLANT WEIGHT LOSS AND TOTAL SERUM PROTEIN PREDICT RELAPSE OF ACUTE MYELOID LEUKAEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

T. Luft[1,], S. Dietrich[1], A. Radujkovic[1], F. Stölzel[2], C. S. Falk[3], A. Benner[4], M. Schaich[5], M. Bornhäuser[5], A. Krämer[1], U. Hegenbart[1], P. Dreger[1]*
[1]University Hospital Heidelberg, Heidelberg, [2]Department of Internal Medicine I, University Hospital Carl Gustav Carus Dresden, Technical University Dresden, Dresden, [3]Institute of Transplant Immunology, IFB-Tx, Hannover Medical School, [4]Biostatistics, German Cancer Research Centre DKFZ, Heidelberg, [5]Technical University Dresden, Dresden, Germany

Introduction: The impact of nutritional status on outcome of allogeneic stem cell transplantation (alloSCT) is controversial. This retrospective study investigates the influence of pre-transplant weight loss and serological indicators of nutritional homeostasis on relapse and death of acute myeloid leukaemia (AML) after alloSCT.
Materials (or patients) and methods: Pre-transplant weight loss along with serum levels of total serum protein (TSP), albumin, C-reactive protein, and leptin were collected in a training cohort (n=149) and correlated with clinical outcome. Metabolic risk groups were defined and tested in an independent validation cohort (n=167).
Results: We identified pre-transplant weight loss exceeding 2% and TSP lower than 70 g/L, as strong independent predictors of relapse and death. Patients in the metabolic high risk group (low TSP and weight loss >2%) had an increased risk for relapse (P=0.0002) and death (P=0.002), but a similar risk for acute GVHD. Weight loss coincided with reduced pre-transplant serum leptin levels. The adverse influence of weight loss and high metabolic risk on relapse and overall survival could be confirmed in the validation cohort. Multivariate analysis of both cohorts revealed a hazard ratio for relapse of 7.78 (2.59-23.36, P=0.0003) in the metabolic high risk group.
Discussion: Altered nutritional homeostasis prior to alloSCT correlates with recurrence of AML after transplantation. Studies addressing pre-transplant nutritional interventions in order to reduce AML relapse rates are warranted.
Disclosure of Interest: None Declared.

PH-P006

LEVELS OF MINIMAL RESIDUAL DISEASE AS AN INDICATOR FOR ASSESSMENT OF THE RISK IN PATIENTS WITH ACUTE LEUKEMIAS AFTER INDUCTION THERAPY AND ALLOGENEIC HEMATOPOIETIC STEM CELLS TRANSPLANTATION (CIC859)

M. Guenova[1,], P. Ganeva[1], G. Balazenko[1], A. Michova[1], B. Spassov[1], V. Varbanova[1], G. G Mihaylov[1], G. Arnaudov[1]*
[1]Specialised Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria

Introduction: The use of modern induction protocols allows for achievement of complete remission (CR) in a considerable number of patients with acute leukemia. Regardless of this the largest part of them relapse because of the persistence of leukemia cells, that cannot be revealed through conventional morphology methods of examination and which are defined by the term minimal residual diseases (MRD). In general relapse remains the leading cause for treatment failure, even after transplantation.
Materials (or patients) and Methods: We present the results from studies of MRD in acute myeloid leukemia patients in the SHATHD Sofia (CIC 859) Considerably larger evet free survival (EFS) and overall survival (OS) was established for patients with lower levels of MRD, assessed after induction treatment.
Results: Analysis of MRD levels at the 100th day after allo SCT-through 8-color flow cytometry in 24 acute leukemia patients reveals also considerably longer EFS (long rank test, P-0=003) and OS (long rank test, P-0=03 (inpatients with levels below 0=05% residual leukemia blasts.
Discussion: The results suggest that MRD evaluation during treatment of acute leukemia patients is significant for their prognostic evaluation both as OS and as time to relapse. On the other hand there is an active debate over the possibilities for risk stratification and therapeutic decisions on ground of MRD levels assessment, and the introduction if novel approaches to overcome leukemia resistance to standard therapeutic regimens and agents as well as strategies aimed at modulation of the allogeneic GVL effect.
Acknowledgements:The own study were conducted with the support of the Scientific studies fund (BU0L-705/07; DO2-35/2009).
Disclosure of Interest: None Declared.

PH-P007

NKP46 EXPRESSION AT DIAGNOSIS PREDICTS POST-GRAFT OUTCOME IN AML PATIENTS

A.-S. Chretien[1,], C. Zemmour[2], C. Fauriat[3], S. Harbi[4], A. Le Roy[5], C. Arnoulet[6], N. Vey[7,8], D. Blaise[9], D. Olive[1,5,10]*
[1]Immunologie des Tumeurs, Inserm U1068, CRCM, [2]Unité de Biostatistique et méthodologie, DRCI, Institut Paoli Calmettes (IPC), [3]Tumor Immunology, Inserm U1068, CRCM, [4]Unité de Transplantation et de Thérapie Cellulaire, Inserm, [5]IBiSA Cancer Immunomonitoring Platform, [6]Département de Biopathologie, [7]Département d'Hématologie, IPC, [8]UM105, Aix-Marseille Université, [9]Unité de Transplantation et de Thérapie Cellulaire, IPC, [10]UM 105, Aix-Marseille Université, Marseille, France

Introduction: NK (Natural killer) cells are involved in tumor immune surveillance, in particular in hematologic malignancies. NK cells receptors (NCR), NKp30, NKp44 and NKp46, are specifically expressed by NK cells and are major determinants of NK cell functionality. The aim of this study was to assess the impact of

NKp46 expression at diagnosis on clinical outcome in acute myeloid leukemia (AML) patients with allogeneic hematopoietic stem cell transplantation (HSCT).
Materials (or patients) and Methods: NKp46 expression on peripheral blood NK cells was prospectively assessed by flow cytometry in 125 AML patients. Median age at diagnosis was 48 years (range 20-65). Among these 125 patients, 56 patients received allogeneic HSCT. The threshold for NKp46 expression was set on dispersion criteria of the population. Clinical outcome was evaluated with regards to NKp46 expression.
Results: In the group of allografted patients, patients whose NK cells highly expressed NKp46 at diagnosis had better overall survival (OS) (*P*=0.0229; HR=3.062; 95% CI = [1.168-8.031]) and relapse free survival (RFS) (*P*=0.0482; HR=2.881; 95% CI = [1.009-8.228]) than patients with low NKp46 expression. Patients with high NKp46 expression had improved survival probabilities at 2 years compared with patients with low NKp46 expression (90% *vs* 54%, respectively). No clinical benefit was observed for non-allografted patients with high NKp46 expression compared to non-allografted patients with low NKp46 expression, suggesting that NKp46 expression is a predictive biomarker of graft outcome rather than a prognostic biomarker.
Discussion: In this study we have identified NKp46 as a potential biomarker predictive of HSCT outcome. Clinical translation of these results could find applications in terms of identification of patients at high risk of adverse outcome. Moreover, these results highlight the potential of therapeutic strategies aiming at maintaining high levels of NKp46 on NK cells after allogeneic HSCT. Finally, this biomarker could be used as a stratification criterion in clinical trials.
Disclosure of Interest: None Declared.

PH-P008

ANALYSIS 52 CONSECUTIVE ALLOTRANSPLANTS OF ACUTE LYMPHOBLASTIC LEUKAEMIA: EBMT RISK SCORE, PRETRANSPLANT REMISSION STATUS AND GRAFT VERSUS HOST DISEASE WERE THE STATISTICALLY SIGNIFICANT DETERMINING FACTORS FOR SURVIVAL

B. Árpád[1,], P. Reményi[2], É. Torbágyi[2], L. Lengyel[1], L. Gopcsa[1], Z. Csukly[1], A. Barta[1], J. Fábián[1], M. Réti[1], T. Masszi[2]*
[1]BMT Unit, [2]Unified St.Istvan & St.Laszlo Hospital, Budapest, Hungary

Introduction: From 2008.04 – 2012.10. in our unit 52 ALL patients were allotransplanted.
Materials (or patients) and Methods: Patients characteristics: CR1/CR2/R: 36/8/6, sibling/VUD (HLAmin 7/8): 29/23, median follow up time: 15.1 months (6-54), B cell origin (16 Ph chromosome+)/ T cell origin: 39/13,. EBMT risk score: 0: 1, 1: 8, 2: 17, 3: 9, 4: 8, 5: 6, 6: 1. Most of the patients (46/52) received 12Gy TBI based conditioning regimen in combination with VP16 or CY. The rest of the patients received BU based MAC regimen. For graft versus host disease profilaxis most of the patients (45 pts) received tacrolimus and short sirolimus (day -1 – day 30), the remaining patients received cyclosporin A + short methotrexate or tacrolimus + mycophenolate mofetil for GVHD profilaxis.
Results: One graft failure occurred. 9/52 patients died before day 100. In 23/52 patients did not developed acute graft versus host disease, 7/52 grade 1., 17/52 grade2., 4/52 grede.3. and 1/52 grade.4. acute GVHD appeared. 20/43 were free from chronic GVHD, 9/43 patients had limited chronic GVHD, 14/43 patients had extensive chronic GVHD . Eleven patients relapsed, from that 9 patients died subsequently due to relapse, 2 patients still alive after salvage chemotherapy and with GVHD induced by donor lymphocyte infusion.
The log rank statistical analysis of the risk factors for survival showed the most robust effect of chronic GVHD (Kaplan Meier estimated survival w/o cGVHD: 42% vs. lim.cGVHD: 89% *P*=0.007 or vs. ext.cGVHD: 57% *P*= 0.14; lim.cGVHD vs. ext.cGVHD hazard risk:2.1, *P*=0.3). Additionally important proved to be the EBMT risk score (KM survival with score 0-3: 56% vs. with score 4-6: 25% HR: 0.33 p= 0.02), the pretransplant remission status (KM survival CR1: 58%, CR2: 37%, relapse 1: 0%, advanced phase: 33%; only significant is the CR1 survival benefit from the others), the use of VP16 in the conditioning regimen (KM survival 65% vs. 40%, *P*= 0.039) and the effect of acute GVHD (KM survival ac.GVHD0-2: 50%, acGVHD3-4: 20%, *P*= 0.034).
Discussion: The most advantageous combination of different factors to get cured are: disease in CR, TBI/VP 16 conditioning regimen, no acute GVHD, but limited chronic GVHD (the most important factor). The most robust effect of limited chronic GVHD of being cured gives room for less toxic conditioning regimens and chronic GVHD in combination. GVHD titration is still the most important and most challenging task of allogeneic stem cell transplantation.
Disclosure of Interest: None Declared.

PH-P009

BUSULFAN, FLUDARABINE, ANTI-THYMOCYTE GLOBULIN AND LOW-DOSE TOTAL BODY IRRADIATION IN THE TREATMENT OF JUVENILE MYELOMONOCYTIC LEUKEMIA WITH MYELOABLATIVE STEM CELL TRANSPLANTATION

R. Moorjani[1], T. H. Truong[1,2,], V. Lewis[1,2], G. M.T. Guilcher[1,2]*
[1]University of Calgary, [2]Oncology, Alberta Children's Hospital, Calgary, Canada

Introduction: Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative neoplasm diagnosed commonly in children under the age of 2 years. Signs and symptoms of this disease include anemia, fatigue, lymphadenopathy, hepatomegaly, splenomegaly and thrombocytopenia. Allogeneic hematopoetic stem cell transplantation (HSCT) is the only known curative therapy, with no standard conditioning regimen. While myeloablative conditioning is considered necessary to eradicate stem cell clones, treatment related mortality rates are high with busulphan, cyclophosphamide and total body irradiation (TBI)-based conditioning regimens, with many transplant physicians reluctant to administer TBI to young children. Recently, treatment of acute myeloid leukemias in adults with a less toxic regimen consisting of fludarabine and myeloablative doses of busulfan (Bu/Flu) have proven to be effective in reducing the high rates of transplant related morbidity and mortality, while promoting engraftment and survival. Despite this growing evidence supporting the use of Bu/Flu in transplantation for myeloid leukemias, concerns persist that this regimen is not sufficiently myeloablative to be curative in a disease such as JMML.
Materials (or patients) and Methods: We report a case series of 3 children diagnosed with JMML between 7 months and 2 years of age who were conditioned with our institutional protocol of busulphan (16mg/kg total), fludarabine (250mg/m^2 total), rATG (5.5mg/kg total) and 400 cGy of TBI followed by allogeneic stem cell transplant.
Results: All patients presented with leukocytosis, monocytosis, thrombocytopenia, anemia, and splenomegaly. Patient 1 was diagnosed with JMML while she was admitted for an acute respiratory infection, whereas patient 2 presented with unexplained fevers and abdominal distension. In contrast, patient 3 was diagnosed during a routine examination, however he presented with more serious symptoms. In addition to leukocytosis, multiple cytopenias and massive hepatosplenomegaly, this patient developed respiratory symptoms soon after diagnosis, suggestive of pulmonary JMML infiltration. Post transplant, patients 1 and 2 are cured of JMML with full donor myeloid chimerisms, 2 and 4 years post HSCT respectively. Both patients had peripheral blood stem products. Patient 1 had acute graft versus host disease of the skin and gut, and RSV and ventilator induced chronic lung injury. Patient 2 did not have any major complications following transplant. The third patient received an umbilical cord blood HSCT, and while initially engrafted with 86% whole blood donor chimerisms, eventually died of hemorrhage post liver biopsy. Post-mortem examination confirmed recurrence of JMML in bone marrow in addition to a T-cell lymphoproliferative disease in the liver.
Discussion: Novel conditioning regimens are needed to reduce transplant related mortality while maximizing tumor eradication and engraftment in patients with JMML undergoing HSCT. Our

experience with three JMML patients show that a less cytotoxic, yet myeloablative conditioning regimen, has acceptable rates of engraftment and toxicity. Two of three patients are cured of JMML.
Disclosure of Interest: None Declared.

PH-P010
TREATMENT OF RELAPSED PEDIATRIC B-CELL PRECURSOR ALL WITH TRANSLOCATION T(12;21)(P13;Q22) BY MEANS OF ALLO-HSCT

N. Nikolaevich Mamaev[1], E. V. Semyenova[2], N. V. Stancheva[2], V. A. Katerina[3], A. V. Evdokimov[3], I. M. Barkhatov[3], E. G. Boychenko[4], T. L. Gindina[5,], V. M. Kravtsova[6], L. S. Zubarovskaya[2], B. V. Afanasyev[1]*
[1]Chair of Hematology and Transfusiology, [2]Children Transplantology, [3]Lab Molecular Transplantology, First Pavlov Medical University of Saint-Petersburg, [4]Children Hematology, First City Children Hospital, [5]Cytogenetic Lab, [6]Clinical laboratory, First Pavlov Medical University of Saint-Petersburg, Saint-Petersburg, Russian Federation

Introduction: Despite favorable prognosis of B- cell precursor pediatric acute lymphoblastic leukemia (ALL) with translocation t(12;21)(p13;q22), about 20% of them have late relapse after standard BFM protocols used in Europe, but not in the USA [Loh et a., 2006]. To our best knowledge, today only a single adult patient with such ALL variant was treated by alloHSCT.
Materials (or patients) and Methods: Here we present the results of alloHSCT in 10 pediatric patients (4 boys, 6 girls at the age of 4 to 17 years, mean – 9,8 years). The first remission duration ranged from 20 up to 70 months (mean – 39,9 months). Six patients were transplanted in the ≥2 hematological remissions, whereas 4 patients were grafted in relapse. Six patients received graft from matched related (*n*=3) or unrelated (*n*=3) donors, haploidentical HSCT was performed in four other pts. Conditioning regimens were myeloablative (8) and RIC (2). Nine patients successfully engrafted. Transplant failure occurred in 1 case wherein we have to perform additional haploidentical HSCT. Response was analyzed by means of serial RQ-PCR TEL/AML level expression. Serial donor chimerism and the count of bone marrow and peripheral blood blasts were measured.
Results: The study demonstrated that four of ten patients (all < 9 years old) had *TEL/AML1* gene expression at all stages of their disease, including pre- and post-transplant period. Donor chimerism as well as bone marrow and/or peripheral blood blasts were changed in concordance with it. In 3 cases *TEL/AML1* gene levels were absent or low before alloHSCT being negative after HSCT. Finally, in one 13 years old patient, there was a long-lasting molecular remission before and after alloHSCT, although a relapse occurred too. In general, seven our transplant patients are alive for 95 – 2545 (a mean of 778 days) including two in post-transplant relapses, whereas 3 died on days 20-263 after transplantation.
Discussion: A group of relapsed B-cell precursor ALL pediatric patients with t(12;21) is heterogeneous. Some of them (presumably younger patients) are resistant to chemotherapy and show high level of *TEL/AML1* gene expression both before and after alloHSCT. On the contrary, the other patients had better response to chemotherapy and molecular remissions. The observed difference in response on to chemotherapy is not clear yet. It might be connected with leukemia origin of mesenchymal cells in several patients which has been recently evidenced [Shalapour *et al*., 2010] and should be evaluated in further studies.
Loh ML, *et al*. /Blood 2006; 107(11): P. 4508-13.
Shalapour S., *et al*. / *J Mol Med* 2010; Vol.88: P. 249-65.
Disclosure of Interest: None Declared.

PH-P011
ANTI-THYMOCYTE GLOBULIN (ATG) COULD IMPROVE THE OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH AGGRESSIVE T CELL TUMORS

W. Chun[1,], Y. Shike[1], W. Liping[1], J. Jieling[2], C. Yu[1], Y. Jun[1]*
[1]Division of Hematology, [2] Division of Hematology, Shanghai Jiaotong University affiliated Shanghai First People's Hospital, shanghai, China

Introduction: Aggressive T-cell lymphomas represent 10% to 15% of non-Hodgkin's lymphomas in adults. Patients with relapsed or refractory disease are generally considered incurable with conventional therapies. ATG had been used in the conditioning regimen to reduce the incidence of GvHD for a long time especially in the matched unrelated donor HSCT. The early experiment result in our hospital showed that ATG inhibited the proliferation of lymphoid tumor cells in a dose-dependent manner especially in the T cell tumors. We used the ATG as the part of the conditioning regimen in the all patients and to evaluate the long-term antileukemia effect, the safety and complication in the patients with relapsed or high-risk T cell lymphomas.
Materials (or patients) and Methods: 18 patients(male 9, female 9) were enrolled into this study. Median patient age at the time of transplantation was 28 years (range, 7–55 years). At the time of transplant, 4 patients reached first or subsequent complete response (CR) with conventional therapy or the salvage therapy, 4 patients had a partial remission(PR), 7 patients had relapsed disease not responding to salvage therapy or progressive disease, and 3 patients had primary refractory disease. Donors were 10/10 HLA matched related (5), 10/10 matched unrelated (3), 8/10 matched unrelated (5) and mismatched related (5). The median number of CD34+ cells within the allografts was 9.31/kg body weight (BW) (range, 4.6–24.85/kg BW). Rabbit antithymocyte globulin (ATG 2.5 mg/kg×4 days) and total-body irradiation (10 Gy in five fractions) were used in all 18 patients. All patients but one also underwent cyclophosphamide (120 mg/kg). The only one who did not receive CTX had experienced with autologous transplantation. Fifteen high risk patients were in additional use of VP 16 or VM26 30-40 mg/kg. Two patients in CR1 and one patient who were 55 years old had not received VP16. Graft-versus-host disease (GVHD) prophylaxis was cyclosporine based, usually in combination with methotrexate. Quantitative chimerism analyzes were performed using short-tandem-repeat-based polymerase chain reaction techniques at regular intervals for every 4 weeks after transplantation in bone marrow at the first six months.
Results: All patients but one achieved a complete remission in the first three months after allogeneic HSCT. One patient achieved PR on month 1 and soon died from progressive disease. The CR rate after transplantation was 94.4%. At a median follow-up time of 13 months, fourteen(77.8%) patients are alive. OS at three years was 71%. Still four patients were died after transplantation, two from relapse and two from treatment related complications. Acute GvHD grades II-IV occurred in eight patients(50%) and grades III-IV in four patients. Two patients suffering from acute GvHD grade IV died due to treatment-related complications. The maximum cumulative incidence of cGVHD was 42.8%. The high levels of CMV DNA were detected in seventeen patients in the first three months after transplant. Two of them gradually envolved into viral haemorrhagic cystitis. One patient suffered from aspergillus pneumonia while another two suffered from bacterial pneumonia. There was no case of venous occlusive disease.
Discussion: Anti-thymocyte globulin (ATG) could improve the outcome of allogeneic hematopoietic stem cell transplantation in patients with aggressive T cell tumors.
Disclosure of Interest: None Declared.

PH-P012

THIOTEPA-BASED VS TBI-BASED MYELOABLATIVE CONDITIONING PRIOR TO ALLOGENEIC STEM CELL TRANSPLANTATION (HSCT) FOR ACUTE MYELOID LEUKEMIA (AML) IN FIRST COMPLETE REMISSION (CR1): A RETROSPECTIVE ANALYSIS FROM THE ALWP OF THE EBMT

S. Eder[1,], M. Labopin[1], W. Arcese[2], R. Or[3], I. Majolino[4], A. Bacigalupo[5], G. de Rosa[6], L. Volin[7], D. Beelen[8], H. Veelken[9], N. P. Schaap[10], J. Kuball[11], J. Cornelissen[12], A. Nagler[13], M. Mohty[14] and ALWP*

[1]EBMT Office Paris, Hôpital Saint-Antoine, Paris, France, [2]Rome Transplant Network "Tor Vergata", University of Rome Stem Cell Transplant Unit, Rome, Italy, [3]Department of Bone Marrow Transplantation, Hadassah University Hospital, Jerusalem, Israel, [4]Haematology & SCT Unit, Ospedale S. Camillo, Rome, [5]Department of Haematology II, Ospedale San Martino, Genova, [6]Division of Hematology, University of Napoli Federico II Medical School, Napoli, Italy, [7]Helsinki University Central Hospital, Helsinki, Finland, [8]Department of Bone Marrow Transplantation, University Hospital of Essen, Essen, Germany, [9]Department of Hematology, Leiden University Medical Center, Leiden, [10]Department of Hematology, Radboud University Medical Centre, Nijmegen, [11]Department. of Haematology, University Medical Centre, Utrecht, [12]Erasmus MC-Daniel den Hoed Cancer Centre, Rotterdam, Netherlands, [13]Chaim Sheba Medical Center, Tel Hashomer, Israel, [14]Service d'Hématologie et Thérapie Cellulaire, Hôpital Saint Antoine, Paris, France

Introduction: Thiotepa is an alkylating compound with an antineoplastic activity and has been increasingly used for HSCT-conditioning. Interestingly, this agent has a very active myeloablative activity and can mimic the effect of radiation. The aim of this study was to compare outcome of patients receiving a myeloablative conditioning consisting of either high-dose TBI or Thiotepa-based chemotherapy.

Materials (or patients) and Methods: Inclusion criteria were adults with AML, first allograft in CR1 from an HLA-matched sibling donor (MSD) or an unrelated donor (UD) between 2000 and 2011 and myeloablative conditioning. We first compared patient and transplant characteristics between the two types of conditioning, and then performed a matched-pair analysis.

Results: The number of patients was 2833 in the TBI group and 102 in the Thiotepa group. Patients who received Thiotepa were older (49y vs 40y, $P<10\text{-}4$), transplanted more recently (2009 vs 2006, $P<10\text{-}4$) and later after the diagnosis of AML (183 days vs 143 days, $P<10\text{-}4$). The percentage of secondary AML was also higher in the Thiotepa group (14% vs 6%; $P=0.0002$). There was no difference regarding patient/donor gender, type of donor and source of stem cells. In this cohort, we were able to match 96 patients who received Thiotepa with 185 patients who received high-dose TBI. Matching factors were: age and year at transplantation, interval from diagnosis to transplant, secondary AML and type of donor (MSD/UD). 54% of patients in both groups were female; donor sex was male in 55% in the TBI group and 66% in the Thiotepa group. Bone marrow was the stem cell source in 26% in the TBI group and 27% in the Thiotepa group; the remaining patients received PBSC. Median dose of TBI was 12 Gy (range, 8-16). In this group, TBI was combined with Cyclophosphamide (84% of cases), Fludarabine (14%) or other compounds (2%). On the other hand, Thiotepa was administered with Cyclophosphamide (45%), Fludarabine (54%) with/without Busulfan and other combinations (1%). Engraftment occurred in 96% of patients using Thiotepa-based conditioning versus 99% after TBI ($P=0.11$). The interval from transplant to neutrophils count>500/μL was 16 days (range, 9-42) versus 17 days (range, 9-81) in the 2 groups, respectively ($P=0.23$). Acute GvHD grade II+ was observed in 25 patients (27%) after Thiotepa-containing regimen versus 42 patients (25%) after TBI ($P=0.78$). 2-years cumulative incidence of chronic GvHD was 48±4% and 41±6% in the 2 groups, respectively (p=0.15). The 2-year cumulative incidences of non-relapse mortality (NRM) was 21±4% versus 27±4% ($P=0.57$) and relapse incidence (RI) was 18±4% versus 21±3% ($P=0.71$) in the Thiopeta and TBI groups, respectively. The 2-year leukemia-free survival (LFS) and overall survival (OS) were 61±5% and 64±5% in the Thiotepa group versus 51±4% and 52±4% in the TBI group (LFS: $P=0.40$; OS: $P=0.25$).

Discussion: This matched-pair analysis suggests that a Thiotepa-based myeloablative conditioning regimen prior to allogeneic HSCT in AML in first CR, can allow achieving similar results to high-dose TBI-based myeloablative conditioning. Also, given the deleterious long term side effects of TBI, it is likely that a Thiotepa-based myeloablative conditioning would represent an attractive and valid alternative to TBI.

Disclosure of Interest: S. Eder: None Declared, M. Labopin: None Declared, W. Arcese: None Declared, R. Or: None Declared, I. Majolino: None Declared, A. Bacigalupo Conflict with: Adienne, G. de Rosa: None Declared, L. Volin: None Declared, D. Beelen: None Declared, H. Veelken: None Declared, N. P. Schaap: None Declared, J. Kuball: None Declared, J. Cornelissen: None Declared, A. Nagler: None Declared, M. Mohty Conflict with: Riemser.

PH-P013

AZACYTIDINE IN THE TREATMENT OF ACUTE MYELOID LEUKEMIAS RECURRING AFTER ALLOGENEIC TRANSPLANTATION: A SINGLE CENTER EXPERIENCE.

M. Malagola[1,], V. Cancelli[1], A. Turra[1], R. Ribolla[1], F. Schieppati[1], F. Cattina[1], C. Bergonzi[1], C. Fili[1], C. Skert[1], D. Russo[1]*

[1]Chair of Hematology, Bone Marrow Transplant Unit, University of Brescia, AO Spedali Civili Brescia, Brescia, Italy

Introduction: Acute Myeloid Leukemias (AML) recurring after allogeneic stem cell transplantation (allo-SCT) have a very dismal prognosis. Conventional salvage treatments may include intensive chemotherapy eventually followed by donor lymphocyte infusions (DLI) or a second allo-SCT. Although with intensive chemotherapy a second complete remission (CR) may be achieved, its duration is often short and its toxicity may preclude any further consolidation or a second allo-SCT. On the other hand, DLI alone are often unable to control a leukemic recurrence. Azacytidine is a demethylating agent which has proven efficacy in high-risk myelodisplastic syndromes and AML, especially those with low disease burden at onset (blast cells infiltration 20 – 30%). Clinical data suggest that Azacytidine may induce responses in up to 30% of these patients and biological data suggest that, apart from the direct demethylating, antiprolipherative and cytotoxic effects on the leukemic cells, it might also influence the donor immune system, enhancing the Graft versus Leukemia (GvL) effect.

Materials (or patients) and Methods: We report here a series of 6 patients submitted to allo-SCT for AML in first ($n=4$) or second ($n=2$) CR. Median age at transplant was 50 years (range 41-62). Three patients received a myeloablative conditioning regimen. The donor was a matched sibling in 2 cases and a matched unrelated in 4 cases. Two of these patients developed grade II aGVHD after allo-SCT, successfully treated with standard steroid. The disease recurrence was observed at a median of 7 months from allo-SCT (range: 3 – 48). At relapse, the disease presented with blast cell marrow infiltration, not exceeding the 20% in all the cases. All the patients had already stopped the immunosuppressive treatment with the exception of one of those who developed aGVHD who was tapering the steroid.

Results: Azacytidine was administered at the conventional dose (75 mg/sqm sc/day for 7 days every 28 days). The first response evaluation was performed after the fourth cycle. Patients showing a response or a stable disease continued the treatment until disease recurrence or progression. The median number of cycles administered is 4 (range 1 – 12). Two patients are not evaluable for response and toxicity as the have just completed the first two treatment cycles. Among the four evaluable patients, 1 achieved a CR that lasted for 18 months and is alive with active disease, 1 maintained a stable disease (SD) for 3 months and then progressed and died and 2 were non responders and died for disease progression. Overall the treatment was well tolerated. Two of the 4 evaluable patients (50%) developed a grade II–III WHO haematological toxicity which was treated with study drug dose reduction. No grade III-IV WHO extra-hematological toxicity was observed.

Discussion: Our experience, although limited, suggest that azacytidine may be effective and is well tolerated in patients with AML relapsing after allo-SCT. In particular, long lasting remission

may be achieved. Prospective trials are warranted to better define which subset of patients may benefit from this treatment strategy best.
Acknowlegmets: this work was supported in part by Lions Club Bassa Brescian, Banca di Credito Cooperativo di Pompiano e Franciacorta, fondi 60% Porf Domenico Russo, fondi 60% Dr Michele Malagola.
Disclosure of Interest: None Declared.

PH-P014
THE EXPERIENCE TO USE COMBINATION OF LOW DOSE CYTARABINE WITH CLADRIBINE BEFORE ALLOGENEIC STEM CELL TRANSPLANTATION

S. Vasiljevitch Gritsaev[1,], A. A. Kuzjaeva[1], I. M. Zapreeva[1], I. I. Kostroma[1], I. S. Martinkevitch[2], Z. V. Chubukina[3], K. M. Abdulkadyrov[1]*
[1]Hematological Clinic, [2]Cytogenetic Laboratory, [3]Immunologic Laboratory, Russian Institute Of Hematology And Blood Transfusion, St.Petersburg, Russian Federation

Introduction: It is a great problem to get complete response in patients with resistant acute myeloid leukemia (AML) before allogeneic stem cell transplantation. There are many patients with resistant AML treated with high dose chemotherapy unsuccessfully. On the other hand it is a possibility to decrease the somatic status and to get much more comorbidities after some course of aggressive chemotherapy which may exclude patient from the group of candidates to stem cell transplantation. It has been shown the capability of cytarabine (Ara-C) combined with purine analogues to improve the results of AML patients treatment. We used combination of low dose Ara-C with cladribine to prepare intensively pretreated patients with resistant AML before allogeneic stem cell transplantation.
Materials (or patients) and Methods: We used the scheme of Ara-C 20 mg sc bid for 14 consecutive days combined with cladribine 5 mg/m/2 IV once a day during the first 5 days. The therapy was begun after informed agreement was signed.
Results: Six patients aged 24-58 years (Me 55 years) were treated with combine chemotherapy.
One of the patients did not get any response after induction chemotherapy 7+3 and 2 courses of high dose chemotherapy. Other 5 patients had relapsed AML after different period of complete response including a patient after autologous stem cell transplantation. Normal karyotype was detected in 2 patients. Other patients had different chromosomal aberrations including a patient with complex karyotype with 2 monosomies which was detected during relapse for the first time. Nobody of patients had *FLT3*, *NPM1* and *c-Kit* mutations. Coexpression of CD4, CD22, and CD7 was determined on the myeloblasts of 3 patients.
Combined chemotherapy was effective in 3/6 patients. The response was confirmed after the first course. The patients with response were treated with additional induction course. Then one patient was treated with high dose consolidation. Allogeneic stem cell transplantation fully matched related and unrelated donors was performed in 2 patients. The period from obtained response till the start of conditioning regimen was 2 and 4 months. Myeloablative regimen Bu + Cph was chosen for both patients. The number of bone marrow blasts was increased to the level higher than 5% in one patient before the initiating of conditioning regimen.Complete response with full donor chimerism is preserved during 3 and 16 months after stem cell transplantation.
Discussion: We conclude that low intensity chemotherapy like combination of low dose Ara-C with cladribine may be a very effective kind of treatment in some patients with resistant variant of AML who were aggressively pretreated. We suppose that to save the response during the pretransplant period it is necessary to use high dose chemotherapy after complete remission was revealed. The mechanism of action of low intensity chemotherapy in patient with resistant AML is unknown. One possible explanation is absence of unfavorable mutations irrespective of cytogenetic findings.
Disclosure of Interest: None Declared.

PH-P015
OUTCOME OF HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA (AML) OR MYELODYSPLASTIC SYNDROME (MDS) WITH ADVERSE CYTOGENETICS

C. Monica Gorcea[1,], C. Lobetti-Bodoni[1], F. Dignan[1], J. Woodward[1], M. Waller[1], E. Tholouli[1]*
[1]Clinical Haematology, Manchester Royal Infirmary, Manchester, United Kingdom

Introduction: The cytogenetic profile is an important prognostic factor in the myeloid malignancies. Monosomy 7 and/or a complex karyotype are known to correlate with poor prognosis and poor disease-free survival when a conventional approach is undertaken. We sought to analyse the role and outcome of hematopoietic stem cell transplantation (HSCT) in poor risk AML and MDS in adults in our centre.
Materials (or patients) and Methods: A retrospective case analysis was performed for 28 patients (10 female, 18 male) who were treated at Manchester Royal Infirmary between Jan 2007 and Dec 2012. We have analysed the outcome of patients with cytogenetically defined poor-risk AML or MDS treated with chemotherapy followed by matched related (MRD), or voluntary unrelated donor (VUD) transplantation in CR1 or CR2. Most of our patients received 1 line of chemotherapy (*n*=16), 11 received 2 lines and only 1 patients required a 3rd line. The median time from diagnosis to HSCT was 8 months (range 1-88). Poor-risk cytogenetics were defined as monosomy 7 and/or complex karyotype as per MRc AML criteria. Primary diagnoses were AML (*n*=13), therapy-related AML (*n*=8), MDS (*n*=5) and therapy-related MDS (*n*=2). The median age was 53 years (range 23 and 72). After initial chemotherapy, 20 patients received a reduced intensity conditioning (RIC) with Fludarabine Melphalan Campath (FMC) or Fludarabine Busulphan Antithymocyte globulin (FluBu- ATG)conditioning regimens and 8 received myeloablative contidioting (MAC) with different conditioning regimens (BuCy; Cy- Total body irradiation(TBI); BuCyCampath or FluCyTBICampath). A total of 16 patients received VUD, 2 umbilical cord blood (UCB) and 10 MRD.
Results: The median follow-up of our cohort was 26 months (range 7-116). The overall survival (OS) at 1 year, 2 years and 5 years was 89%, 63% and 36% respectively with a transplant-related mortality (TRM) of 11% at 1 year and 15% at 2 years. The TRM was accounted for by cytomegalovirus reactivation or sepsis with subsequent multi-organ failure. The non-TRM was predominantly secondary to leukaemic relapse. The progression-free survival (PFS) was 82%, 63% and 30% at 1, 2 and 5 years respectively. Of the total of 28 patients, 8 developed acute graft-versus-host disease (GVHD) of the skin, 3 of gastro-intestinal tract and 3 of the liver with no GVHD-related mortality.
Discussion: Our findings show that HSCT is a valid option in AML and MDS with adverse cytogenetics with potential long term disease remission and improved overall survival.
Disclosure of Interest: None Declared.

PH-P016

IMPACT OF NPM1 MUTATION ON THE OUTCOME OF PATIENTS WITH NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA AND ALLOCATED TO AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION IN FIRST COMPLETE REMISSION

A. Garrido[1,], S. Brunet[1], M. Hoyos[1], J. Esteve[2], J. M. Ribera[3], L. Escoda[4], M. Tormo[5], R. Guardia[6], P. Torres[7], M. Arnan[8], M. P. Queipo de Llano[9], A. Garcia[10], J. M. Martí[11], C. Pedro[12], M. A. Sampol[13], J. Bargay[14], O. Salamero[15], L. Font[16], I. Heras[17], J. Gonzalez[18], A. Oriol[3], M. Pratcorona[2], J. Sierra[1] and CETLAM cooperative group*

[1]Hematology Department, Hospital de la Santa Creu i Sant Pau, [2]Hematology Department, Hospital Clínic Barcelona, Barcelona, [3]Hematology Department, ICO Badalona- Hospital Germans Trias i Pujol, Badalona, [4]Hematology Department, Hospital Joan XXIII, Tarragona, [5]Hematology Department, Hospital Clínico Valencia, Valencia, [6]Hematology Department, ICO Hospital Josep Trueta, Girona, [7]Hematology Department, Hospital Juan Canalejo, A Coruña, [8]Hematology Department, ICO- Hospital Duran i Reynals, Barcelona, [9]Hematology Department, Hospital Clínico Málaga, Málaga, [10]Hematology Department, Hospital Arnau de Vilanova, Lleida, [11]Hematology Department, Mutua de Terrassa, Terrassa, [12]Hematology Department, Hospital del Mar, Barcelona, [13]Hematology Department, Hospital Son Espases, [14]Hematology Department, Hospital Son Llatzer, Palma de Mallorca, [15]Hematology Department, Hospital de la Vall d'Hebrón, Barcelona, [16]Hematology Department, Hospital Verge de la Cinta, Tortosa, [17]Hematology Department, Hospital General de Murcia, Murcia, [18]Hematology Department, Hospital Virgen del Rocío, Sevilla, Spain

Introduction: Improvements in genetic characterization of acute myeloid leukemia (AML) have allowed risk-adapted post remission treatment for AML. In this regard, the main disease aspects considered are cytogenetics and molecular profile, mainly NPM1 and FLT3 gene mutations. We have analyzed the results of autologous hematopoietic cell transplant (AHCT) in patients with normal karyotype (NK) AML without FLT3 internal tandem duplication (FLT3-ITD) or other adverse features, depending on the presence or not of NPM1 mutation.

Materials (or patients) and Methods: Patients had been enrolled in the AML-99 and AML-03 CETLAM trials. We included patients with NK, no FLT3-ITD, who had achieved complete remission (CR) after a single induction course and, in the most recent trial, without MLL rearrangements and minimal residual disease (MRD) after consolidation chemotherapy. In the two trials the intention in these patients was to perform an AHCT, regardless the availability of an HLA-matched donor.

One-hundred eleven patients fulfilled the mentioned criteria. Characteristics of these patients were: 54 male (49%) and 57 female (51%); 63 patients ≤50 years-old (yo) (57%) and 48 >50 yo. (43%); NPM1 mutation was observed in 53 patients (48%) and was absent in 58 (52%).

Fifty nine of the 111 patients finally received the AHCT. Characteristics of transplanted patients were: 28 male (47.5%) and 31 female (52.5%); 39 patients ≤50 yo (66.1%) and 20 >50 yo (33.9%); NPM1 was mutated in 35 of these patients (59.3%) and non-mutated in 24 patients (40.7%).

The causes of the 52 patients who did not receive the planned AHCT were: stem cell mobilization failure (*n*=25, 48%), allogeneic procedure (*n*=2, 4%), prolonged aplasia (*n*=4, 8%), consolidation chemotherapy due to NPM1 mutation (protocol deviation, *n*=4, 8%), relapse before AHCT (*n*=8, 15%) and other non-specified causes (*n*=9, 17%).

Results: Five-year overall survival (OS), disease free survival (DFS) and cumulative incidence of relapse (CIR) in the 111 patients (intention to treat AHCT) were: 61±5%, 51±5% and 45±3% respectively. When comparing patients with or without NPM1 mutation, OS was 71±7% vs 51±7% (p=0.068), DFS 61±7% vs 41±7% (p=0.008) and CIR was 35±7 vs 54±8 (0.004), respectively.

Similarly, 5 years OS, DFS and CIR in the 59 patients who finally received the AHCT were 59±7%, 48±7% and 47±5%, respectively; the values according to NPM1 status were: OS 65±9% in patients with NPM1 mutated vs 48±11% in NPM1 not mutated (*P*=0.179), DFS was 55±9% vs 39±11% (*P*=0.071) and CIR was 38±8% vs 61±11% (*P*=0.031).

Discussion: Patients with NK, NPM1 mutation and FLT3 wt should be considered as a favourable prognosis. Patients with this characteristics and AHCT as an intention to treat had better DFS and inferior CIR compared with those NPM1 not mutated. Furthermore, patients with NPM1 mutation who finally were transplanted had a significant inferior CIR. AHCT had a role in intensification therapy in these patients.

Disclosure of Interest: None Declared.

PH-P017

5-AZACYTIDINE IN MYELOID MALIGNANCY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

V. Ovechkina[1,], S. N. Bondarenko[1], E. V. Morozova[1], A. D. Kulagin[1], O. A. Slesarchuk[1], N. N. Stancheva[1], A. A. Ratc[1], L. S. Zubarovkaya[1], B. V. Afanasyev[1]*

[1]R.Gorbacheva Memorial Institute of Children Oncology, Hematology and Transplantation, First State Pavlov Medical University of St. Petersburg, St Petersburg, Russian Federation

Introduction: Relapse of myeloid malignancies after allogeneic hematopoietic stem cell transplantation (alloHSCT) is associated with a very poor prognosis. The aim of this study was to assess the effect of 5-azacitidine (5-aza) administration as prophylaxis and treatment of relapse after alloHSCT.

Materials (or patients) and Methods: Results of treatment with 5-aza after alloHSCT for 20 patients with myeloid malignancies (AML 80% (16), MDS 20% (4)) was evaluated. 2 patients were grafted from matched family donor, 16 - matched unrelated donor, 2- haploidentical). Median age was 26 years, range 4-56, 11 male, 9 female. Conditioning regimen was myeloablative in 4 (20%) cases, reduced intensity conditioning was used in 16 (80%) cases. In 50% (10) cases the administration of 5-aza was prophylactic, because of the high relapse risk: 6 patients had advanced disease at the moment of alloHSCT, the unfavorable cytogenetic was detected in 3 cases, 1 patient had the minimal residual disease at the moment of alloHSCT. Median time for administration of 5-aza was day+30 – day+60 after alloHSCT; the main criteria was the recovery of hemopoiesis; 5-aza was injected subcutaneously 35mg/m^2/daily, 7 days of 28-day cycle, 4 cycles. In case of relapse (bone marrow blasts > 5%) the median number of 5-aza cycles administrated was 2, range 1-6. As the therapy of relapse 5-aza was combined with donor lymphocyte infusion or chemotherapy in 80% (8). Median time of administration was day+165 (36-270).

Results: Median duration of follow-up in both groups was 336 days (65-1270). In the group of prophylactic administration 80% patients are still alive in complete remission, 20% died because of late relapses, no facts of treatment related mortality (TRM) were registered. Median duration follow-up in this group was 351 day (133-1035). 5-aza was used as a treatment of relapse in 10 (50%) patients. Remission was achieved in 20% (2 patients), 8 patients had progressive disease. 2 patients are still alive, median of follow-up in this group is 322 (65-1270) days. 8 patients died: 6 (75%) from relapse, 2 (25%) from TRM (sepsis, myocardial infarction). 5-aza therapy was well-tolerated, no severe hematological and not-hematological toxicity was observed.

Discussion: Based on our preliminary analysis 5-aza is effective for prevention of relapse and improvement of overall and disease-free survival in a high-risk group. Administration of 5-aza as a therapy for relapse had relatively low efficacy. Further larger prospective, randomized trials are needed for more solid conclusions.

Disclosure of Interest: None Declared.

PH-P018

OUTCOMES OF AML PATIENTS WHO RELAPSE AFTER ALLOGENEIC STEM CELLTRANSPLANTATION

L. Cudillo[1,], R. Cerretti[1], A. Picardi[1], G. De Angelis[1], F. Di Piazza[1], E. Ceresoli[1], M. G. Cefalo[2], B. Mariotti[1], A. Biagi[1], T. Dentamaro[3], A. Mengarelli[4], A. Chierichini[5], A. Ferrari[6], M. C. Tirindelli[7], W. Arcese[1]*

[1]Stem Cell Transplant Unit, [2]Hematology, Tor Vergata University, [3]Hematology, S.Eugenio Hospital, [4]Hematology, Department of Hematology, Regina Elena Institute, [5]Hematology, S.Giovanni Hospital, [6]Hematology, S.Andrea Hospital, [7]Hematology, Campus Biomedico University, Roma, Italy

Introduction: Leukemia relapse remains a major cause of failure and death after allogeneic stem cell transplantation (SCT). Herein, we report the retrospective analysis on outcome of AML patients who relapsed after SCT.

Materials (or patients) and Methods: Between 2006 and 2012, 132 AML patients in early (1st and 2nd CR; *n*=102) or advanced (*n*=30) phase underwent SCT for high risk disease characteristics. Of these patients, 33 (25%) subsequently relapsed. Demographic and transplant characteristics were as follows: 21 males and 12 females; median age 52 years (range 18-64 yrs), 17 of 102 (17%) patients had been transplanted in early phase and 16 of 30 (53%) in advanced phase. The sources of graft were HLA-id sibling in 21 patients, haploidentical donor in 7 and matched unrelated donor in 5. The conditioning regimen was myeloablative in 18 patients and at reduced intensity in 15. Fifteen patients experienced acute GVHD of grade I-II, while limited chronic GVHD was observed in only 2 of 33 evaluable patients.

Results: The relapse, which was hematologic in 31 and extramedullary in 2, occurred at median time of 5 months from SCT (range 1.5-39). The immunosuppressive treatment was immediately discontinued to all patients still on therapy. Then, chemotherapy alone was given to 18 patients, donor lymphocyte infusions (DLI) ± chemotherapy ± 2nd SCT in 7 patients, intrathecal chemotherapy + radiotherapy in 2 patients with isolated meningeal relapse and mild therapy and/or supportive care alone in 6 patients. GVHD occurred in 4 patients after withdrawal of immunosuppression and in 3 after DLI infusions. After salvage therapy 26 (78%) patients showed a resistant disease and 7 (21%) patients (hematologic, *n*=5; extramedullary, *n*=2) entered CR. Four of 5 patients with hematologic recurrence relapsed again at a median time of 4.5 months (range 3-10). Thirty patients (91%) died: 29 of refractory/relapsed disease and 1 patient with extensive chronic GVHD died in CR of infectious complication. The median time of overall survival after relapse was 3 months (range 0.1-57). Only 3 patients are alive in CR (2 after isolated meningeal relapse and 1 after marrow relapse), at 19, 35 and 57 months from relapse, respectively.

Discussion: AML relapsing after SCT is a particularly aggressive disease, patient outcome is extremely poor and salvage treatment resulted in sustained CR in a small cohort only. Monitoring of minimal residual disease and pre-emptive therapy could offer a better approach in this unfavourable patient group.

Disclosure of Interest: None Declared.

PH-P019

HIGH PRE-TRANSPLANT CD4 T LYMPHOCYTE COUNT AND DONOR HLA MISMATCH MAY CONTRIBUTE TO RELAPSE OF ACUTE LEUKAEMIA AFTER MYELOABLATIVE HAEMOPOIETIC STEM CELLS TRANSPLANTATION (HSCT)

N. Jacobsen[1,], C. Heilmann[2], N. Keiding[3], H. Sengeløv[1], L. P. Ryder[4]*

[1]Department of Haematology, [2]Department of Paediatrics, Rigshospitalet, [3]Section of Biostatistics, Faculty of Health Sciences, University of Copenhagen, [4]Department of Clin. Immunol/Tissue Typing, Rigshospitalet, Copenhagen, Denmark

Introduction: It has been assumed that HSCT recipients with an unrelated or HLA mismatched donor run a reduced risk of post-transplant relapse of acute leukaemia. We here suggest that this may be otherwise. In addition, we report that AML recipients with a high pre-conditioning CD4 T cell count have an increased relapse risk.

Materials (or patients) and Methods: This is a single centre study on children and adults with AML (*N*=89) or ALL (*N* = 100) in 1st or 2nd remission who received non-T-depleted myeloablative HSCT between 1998 and 2006. Mean recipient age was 26 yrs. (range 1-56). Donors were either an HLA identical sibling (Id. Sib.) or an alternative (unrelated) donor. Alternative donors were either identical at 10/10 loci by high resolution HLA typing or had documented or non-excluded HLA mismatch. The pre-transplant conditioning was TBI 12 Gy (87%) or i.v. Busulfex (13%) with cyclophosphamide or VP16. Antithymocyte globulin (ATG, ATGAM 20 mg pr.kg pr. dose, or Thymoglobuline 2.5 mg pr.kg pr. dose) was given for a total of three doses day -3 to day -5 before HSCT. ATG was given to none of 77 patients with Id. Sib. donor, and to 93 of 112 patients with alternative donors. Blood CD4 and CD8 T-cells were assessed by flow cytometry prior to start of conditioning and their effects on relapse were analyzed as continuous variables using Cox multivariate regression.

Results: During analysis of risk factors for post-transplant relapse of acute leukaemia we noted an increased risk associated with recipient pre-transplant CD4 T-cell count (high worse than low), and with the use of an alternative donor. In contrast, the use of ATG during conditioning reduced the risk. We further analysed these effects in an unselected group of 189 recipients with AML or ALL in 1st or 2nd remission. Main results include: 1) The increased relapse risk associated with CD4 was restricted to AML recipients. 2) In recipients with AML and Id. Sib. donor (*N* = 44) the relapse hazard rate (HR) was 5.8 (*P* = 0.028). No similar effect was found in ALL. 3) In patients with any donor (Id. Sib. or alternative) the effect of CD4 was still seen in AML patients, but only in recipients who did not receive ATG during pre-transplant conditioning (*N* = 53. HR = 8.04, *P* = 0.004). The adverse effect was completely abrogated in ATG conditioned AML patients. In ALL patients no effect of CD4 was observed regardless of ATG (*N* = 100). 5) There was an increased relapse risk associated with donors with non-excluded or documented HLA mismatch. This adverse donor effect was present both in AML and ALL and was also abrogated by ATG (AML + ALL without ATG: *N*= 96, HR = 16.36, *P* = 0.0001).

Discussion: High pre-conditioning recipient CD4 T lymphocyte count was associated with increased relapse in AML. This effect was abrogated by ATG during conditioning. An adverse effect was associated with alternative donors. We therefore suggest 1) that CD4 T lymphocytes of recipient origin survive conditioning regimens, if ATG is omitted. 2) Surviving recipient CD4 T lymphocytes may contribute to a reduced graft versus leukaemia effect in AML. 3) HLA mismatch may potentiate this adverse effect. 4) When mismatched donor HSCT in the past has been associated with a reduced relapse risk, this effect could in some cases have been a result of ATG rather than the mismatch.

Disclosure of Interest: None Declared.

PH-P020

LINES OF PRE-TREATMENT AND BLAST COUNT PRIOR TO HCT IS PREDICTIVE FOR SURVIVAL IN REFRACTORY AML PATIENTS TREATED WITH FLAMSA-RIC

C. Pfrepper[1,], A. Klink[2], G. Behre[1], T. Schenk[2], G.-N. Franke[1], M. Jentzsch[1], S. Schwind[1], H. K. Al-Ali[1], A. Hochhaus[2], D. Niederwieser[1], H. Sayer[2]*

[1]Hematology, Oncology, Hemostasis, University Hospital Leipzig, Leipzig, [2]Hematology, Oncology, University Hospital Jena, Jena, Germany

Introduction: Hematopoietic stem cell transplantation (HCT) is the standard treatment for high risk acute myeloid leukemia (AML) in first or second complete remission (CR). Unfortunately not all patients achieve complete remission prior to HCT. The aim of this study was to find predictors for survival after HCT for refractory AML after FLAMSA-RIC conditioning.

Materials (or patients) and Methods: We retrospectively analyzed the outcome of 49 consecutive patients (median age 49; range 21-65 years) transplanted at the University Hospitals of Jena

and Leipzig for refractory AML between 2006 and January 2013. All patients had active disease prior to HCT, 46 patients with a median of 25 (range 5.3 – 90) % bone marrow blasts and three patients with extramedullary myeloid blast infiltration or blasts in peripheral blood. Primary induction failure (PIF) was the reason for HCT in 20 patients while 29 patients had first or higher refractory relapse. Thirty one patients had one or two lines of pre-treatment while 18 patients had more than two lines of pre-treatment. Conditioning for HCT was performed with chemotherapy consisting of fludarabine (4x30mg/m^2), cytarabine (4x2g/m^2) and amsacrine (4x100mg/m^2) followed 4 days later by 4 Gy total body irradiation in 38 patients or busulfan (8x1mg/m^2) in 11 patients, combined with cyclophosphamide (120mg/m^2). Thymoglobine (3x2mg/m^2) was given when unrelated donors were used (FLAMSA-RIC).
Results: Estimated overall survival (OS) and event free survival (EFS) at 3 years after a median follow up of 36 (range 6-71) months was 16% and 11%, respectively. Causes of death were relapse in 59%, infection in 14% and graft versus host disease (GvHD) in 10% of all patients. Twenty eight patients (57%) achieved CR four weeks after HCT while nine patients had partial remission (PR), nine patients had stable disease (SD) and three patients had either no evaluable bone marrow examination or died before due to infections. Another seven patients with PR and SD achieved CR (overall CR rate 71%) from four weeks to day 90 after HCT following reduction of immunosuppression.
Risk factors for OS were blast count of ≥ 20% prior to HCT with an OS at 3 years of 4 % compared to 34% in patients with blast counts of < 20% (*P*=0.02) and a trend for patients with ≥3 vs. <3 lines of pre-treatment (5.6 vs. 22%; *P*=0.08). Pre-treatment lines were also a risk factor for EFS. Patients with <3 lines of pre-treatment had a better EFS of 18% than patients with ≥3 lines (0%; *P*=0.05). Limited chronic GvHD resulted in EFS of 29% compared to 15% in patients without chronic GvHD (*P*=0.06). This was due to a lower RI of 48% compared to 76% (*P*=0.06). Combining patients with low blast count and less than 3 lines of pre-treatment (*n*=15) resulted in an OS of 38% at 3 years, compared to 7% in the other group (*P*=0.02). Occurrence of acute GvHD and cytogenetic risk factors did not influence OS, EFS or RI significantly.
Discussion: In this study we document an OS of 16% at 3 years for patients with refractory AML after HCT with FLAMSA-RIC. Outcome is dependent on blast count prior to HCT, lines of pre-treatment and the incidence of chronic limited GvHD.
Disclosure of Interest: None Declared.

PH-P021
THE PROGNOSTIC SIGNIFICANCE OF INTERMEDIATE AND UNFAVORABLE CYTOGENETICS TO PREDICT OUTCOME IN DENOVO ACUTE MYELOID LEUKEMIA (AML) AFTER STEM CELL TRANSPLANTATION (SCT)

M. Yaghmaie[1,], K. Alimoghaddam[1], M. Jahani[1], S. A. Moosavi[1], B. Bahar[1], A. A. Hamidieh[1], M. Vaezi[1], E. Gheysari[1], E. Torkamaniha[1], N. Gerayeli[1], A. Jalali[1], A. Ghavamzadeh[1]*
[1]Tehran University of Medical Sciences, Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran, Iran, Republic Of Islamic

Introduction: Cytogenetic abnormalities detected at diagnosis are recognized as important in predicting response to chemotherapy in AML. However, there is controversy concerning the prognostic significance of karyotype for outcome after SCT.
Materials (or patients) and Methods: In this single-institution report, we conducted a retrospective analysis of the impact of intermediate and unfavorable cytogenetics according to MRC classification at the time of primary diagnosis on outcome after SCT in de novo AML. The study included 343 patients who underwent SCT (74 % Allogenic SCT, 25.4% Autologous SCT and 0.6% syngenic) at hematology, oncology and stem cell transplantation research center, Shariati Hospital between Oct. 1993 and July 2013.
Results: Intermediate and unfavorable cytogenetics were found in 303 (88.3%) and 40 patients (11.7%), respectively. The median follow-up of survivors was 28 months. The 3-year OS for patients with intermediate and unfavorable cytogenetics who underwent allo-SCT were 66.7%(SE 3.6%) and 30.3%(SE 10.6%), respectively (*P*= 0.001) and for the patients who underwent auto-SCT in these two cytogenetic groups were not statistically significant.The cumulative incidence function (CIF) of relapse at 36 months after allo-SCT in the intermediate and unfavorable groups were 26%(95% CI:20-32.5%) and 68.6%(95% CI:40.8-85.3%), respectively (*P*<0.001) and CIF of TRM was 15.2%(95% CI:10.4-20.8%) and 3.7%(95% CI:0.3-16.2%) in the intermediate and unfavorable cytogenetic groups, respectively(*P* =0.149). The CIF of relapse and TRM in these two cytogenetic groups who underwent Auto-SCT was not statistically significant. The acute GvHD difference between intermediate and unfavorable groups was not statistically significant (45.8% in intermediate versus 40.7% in unfavorable; *P*=0.618), but the incidence of chronic GvHD between patients within 100 days after allo-SCT were 22.1% in intermediate and 8.7% in the unfavorable group (*P*=0.155). The HR of cGvHD in the unfavorable versus intermediate group was 0.36(95% CI:0.09-1.47; *P* =0.155) and the HR of death without cGvHD in the unfavorable versus intermediate group was 3.26 (95% CI:1.61-6.58; *P* =0.001).
Discussion: Our study showed that outcome after allo-SCT in de novo AML differs depending on cytogenetic risk-group; and confirmed the classification of chromosomal aberrations into intermediate and unfavorable groups was a better predictor of relapse rate and OS than almost any other tested parameters. In the unfavorable group, the relapse rate was extremely high, and allo-SCT should be performed in CR1. The impact of cytogenetic risk group was not seen on TRM in our study. Apart from cytogenetic group, chronic GvHD was significantly higher in the intermediate risk group that can decrease the probability of relapse and increase the survival in this cytogenetic group after allo-SCT. In conclusion, the presence of unfavorable cytogenetic abnormality versus those with normal karyotype or intermediate cytogenetic abnormality associated with worse outcome after allo-SCT in AML patients and their treatment remains an unmet medical need. Further studies analyzing the impact of particular chromosomal aberrations in AML on patient's outcome after allo-SCT would allow for refined risk stratification in adults with de novo AML and update indications for allo-SCT in de novo AML.
Disclosure of Interest: None Declared.

PH-P022
AN INTENT-TO-TRANSPLANT SINGLE CENTER ANALYSIS IN ELDERLY PATIENTS WITH HIGH RISK ACUTE LEUKEMIA OR MYELODYSPLASTIC SYNDROME: THE SAN RAFFAELE HOSPITAL EXPERIENCE

M. Bernardi[1,], M. Giovanni Carrabba[1], C. Messina[1], J. Peccatori[1], M. T. Lupo Stanghellini[1], E. Sala[1], F. Pavesi[1], S. Claudiani[1], A. Assanelli[1], C. Corti[1], R. Greco[1], F. Giglio[1], F. Ciceri[1]*
[1]Onco-Haematology, San Raffaele Scientific Institute, Milan, Italy

Introduction: intensive programs with curative intent for high risk acute leukemia (AL) or myelodysplastic syndrome (MDS) include allogeneic (allo) stem cell transplantation (SCT) or autologous (auto) SCT as an alternative for pts unfit for alloSCT or without a donor. These programs are often considered unfeasible in elderly pts for the high risk of toxicity and mortality related to treatment. We report a retrospective intent-to-transplant analysis in our pts older than 65 years with high risk AL or MDS.
Materials (or patients) and Methods: between 09/2003 and 04/2013, 77 consecutive pts with newly diagnosed AL or MDS were candidated to alloSCT (27) or to autoSCT (50), at our Center. Median age 70 (66-80), PS ≤ 2, left ventricular ejection fraction ≥ 50%, renal and hepatic function tests within normal range. Diagnosis: acute leukemia 60 (AML 58, ALL 2), MDS 17, all with unfavourable prognostic characteristics. Standard induction chemotherapy was programmed for 69 pts, 8 pts with MDS were addressed to upfront alloSCT. Conditioning before alloSCT: reduced intensity in all cases, mostly fludarabine-treosulfan based. Conditioning before autoSCT: BEAM or fludarabine-treosulfan-cytarabine combination.

Results: 65/69 pts (94%) received one or two cycles of standard induction chemotherapy, 43 pts (66%) obtained the complete remission (CR), treatment related mortality was 15% (10 pts), 33 pts then received at least one cycle with high-dose cytarabine, 10 pts further standard chemotherapy. Overall, 29 pts (38%) received a SCT, alloSCT 14/27 (52%), autoSCT 15/50 (30%). Disease status at SCT: CR1 22, persistence of disease 2, upfront 5. Causes for failure of the program (48 pts): death before induction 3 cases, death during chemotherapy 11, death during donor search 2, no donor 1, clinical reasons 3, patient refusal 1, disease refractory to induction 11, early relapse 4, failure of autologous leukapheresis (LK) 12. Day-60 transplant related mortality was 10% (3/29, 2 alloSCT, 1 autoSCT). At last follow up 25 pts (32%) were alive, 19 (25%) in CR. Median and estimated 3 yrs OS of all pts (77) were 392 days (1-3668) and 28%, respectively, median and estimated 3 yrs OS of transplanted pts (29) were 1155 days (161-2437) and 55%, respectively.

Discussion: our analysis revealed that alloSCT was feasible more frequently than autoSCT, that is in one-half vs one-third of pts. Three principal causes explained the failure of the autoSCT program in most pts (83%): disease refractory to induction, death during induction and failure of LK. Causes of program failure for alloSCT pts were more heterogeneous. Survival of pts who received the programmed SCT, allo or auto considered together, was encouraging. In conclusion, pts with advanced age and high risk AL/MDS can be treated and gain survival with an intensive therapeutic program including a SCT. To increase the feasibility of a transplant program in these setting it is crucial to reduce chemotherapy toxicities, improve the rate of complete remission and of successful autologous LK.

Disclosure of Interest: None Declared.

PH-P023

BONE MARROW MESENCHYMAL STROMAL CELLS PROTECT ACUTE LEUKEMIA CELLS FROM CYTOTOXIC AGENTS VIA GALECTIN-3 MEDIATED WNT/B-CATENIN SIGNALING

H. Kaimin[1,], G. Yanjun[1], L. Lixia[1], L. Lizhen[2], W. Binsheng[1], H. He[2]*
[1]School of Medicine, Zhejiang University, [2]Bone Marrow Transplantation Center, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

Introduction: Acute leukemia remains one of the greatest challenges in oncology. Increasing evidence indicates that the bone marrow microenvironment (BMM) plays a pivotal role in both the pathogenesis and relapse of leukemia. Recent studies confirmed galectin-3 (gal-3), a multifunctional member of the β-galactoside-binding protein family, is critical in leukemia drug resistance and patient's prognosis. Here we determined the role of gal-3 in the promotion of BMM-induced acute leukemia cells' (ALCs') survival, and the mechanisms involved.

Materials (or patients) and Methods: Our study applied the co-culture system with mesenchyml stromal cells (MSCs) to mimic the leukemia BMM *in vitro*. We validated our hypothesis in different sub-types of ALC lines including Reh, Sup-B15, Jurkat and Kasumi-1. Bone marrow was obtained from healthy adult donors with informed consent and MSCs were cultured and identified as previously reported.

Results: CCK-8 test demonstrated MSCs improved viable ALC number with or without cytotoxic agents Idarubicin (IDA) or Etoposide (Vp-16). PI/Annexin V assay confirmed reduced apoptotic level of MSCs-conditioned ALCs induced by IDA or Vp-16 ($P<0.05$) (Figure 1A). To clarify the underlying mechanisms we performed Western-blot and real-time polymerase chain reaction. We found that compared to ALCs cultured alone, both mRNA and protein expression of gal-3 were significantly up-regulated in MSCs-conditioned ALCs ($P<0.05$). Increased gal-3 levels correlated with Akt phosphorylation, β-catenin stabilization and higher expression of β-catenin target gene cyclin D1 ($P<0.05$) (Figure 1B and 1C). Then we used gal-3 small interfering RNA (siRNA) to silence its expression in ALCs to determine whether gal-3 modulates the protective effects of MSCs in ALCs. Compared with MSCs-conditioned vector-transfected Kasumi-1, we detected an increased apoptotic percentage against IDA and a decrease of β-catenin protein level in gal-3 siRNA-transfected ones (Figure 1D).

[PH-P023]

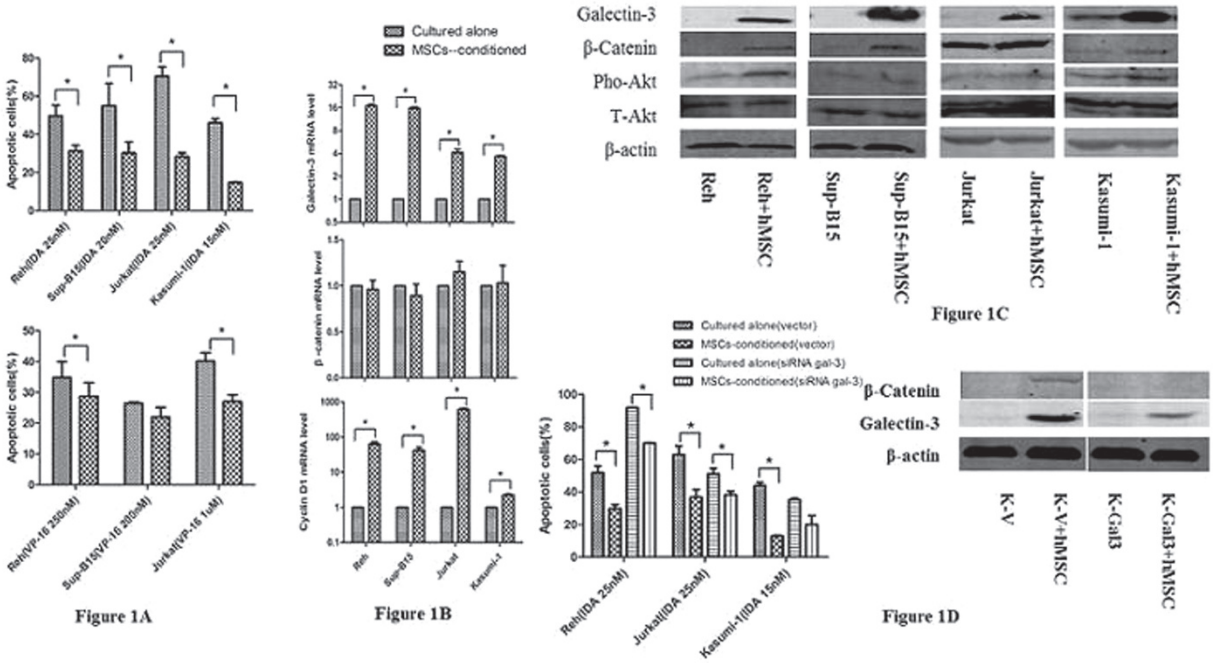


Figure 1A

Figure 1B

Figure 1C

Figure 1D

Discussion: Altogether, our findings reveal that gal-3-induced Wnt/β-catenin signaling is involved in MSCs-mediated drug resistance. Silencing gal-3 sensitized the ALCs to chemotherapy and decreased β-catenin stabilization, suggesting gal-3 can be a novel therapeutic target in acute leukemia.
Disclosure of Interest: None Declared.

PH-P024

CD44V6 AS A NEW TARGET FOR AML AND MM THERAPY

B. Nicolis Di Robilant[1,], M. Casucci[1], L. Falcone[1], B. Camisa[2], B. Gentner[3], G. Oliverira[2], L. Naldini[3], C. Bordignon[4], F. Ciceri[5], C. Bonini[5], A. Bondanza[1]*
[1]Leukemia Immunotherapy Group, Division of Regenerative Medicine, Stem Cells and Gene Therapy, [2]Experimental Hematology Unit, Division of Regenerative Medicine, Stem Cells and Gene Therapy, [3]San Raffaele-Telethon Institute for Gene Therapy (TIGET), San Raffaele Scientific Institute, [4]MolMed S.p.A., [5]Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milan, Italy

Introduction: Targeting the interactions between tumor cells and their microenvironment is an exciting new frontier in cancer therapy. The hyaluronate receptor CD44 was shown to be required for retroviral-induced leukemogeneis in syngeneic mouse models. Conversely, CD44 mAbs interfere with human leukemia initiation in immunocompromised mice by inhibiting leukemia stem cell homing to the bone marrow (BM). The therapeutic potential of CD44 mAbs is also under clinical investigation in humans. Much less is known on the role of the differently spliced CD44 variant isoforms. The expression of exon 6 (CD44v6) conveys additional properties to standard CD44, like binding to osteopontin and cooperation with different tyrosine kinase receptors (RTKs). Interestingly, CD44v6 expression by AML and MM correlates with a bad prognosis. Since CD44v6 expression is much more tumor-restricted than CD44, targeting this isoform may have a better efficacy/toxicity profile than targeting the standard molecule. The aim is to preclinically validate CD44v6 as a therapeutic target in AML and MM.
Results: By FACS analysis and RT-qPCR, we established CD44v6 over expression in a relevant fraction of leukemic blasts from AML pts (15/25, 60%) with preference for the M4-5 FAB subtypes, and in the majority of malignant plasmacells from MM pts (13/15, 87%). CD44v6 was also over expressed on THP-1, Kasumi and U937 human AML cells, and on MM1.S, XG-6 and XG-7 MM cells. To address the specific role of CD44v6 in BM homing, we pretreated MM1.S cells with either a CD44 mAb (SFF-2) or a CD44v6 mAb (VFF-18) and infused them i.v. in NSG mice. Unexpectedly, while SFF-2 almost completely inhibited early (18hrs) homing to the BM compared with an irrelevant mAb, VFF-18 had no effect. To rule out confounding variables associated with specific mAb clones, we silenced CD44v6 expression in MM1.S cells by lentiviral-mediated shRNA transduction and confirmed no difference in BM homing compared with control LV-transduced cells. Longer follow-ups (4-6 weeks) however revealed that, despite unaltered rates of *in vitro* proliferation, CD44v6-silenced MM1.S cells were severely hampered in their tumorigenic capacity *in vivo* ($P<0.001$). These results were confirmed by using THP-1 cells ($P<0.001$) and primary leukemic blasts ($P<0.01$). Hypothesizing that CD44v6 may be crucial for *in vivo* tumorigenesis by cooperating with RTKs, we set-up a co-culture system with BM-derived mesenchymal stromal cells (MSCs). MSCs protected a wide range of tumor cells, including primary leukemic blasts, from spontaneous apoptosis ($P<0.05$) and from apoptosis induced by Ara-C or daunorubicin ($P<0.01$), or bortezomib in the case of MM cells ($P<0.001$). Comparable results were obtained by using MSC supernatants, hinting to a causative soluble factor, which was neither VEGF nor HGF as demonstrated by inhibition experiments with bevacizumab and crizotinib, respectively. Noteworthy, MSCs or their supernatants prompted a significant up-regulation of CD44v6 expression levels ($P<0.01$). Most importantly, adding CD44v6 mAb to conventional chemotherapy (Ara-C) significantly reduced circulating leukemic blasts *in vivo*.
Discussion: These results clearly indicate that CD44v6 is dispensable for BM homing, but responsible for AML and MM addiction to microenvironmental signals. Combining CD44v6 targeting with cytotoxic chemotherapy might interfere with this vicious circle and result in higher and/or more durable response rates.
Disclosure of Interest: None Declared.

PH-P025

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ADULTS WITH ACUTE MYELOID LEUKEMIA (AML): THE TUNISIAN RESULTS

L. Torjemane[1,], N. Abdejlil [1], A. Lakhal[1], W. Barhoumi[1], L. Ben Hamed [2], H. Kaabi[2], H. Slama[2], A. Laamouri[3], S. Ladeb[1], T. Ben Othman[1]*
[1]Hematology, Centre National de Greffe de Moelle Osseuse de Tunis, [2]Centre National de Transfusion Sanguine de Tunis, Bab Saadoun, [3]Laboratoire de cytogénétique, Institut Pasteur de Tunis, Tunis, Tunisia

Introduction: In Tunisia, allo-HSCT for adult AML is recommended for intermediate and poor-risk AML if an HLA-identical sibling donor was available. Here, we report our results since we introduced the Bu(iv)-Cy regimen.
Materials (or patients) and Methods: Between January 2005 and December 2012, 73 patients were transplanted for AML. The median age was 31 years (range; 18-45 years). According the cytogenetic stratification, 68 valuable patients were in the poor-risk (n=22; 32%), intermediate (n=44; 64%) or the favorable risk group (n=5). Induction treatment was the standard cytarabine/mitoxantrone 7+3. The transplant was performed in first CR (n = 57), in second CR (n=7) or in failure (n=9). Conditioning regimen was Bu (iv) - Cy (97%) or ICT- Cy (3%). GVHD prophylaxis associated Cyclosporine A and methotrexate. The graft was bone marrow in 49 patients (67%) providing a median of 2.15 x10^8 MNC/ kg (range; 0.7 - 4.07) and peripheral blood stem cells in 24 patients (33%) providing a median of 4.75 x 10^6 CD34+ cells/kg (range; 2.46- 6.79). The median time from diagnosis to transplant was of 5 months (range; 3 -13 months).
Results: Engraftment was achieved in 72 patients (98%). Only one early death occurred and was related to a toxic acute renal failure (2%). The rate of overall treatment–related mortality was 8% (n = 6). Causes of death were refractory GVHD and non-infectious pulmonary complications. The cumulative incidence of relapse was of 29%. The median time to relapse was 10 months (range; 2 - 25months). The cumulative incidence of acute GVHD, cytomegalovirus infections and chronic GVHD were 31 %, 36% and 50% respectively. After a median survival of 26 months (range; 20 days -96 months), 48 patients were alive. The overall survival and the disease-free survival were of 63% and 60 % at 3 years respectively. The overall survival rates were of 75 %, 42 % and 12 % for patients transplanted in CR1,CR2 and failure respectively (P = .0001). Pre-transplant disease status was the only risk factor that significantly affects survival.
Discussion: Allogeneic HSCT with sibling donor in adult AML patients is relatively safe. The pre- transplant disease status is discriminant for survival.
Disclosure of Interest: None Declared.

PH-P026

THE OUTCOMES OF UNMANIPULATED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION PERFORMED DURING THE FIRST COMPLETE REMISSION IN ADULT PATIENTS WITH PH-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA INDICATE NO DIFFERENCE BETWEEN THE HIGH- AND LOW-RISK GROUPS

X.-D. Mo[1,], L.-P. Xu[1], D.-H. Liu[1], X.-H. Zhang[1], H. Chen[1], Y.-H. Chen[1], W. Han[1], Y. Wang[1], F.-R. Wang[1], J.-Z. Wang[1], K.-Y. Liu[1], X.-J. Huang[1]*
[1]Peking University People's Hospital, Institute of Hematology, Beijing, China

Introduction: The present study aimed to evaluate the therapeutic effects of haploidentical hematopoietic stem cell transplantation

(haplo-HSCT) performed during the first complete remission (CR1) in adults with Philadelphia chromosome-negative (Ph-negative) acute lymphoblastic leukemia (ALL).
Materials (or patients) and methods: Patients were classified as having high-risk if they met one of the following criteria at diagnosis: (1) adverse cytogenetic [t(4;11), or complex karyotype (≥5 abnormalities)]; (2) older age (≥35 years); (3) high-leukocyte counts (≥30 × 10^9/L for B-precursor ALL or ≥100 × 10^9/L for T-precursor ALL), or (4) delayed CR1 (remission required more than 28 days of induction therapy). All other patients were classified as low-risk.
Results: The median time from diagnosis to transplantation was 180 days. All patients achieved neutrophil engraftment within 30 days after HSCT, and by 100 days, the cumulative incidence of platelet engraftment was 88.5%. The 100-day cumulative incidence of grade II–IV and III–IV acute graft-versus-host disease (GVHD) was 40.2% and 6.7%, respectively. The 3-year cumulative incidence of total and extensive chronic GVHD (cGVHD) was 61.7% and 28.4%, respectively. The 3-year cumulative incidence of relapse and non-relapse mortality (NRM) was 18.3% and 19.9%, respectively. Overall survival (OS) and disease-free survival (DFS) at 3 years were 69.5% and 63.5%, respectively. Patients with limited cGVHD experienced a significantly better OS and DFS than those without cGVHD or those with extensive cGVHD. The outcomes were comparable between the high- and low-risk groups (Table 1).

[PH-P026]

PH-P027

ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS OLDER THAN 65 YEARS WITH HIGH RISK ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROME: A RETROSPECTIVE ANALYSIS ON 72 PATIENTS

E. Sala[1,], M. G. Carrabba[1], M. T. Lupo Stanghellini[1], C. Messina[1], F. Pavesi[1], S. Claudiani[1], F. Giglio[1], G. V. Levati[1], A. Assanelli[1], C. Corti[1], J. Peccatori[1], C. Fabio[1], M. Bernardi[1]*
[1]Hematology and Bone Marrow Transplant Unit, San Raffaele Scientific Institute, Milan, Italy

Introduction: Allogeneic haematopoietic stem cell transplantation (ASCT) is the only curative option for patients (pts) with high risk (HR) Acute Myeloid Leukemia (AML) and Myelodysplastic Syndromes (MDS). Elderly subjects (> 65 yrs), who represent the majority of AML/MDS pts, are often excluded from ASCT programs due to the related high risk of toxicities and mortality. Recent development of reduced-intensity conditioning regimens and improvement of supportive measures have made ASCT a feasible option for selected older pts. Published data suggest that ASCT from HLA-matched related donors improves the outcome of this population.
Materials (or patients) and Methods: We analyzed data from 72 consecutive pts with HR AML/MDS who underwent ASCT in our Institution between 08/2004 and 04/2013. Transplanted pts included in this study fulfilled the following criteria: (1) cytologically proven diagnosis of primary or secondary MDS or AML; (2) age ≥ 65 years; (3) Performance Status (ECOG) ≤ 2; (4) completion of the transplant procedure.

Table 1. Cumulative incidences of clinical outcomes in low- and high-risk groups at 3 years

	Standard-risk group (n=79)		High-risk group (n=85)		*P* value
	Cumulative incidence (%)	95% CI (%)	Cumulative incidence (%)	95% CI (%)	
aGVHD					
100-day Grade I–IV	65.8	55.2–76.4	61.2	50.7–71.7	0.864
100-day Grade II–IV	41.8	30.8–52.8	38.8	28.4–49.2	0.892
100-day Grade III–IV	5.1	0.2–10.0	8.2	2.3–14.1	0.406
cGVHD					
3-year total	62.4	51.0–73.8	61.1	50.3–71.9	0.612
3-year extensive	26.5	16.3–36.7	30.1	20.0–40.2	0.653
3-year relapse	21.3	11.8–30.8	15.7	7.8–23.6	0.407
3-year NRM	19.4	10.5–28.3	20.3	11.6–29.0	0.812
3-year OS	67.4	56.4–78.4	71.4	61.6–81.2	0.807
3-year DFS	61.4	50.1–72.7	65.2	54.8–75.6	0.819

aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; NRM: non-relapse mortality; OS: overall survival; DFS, disease-free survival.

Discussion: Comparable transplantation outcomes between high- and low-risk Ph-negative ALL CR1 patients may be related to the stronger graft-versus-leukemia effect, the shorter time required for donor selection, and a convenient repeated access to donor cells when donor-derived cellular therapy is needed for the relapse.
Disclosure of Interest: None Declared.

Results: Diagnosis: 57 AML and 15 MDS. Median age was 68 years (range 65-76 years). Pts' and transplant characteristics are listed in Table 1. Median follow-up (FU) after ASCT among surviving pts was 36 months (range, 8 - 48 months). 69 out of 72 pts (96%) were in CR at day +30 after ASCT. At the last FU 35 out of 72 pts (49%) were alive, 32 (44%) in CR, 36 pts (51%) died for the

following causes: disease relapse/progression in 17 cases (47%), GvHD in 6 (17%), infection in 13 (36%), 1 patient was lost at follow up. Transplant Related Mortality was 22% at 1 year and 26% at 3 years after transplant. Relapse Incidence (RI) was 17% at 1 year and 27% at 3 years. 1-year Overall Survival (OS) was 65%, 3-years OS was 41%.

Table 1

Status at ASCT	
CR	38 (53%)
PD	25 (35%)
upfront	9 (12%)
HCT-CI	
Median	1
Range	0-9
Donor type	
MRD	11 (15%)
MUD	18 (25%)
HAPLO	41 (57%)
CBU	2 (3%)
Conditioning	
Treo-Flu	40 (56%)
Treo-Flu-TBI	16 (22%)
Bu-Flu	6 (8%)
Treo-Flu-Mel	4 (5%)
Treo-Clofarabine	4 (5%)
Others	2 (4%)
GvHD prophylaxis	
Rapamycine/MMF	37 (51%)
CSA/MTX	27 (38%)
Ex vivo T-depletion	8 (11%)
Number of ASCT	
1st	68 (94%)
2nd- 3rd	4 (6%)

Discussion: Our retrospective analysis supports ASCT as a potentially curative option for selected elderly pts. Of note, most pts (85%) received the transplant from an alternative donor. We routinely used clinical standard parameters to evaluate the fitness of pts candidate to ASCT. Recently more standardized criteria (e.g. EBMT risk score, Pretransplantation Assessing of Mortality (PAM) score and Disease Risk Index (DRI) score) had become available and have been introduced in our pre-transplant evaluations. Prospective clinical trials are necessary to confirm and validate our promising results.
Disclosure of Interest: None Declared.

PH-P028

MYELODYSPLASTIC SYNDROME RELATED CYTOGENETIC ABNORMALITIES DO NOT HAVE NEGATIVE IMPACT ON SURVIVAL AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES (AML-MRC)

M. Yuasa[1,], N. Uchida[1], D. Kaji[1], H. Ota[1,2], K. Ishiwata[1], M. Tsuji[1], H. Yamamoto[1], G. Yamamoto[1], Y. Asano-Mori[1], S. Maikino[2], A. Yoneyama[3], K. Masuoka[4], A. Wake[5], K. Izutsu[1], S. Taniguchi[1]*
[1]Department of Hematology, [2]Department of Transfusion Medicine, [3]Department of Infectious Diseases, Toranomon Hospital, Minatoku, [4]Department of Hematology, Mishuku Hospital, Meguroku, [5]Department of Hematology, Toranomon Hospital, Kawasaki, Japan

Introduction: Acute myeloid leukemia with myelodysplsia–related changes (AML-MRC) is characterized by multilineage dysplasia (MLD), history of myelodysplastic syndrome (MDS) or MDS/myeloproliferative neoplasm (MPN), and MDS-related cytogenetic abnormalities. Prognosis of patients with AML-MRC is generally poor with standard chemotherapy especially in those with MDS-related cytogenetic abnormalities. Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an option to improve prognosis for those with AML-MRC, clinical outcome and impact of cytogenetics has not been extensively studied.
Materials (or patients) and Methods: We retrospectively analyzed 145 consecutive patients with adult AML-MRC who underwent allo-HSCT for the first time at Toranomon Hospital from Jan. 2006 to Dec. 2012 and evaluated the significance of morphological and cytogenetic features on clinical outcome after allo-HSCT.
Results: One hundred and twenty-three (85%) had MLD, 22 (15.1%) had MLD as sole AML-MRC criterion, 63 (43%) had MDS-related cytogenetics, and 86 (59%) patients had history of MDS or MDS/ MPN. Fifty-one (35%) had normal karyotype, 31 (21%) had 1 abnormality, 11 (8%) had 2, 52 (36%) had 3 or more at diagnosis. The median age at transplantation was 61 years (range, 21 - 82), with a median HCT-CI score of 3 (0-8). 101 patients (69.7%) were male, and 135 (93.1%) were not in remission. 110 (75.8%), 3 (2.0%), 15 (10.3%), 17 (11.7%) underwent single cord blood transplantation (single CBT), double CBT, related HSCT, and unrelated HSCT, respectively. Myeloablative conditioning was used in 79 (54.5%). GVHD prophylaxis was Tacrolimus (TAC) based in 127 (87.6%), and cyclosporin (CsA) based in 18 (12.4%). The median total nucleated cell count and $CD34^+$ cell of cord blood unit infused was 2.57 (1.62-5.10) $*10^7$kg and 0.86 (0.29-2.97) $*10^5$/kg. 112 (77.2%) achieved neutrophil engraftment at median of 20 days (11-43). Cumulative incidence of grade II-IV and III-IV acute GVHD were 50.8% and 22.4%, respectively. Chronic GVHD of limited and extensive type developed in 25 (17.2%), and 12 (11.7%). Fourty-six patients are alive at a median follow-up of 850 (62-2624) days, median overall survival was 214 days, and median disease free survival was 145 days. There was no significant difference in 2 year OS between those who had or did not have MLD (31.5% vs.22.8%, log rank *P*=0.50), MLD as sole AML-MRC criterion (33.0% vs 33.2%, log rank *P*=0.80), MDS-related cytogenetics (30.5% vs 35.2%, log rank *P*=0.31), or history of MDS or MDS/ MPN (31.1% vs 36.2%, *P*=0.78). There was no significant difference in relapse incidence at 2 year those who had or did not have MLD (19.2% vs.25.0%, log rank *P*=0.30), MLD as sole AML-MRC criterion (14.7% vs 20.4%, log rank *P*=0.56), MDS-related cytogenetics (20.8% vs 18.72%, log rank *P*=0.61), or history of MDS or MDS/ MPN (20.3% vs 18.3%, *P*=0.93).
Discussion: This retrospective study suggests that allo-HSCT is a promising option for patients with AML-MRC. Presence of MLD, or MDS-related cytogenetics did not have negative impact on survival or relapse incidence after allo-HSCT.
Disclosure of Interest: None Declared.

PH-P029

COMPARISON OF DIFFERENT PRETRANSPLANT PREDICTIVE SCORES IN PATIENTS WITH REFRACTORY ACUTE MYELOID LEUKEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION INCLUDING HIGHDOSE MELPHALAN: RESULTS OF A DOUBLE-CENTER OBSERVATIONAL STUDY

N. Kristin Steckel[1,], M. Ditschkowski[1], C. Groth[2], G. Silling[2], U. Krug[2], R. Trenschel[1], H. Ottinger[1], C. Müller-Tidow[2], W. E. Berdel[2], M. Koldehoff[1], A. H. Elmaagacli[3], D. W. Beelen[1], M. Stelljes[2]*
[1]Clinic of Bone Marrow Transplantation, West German Cancer Center, University Hospital Essen, Essen, [2]Medicine A, Hematology, University Hospital Muenster, Muenster, [3]Hematology, Asklepios Clinic Hamburg, Hamburg, Germany

Introduction: Patients (pts) with refractory acute myeloid leukaemia (AML) have a particularly dismal prognosis using conventional supportive therapy and chemotherapy. In this double-center retrospective observational study, sequential high-dose melphalan (HD-Mel) was used as part of conditioning regimen for allogeneic hematopoietic stem cell transplantation (aSCT).
Materials (or patients) and Methods: 162 adult pts (median age 55, range 17 to 71 years) with refractory AML were transplantat. For all pts pretransplant assessment of transplantation (PAM) score, European BMT (EBMT) score, and Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI) was calculated. 107 pts (66%) underwent a total body irradiation (TBI) based conditioning regimen and 55 pts (34%) a chemotherapy based regimen after a median interval of 5 days (range 1-18 days) following HD-Mel application. HD-Mel dose was 140 mg/m^2 in 150 pts, and 200 mg/m^2 in 12 pts. 48 pts (30%) were transplanted with an identical sibling donor (ISD), and 114 pts (70%) with matched unrelated

donors (MUD). Pts had mostly intermediate (51%) or adverse (46%) cytogenetic risk subgroups.
Results: In 150 pts (93%) a stable engraftment was observed and a complete chimerism could be detected in 124 pts (83%). Acute GvHD grades 0-I developed in 98 pts (60%), whereas higher grades II-IV occurred in 64 pts (40%). The median calculated PAM score was 31 (range 22-89). The corresponding median overall death probability within 2 years after aSCT was 70% (range 44-95%). Median HCT-CI was calculated with 2 (range 0-9) characterizing a low risk comorbidity. Finally the European BMT (EBMT) score was calculated with more than 5 (range 3-7) in 116 pts (72%) transplanted in this cohort. The cumulative incidence of treatment failure estimate was 68% (95% CL: 60-76%) resulting in a cumulative relapse estimate of 35% (95% CL: 28-43%), and an overall non-relapse mortality (NRM) of 38% (95% CL: 30-46%). After a median follow-up of 35 months after aSCT among surviving pts, the 3-year overall survival estimate (OS) was calculated 37% (95% CL: 35-45%), and 5-year OS 30% (95%>CL: 25-40%). Time-dependent multivariate analysis on the primary endpoints OS, relapse-free survival (RFS), relapse incidence, and NRM demonstrated an influence of chronic GvHD on EFS (hazard ratio [HR] 0.443, *P*=0.0003), NRM ([HR] 0.369, *P*=0.0032), and relapse incidence ([HR] 0.554, *P*=0.0467). Furthermore, MUD donors tended to have a favourable impact on relapse risk ([HR] 0.590, *P*=0.0595) compared to sibling donors. PAM score demonstrated significant influence on OS (*P*=0.0381) and RFS (*P*=0.0121). For low and high risk groups stratified PAM score was shown to have significant influence on OS ([HR] 1.175, *P*=0.0056), RFS ([HR] 1.003, *P*=0.0160), and NRM ([HR] 1.283, *P*=0.0049), respectively. No influence could be observed for EMBT score and HCT-CI.
Discussion: The results of this large study confirm an association between PAM score risk stratification and outcome after aSCT following sequential conditioning regimen with HD-Mel in patients with refractory AML. We could show, that by using a stratified PAM score with the threshold of 30 points a separation between prognostic risk groups is possible. We can summarize that this protocol is a feasible therapy option for patients with refractory AML.
Disclosure of Interest: None Declared.

PH-P030
INFLUENCE OF HIGH-DOSE ATG ON GVHD, POST-TRANSPLANT IMMUNE RECONSTITUTION AND RELAPSE OF PATIENTS RECEIVING ALLOGENEIC BLOOD STEM CELL TRANSPLANTATION WITH SEQUENTIAL CONDITIONING FOR HIGH-RISK MYELOID LEUKEMIA
A. Groten[1,], T. Schroeder[1], A. Dienst[1], K. Nachtkamp[1], E. Rachlis[1], U. Germing[1], R. Haas[1], M. Kondakci[1], G. Kobbe[1]*
[1]Hematology, Oncology and Clinical Immunology, University Hospital Duesseldorf, Duesseldorf, Germany

Introduction: Regimens like the "FLAMSA-protocol" that combine intensive induction and dose-reduced conditioning regimens for allogeneic blood stem cell transplantation (alloBSCT) have shown to give promising results in the treatment of patients with high-risk myeloid leukemia (HR-ML). We retrospectively looked for the influence of *in-vivo* T-cell depletion using ATG on GvHD, post-transplant immune reconstitution, relapse and survival.
Materials (or patients) and Methods: One hundred and five patients (pts, 58m, 47f, age 52 years, 20-68) with HR-ML (52 de-novo AML, 26 sAML, 18 MDS, 9 MPS) received Fludarabine (4×30mg/m^2), Amsacrine (4×100mg/m^2), Ara-C (4×2g/m^2, FLAMSA), age adapted HD-Mel (100-200mg/m^2) and alloBSCT (103 PBSC, 1 CD34+CB, 1 BM, median CD34+cells/kg 7.8 × 10^6, 2-38) from 39 related and 66 unrelated donors. A total of 73 patients (70%), most of them receiving grafts from unrelated donors, received ATG Fresenius (60mg/kg in 61 and 30mg/kg in 12 pts). Twenty-eight pts (27%) had untreated MDS (16), sAML (9) or MPS (3). Among 77 previously treated pts 22 (27%) were in 1st CR, 12 (16%) in 1st PR, 9 (12%) had primary refractory disease and 34 pts (44%) had relapsed (21 untreated, 13 treated: 5 in 2nd CR/PR, 8 refractory).
Results: One hundred and one pts had donor reconstitution of hematopoiesis after a median of 14 days (8-46), 3 died early and 1 had graft failure. At day +28, 101 pts (96%) were in CR. Median follow-up is 3.7 years (0.5-9.8), Overall survival (OS) at 1&4 years was 65.5% (60.8-70.2) and 58.8% (53.7-63.9). The respective event free survival (EFS) was 55.0% (50.1-59.9) and 36.3% (31.1-41.5). aGvHD occurred in 52 pts (50%) and cGvHD was seen in 44 pts (47%) surviving more than 100 days after SCT. Estimated relapse rate (RR) at 1&4 years was 34.4% (29.4-39.4) and 54.6% (48.8-60.4). Estimated non-relapse-mortality rate (NMR) at 1&4 years was 14.9% (11.3-18.5) and 17.5% (13.2-21.8), respectively. Looking for prognostic factors we found that OS, EFS and RR were influenced by disease status, previous therapy and cytogenetics. Age and HCTCI were predictors for NRM. ATG reduced cGvHD (*P* <.001), but delayed hematopoietic and immune reconstitution (*P*<.001 for WBC and PLT reconstitution, *P*<.001 and p=.002 for lymphocyte count at day + 28 and +100, *P* <.001 for CD3CD4-cell count day 50-400) and increased RR (*P*=.014). Due to the fact that therapy of relapse was effective in many patients the difference in OS (4yOS 55% vs 68%) was not significant.
Discussion: Sequential conditioning using FLAMSA-Melphalan is safe and effective in patients with HR-ML. Final results are still compromised by disease recurrence in a considerable number of patients. Our results indicate, that in the setting of HR-ML, the benefits of high-dose ATG in terms of reduced cGvHD are accompanied by slow immune reconstitution and an increased relapse rate. Therefore studies are needed to tailor ATG-dose to disease risk.
Disclosure of Interest: A. Groten Conflict with: Fresenius, Travel support, T. Schroeder: None Declared, A. Dienst: None Declared, K. Nachtkamp: None Declared, E. Rachlis: None Declared, U. Germing: None Declared, R. Haas: None Declared, M. Kondakci: None Declared, G. Kobbe Conflict with: Fresenius, Travel support.

PH-P031
ALLOGENEIC TRANSPLANTATION IN SECONDARY AML: OUTCOME ANALYSIS IN 71 CONSECUTIVE PATIENTS
S. Claudiani[1,], M. T. Lupo Stanghellini[1], M. Morelli[1], E. Sala[1], M. Marcatti[1], A. Assanelli[1], M. Carrabba[1], S. Trinca[1], S. Marktel[1], F. Lunghi[1], E. Guggiari[1], F. Giglio[1], C. Corti[1], J. Peccatori[1], F. Ciceri[1], M. Bernardi[1]*
[1]Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milano, Italy

Introduction: Patients (pts) with secondary acute myeloid leukemia (sAML) have a very poor response to chemotherapy and dismal prognosis (2 years survival is ≤ 10%). Allogeneic stem cell transplantation (allo-SCT) is the only potentially curative treatment in this setting.
Materials (or patients) and Methods: We analyzed 71 consecutive pts affected by AML secondary to MDS (*n*=25), to myeloproliferative disorders (11) and AML with bi- or tri-linear dysplasia at diagnosis (*n*=35), treated with allo-SCT at our Institution from March 2004 to November 2013. 2/71 pts were transplanted upfront. Median time from diagnosis to transplant was 5,1 months.
Median age at transplant was 60 years (range 28-72), 49/71 males. The median follow-up was 13.5 months.
Cytogenetic analysis showed normal karyotype in 36 pts (50%), complex karyotype in 13 (18%), monosomal karyotype in 5 (7%), other abnormalities in 18 (25%). Only 33 pts were evaluable for molecular alterations and 22 had none. Conditioning was myeloablative in 69 cases (treosulfan based 52/69; clofarabine was part of the conditioning regimen in 6 cases and low dose TBI in 15 pts) while only 3 pts received non-myeloablative conditioning. Graft versus host disease (GvHD) prophylaxis included: *ex vivo* T-cell depletion (11 pts), *in vivo* T-cell depletion with ATG (47 pts); immunosuppressive treatment with rapamycin-mycophenolate mofetil (23 pts), cyclosporine-methotrexate (31 pts), cyclosporine alone (4 pts), cyclophosphamide-rapamycin-mycophenolate mofetil (2 pts). 19 pts received a HLA-identical sibling allo-SCT, 19 an unrelated one, 2 pts a cord blood transplant and 31 an haploidentical one; donor source was mobilized peripheral blood stem cell in the majority of cases (64/69). At transplant 37 pts were in 1st complete remission (CR), 8 in CR2, 26 in active disease.

Results: A median of 7 x10^6 CD34+/Kg and 2.7 x10^8 CD3+/Kg cells were infused. 5 pts were not evaluable for engraftment. The overall engraftment rate was 89%; median time to neutrophil and platelet engraftment was 17 and 16 days respectively. 2 pts experienced primary graft failure and 1 patient never reached platelet engraftment. Transplant related mortality (TRM) at 100 days, 1 and 2 years were respectively 15.4%, 22.6% and 27.15%. At the last follow-up 28/71 patients experimented relapse at a median of 5,7 months (and died because of it after a median of 15,6 months) and 23/71 were alive in CR with a median follow-up of 35.4 months. Acute Graft-versus-Host-Disease (GvHD) occurred in 21/71 pts of grade II-IV, in 14/21 of grade III-IV. Chronic GvHD occurred in 12 pts (according to NIH evaluation 7/12 moderate and 5/12 severe). 2 years OS and RFS were 42.7% and 36% respectively. In multivariate analysis only grouped complex plus monosomal karyotypes and phase other than CR correlated with inferior OS (*P*= 0.03, RR 2.77 and p= 0.03, RR 2.73 respectively) and RFS (*P*= 0.003, RR 3.59 and *P*= 0.04, RR 2.5 respectively). No differences among donor types, ages and comorbidity scores were found.
Discussion: Our study (which includes a relevant proportion of pts >60 years old) supports allo-SCT as a feasible and potentially curative option for sAML. Allo-SCT provides better outcome to very high risk pts in 1st CR; it is mandatory to consider transplant in the treatment algorithm of such pts.
Disclosure of Interest: None Declared.

PH-P032
REMOVAL OF AML-LEUKEMIC STEM CELLS FROM THE GRAFT BY CD96 ANTIBODY TH-111

M. Gramatzki[1,], C. Kellner[1], M. Peipp[1], N. Schub[1], A. Humpe[1], M. Staudinger[1]*
[1]Division of Stem Cell Transplantation and Immunotherapy, University Hospital of Kiel, Kiel, Germany

Introduction: In AML patients despite intensive chemotherapy and additional hematopoietic stem cell transplantation, residual leukemic stem cells (LSC) may lead to relapse. Therefore, elimination of LSC by targeted therapy may represent a promising therapeutic approach. Recently, CD96 was identified as marker antigen on AML LSC (Hosen *et al*., PNAS 104:11008, 2007). Here, strategies for engineering autologous stem cell grafts as well as for *in vivo* targeting of residual AML stem cells by addressing CD96 for magnetic cell sorting (MACS) or antibody dependent cellular cytotoxicity (ADCC) are described.
Materials (or patients) and Methods: CD96 antibodies containing a human IgG_1Fc portion optimized for Fc receptor binding and wild type or affinity maturated CD96 binding domains were constructed by recombinant DNA technology. Parental CD96 antibody TH111 was raised in our laboratory (Gramatzki *et al*., *Exp. Hematol*. 26:1209, 1998) as well as recombinant CD96 antibodies were enriched by affinity purification from supernatants of hybridoma TH111 or transgenic cell cultures, respectively. For MACS based cell sorting of CD96-positive target cells, parental CD96 antibody was biotinylated using a biotinylation assay targeting free amines. Analysis of antibody binding, and biotinylation as well as subsequent differentiation of cell populations before and after cell sorting was done by flow cytometry. The potential of stem cells to proliferate and differentiate was analyzed by colony forming assays. Lytic properties of recombinant affinity optimized CD96 antibodies were evaluated using purified NK cells of healthy donors and CD96-positive target cells in standard chromium release assays.
Results: To evaluate the efficacy of purging LSC by MACS technology, stem cell containing grafts (*n*=10) were spiked with CD96-positive AML cells. Up to a 1000-fold depletion of targeted cells was achieved using biotinylated CD96 antibody TH111 in combination with anti-Biotin-microbeads (Miltenyi, Bergisch Gladbach, Germany). Viability, cell count and the potential of HPC to proliferate and differentiate were not affected by the cell sorting procedure. Eradication of AML stems cells is also an issue after allogeneic stem cell transplantation. To target CD96-positive AML-LSC by ADCC, chimeric antibodies containing wild type or affinity maturated variable regions in combination with an optimized human IgG_1 Fc were generated. As shown by flow cytometry, the antigen binding affinity of the recombinant maturated antibody was enhanced (EC50 0.6 µg/ml vs. 2 µg/ml). Moreover, also NK cell mediated lytic properties against CD96-positive target cells were found elevated (EC50: 0.02 µg/ml vs. 0.15 µg/ml) as analyzed in standard ADCC assays.
Discussion: By removing AML-LSC this CD96 purging strategy may allow a revitalization of autologous hematopoietic stem cell transplantation for certain patients with AML. Furthermore, CD96 antibody optimized for antigen as well as Fc receptor binding aims at using CD96 *in vivo*, possibly also in the allogeneic situation.
Disclosure of Interest: None Declared.

PH-P033
OUTCOME OF CONDITIONING INTENSITY IN ACUTE MYELOID LEUKEMIA WITH MONOSOMAL KARYOTYPE IN PATIENTS OVER 45 YEAR-OLD: A STUDY FROM THE ACUTE LEUKEMIA WORKING PARTY (ALWP) OF THE EUROPEAN GROUP OF BLOOD AND MARROW TRANSPLANTATION (EBMT)

X. Poiré[1,], M. Labopin[2], J. J. Cornelissen[3], L. Volin[4], C. Richard Espiga[5], J. H. Veelken[6], N. Milpied[7], J. Y. Cahn[8], I. Yakoub-Agha[9], G. W. van Imhoff[10], M. Michallet[11], L. Michaux[12], A. Nagler[13], M. Mohty[14] and Acute Leukemia Working Party*
[1]Hematology, Cliniques Universitaires Saint-Luc, Brussels, Belgium, [2]EBMT Paris Office, Paris, France, [3]Erasmus MC-Daniel den Hoed Cancer Centre, Rotterdam, Netherlands, [4]Helsinki University Central Hospital, Helsinki, Finland, [5]Hospital U. Marqués de Valdecilla, Santander, Spain, [6]Leiden University Hospital, Leiden, Netherlands, [7]CHU Bordeaux, Pessac, [8]Hopital A. Michallon, Grenoble, [9]Hôpital HURIEZ, Lille, France, [10]University Medical Center Groningen (UMCG), Groningen, Netherlands, [11]Centre Hospitalier Lyon Sud, Lyon, France, [12]Cliniques Universitaires Saint-Luc, Brussels, Belgium, [13]Chaim Sheba Medical Center, Tel-Hashomer, Israel, [14]Hopital Saint Antoine, Paris, France

Introduction: Acute myeloid leukemia with monosomal karyotype (MK AML) carries a very poor prognosis, even after allogeneic stem cell transplantation (SCT) due to a high relapse rate. However, SCT remains the only curative option in this high-risk population. Because myeloablative conditioning regimen (MAC) is associated with less relapse, we hypothesized that more intensive conditioning regimen might be beneficial for MK AML patients.
Materials (or patients) and Methods: We retrospectively reviewed 303 patients diagnosed with MK AML, either de novo or secondary, over 45 year-old and transplanted between 2000 and 2011 with either a MAC or a reduced-intensity conditioning regimen (RIC). We compared outcomes between those 2 conditioning regimen and correlated them with available prognostic features.
Results: One hundred five patients received a MAC and 198 a RIC. Median age at SCT was 57 year-old, significantly lower in the MAC group (53 year-old) than in the RIC group (59 year-old). Median follow-up was 42 months (range 3-156 months). The 3-year overall survival (OS), leukemia-free survival (LFS) and relapse rate (RR) were not statistically different between both groups with overall values of 34%, 29% and 51%, respectively. On the contrary, the 3-year non-relapse mortality (NRM) was significantly higher in MAC recipients (28%) compared to RIC patients (16%, *P*=0.004). These results were confirmed in a multivariate analysis. Incidence of grade II to IV acute graft-versus-host disease (GvHD) was significantly higher after a MAC (30.5%) than after a RIC (19.3%, *P*=0.02). Incidence of chronic GvHD was comparable between both group (35%) and did not impact on LFS. Interestingly, within our MK AML cohort, hypodiploidy was significantly associated with lower OS, lower LFS and higher RR.
Discussion: Due to less toxicity and comparable OS, LFS and RR, RIC appears as a good transplant option in the very high-risk population of patients diagnosed with MK AML.
Disclosure of Interest: None Declared.

PH-P034

PATIENTS WITH PRIMARY REFRACTORY OR RELAPSED ACUTE MYELOID LEUKEMIA MAY ACHIEVE LONG-TERM REMISSION FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION – A SINGLE CENTER EXPERIENCE

P. Hemmati[1,], T. T. Terwey[1], I.-K. Na[1], P. le Coutre[1], L. G Vuong[1], B. Dörken[1], R. Arnold[1]*

[1]Haematologie, Onkologie und Tumorimmunologie, Charité - Universitaetsmedizin Berlin, Berlin, Germany

Introduction: Patients with primary refractory or relapsed acute myeloid leukemia (AML) have a dismal outcome with chemotherapy alone. Therefore, many of these patients are referred to allogeneic stem cell transplantation (alloSCT) as an ultimate approach. We investigated the long-term outcome of these patients transplanted at our center between 1997 and 2012.

Materials (or patients) and Methods: Altogether, 96 patients (37 female, 59 male) with primary refractory or relapsed AML were retrospectively analyzed. The median age was 48 (range: 19-70) years. 58 patients (60%) had de-novo AML, 28 patients (40%) had secondary or therapy-related AML. The median time from initial diagnosis to transplantation was 7 (range: 2-95) months. According to the SWOG/ECOG criteria cytogenetic risk was favorable, intermediate, or poor in 6 (6%), 40 (42%), or 44 (46%) patients. Out of these, 21 patients (22%) had a complex karyotype and 12 patients (13%) fulfilled the criteria of a monosomal karyotype. Conditioning was myeloablative (12 Gy TBI, 120 mg/kg CY) in 35 patients (36%), whereas 61 patients (64%) were transplanted following a reduced intensity conditioning regimen (150 mg/m^2 FLU, 8 mg/kg BU, 40 mg/kg ATG, or Flamsa-RIC). 33 patients (34%) had a 10/10 matched related donor. 50 patients (52%) had a 10/10 matched unrelated donor whereas for 13/96 patients (14%) a mismatched unrelated donor was chosen. In 91 patients (95%) peripheral stem cells were used as a stem cell source. 5 patients (5%) received a bone marrow graft.

Results: After a median follow-up of 80 (range: 12-182) months for the surviving patients, 23 patients (24%) are alive and in continuous complete remission. 47 patients (49%) died from relapse, whereas 26 patients (27%) died from infections or GvHD. In univariate analysis the presence of BM-blasts >20% was associated with a lower disease-free survival (DFS), whereas a poor-risk karyotype was associated with a higher relapse incidence. Reduced physical performance and the presence of relevant comorbidities predicted a higher non-relapse mortality (NRM). Multivariate analysis revealed that increased BM-blasts, a poor-risk karyotype, and poor physical performance are independent predictors for DFS, relapse, or NRM.

Discussion: Despite a high risk for relapse or NRM, in selected patients with primary refractory or relapsed AML unresponsive to salvage re-induction therapy long-term remission may be achieved by alloSCT. However, distinct risk-factors such as overall physical performance, comorbidities and genetic markers need to be taken into account during the decision process.

Disclosure of Interest: None Declared.

PH-P035

OUTPATIENT-BASED SALVAGE REGIMEN WITH 10 DAYS OF DECITABINE WITH OR WITHOUT DLI FOR RELAPSED MDS AND AML AFTER ALLOGENEIC HSCT

A. Muetherig[1,], J. Moritz Middeke[1], C. Thiede[1], K. Trautmann[1], C. Röllig[1], J. Schetelig[1], G. Ehninger[1], M. Bornhäuser[1], U. Platzbecker[1]*

[1]Department of Internal Medicine I, University Hospital TU Dresden, Dresden, Germany

Introduction: Relapse after allogeneic hematopoietic stem cell transplantation (HSCT) is mostly associated with a dismal prognosis in patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). In fact, the considerable toxicity of conventional chemotherapy often limits the potential success of conventional reinduction therapy. A 10-day regimen with decitabine (DAC), a demethylating agent, has been recently shown to be associated with a considerable response rate in patients with relapse after conventional chemotherapy. This approach has not been reported after allogeneic HSCT so far.

Materials (or patients) and Methods: We report on ten patients with either AML (*n*=9) or MDS (*n*=1) and a median age of 55 years (range 37 - 64) relapsing a median of 13 months after HCST (range 4 - 79). The median number of prior therapies was 3 (range 2-5). Notably, four patients had even progressed to a prior therapy with azacytidine. All patients received an outpatient-based 10 day regimen of DAC at a dose of 20 mg/m^2 every 4 weeks. Dose was adopted subsequently depending on response and toxicity. Donor lymphocyte infusion (DLI) was given on day 11 of the DAC schedule only in case of absence of GVHD.

Results: A median of 2 treatment cycles (range 1 – 5) were administered. In six patients DAC was followed by at least one DLI infusion while 2 patients underwent subsequent second transplantation following a conditioning with 2 Gy TBI. The median blood donor chimerism increased from 70 % (range 18 – 95) before treatment to 98 % (range 64 – 100) after the first cycle of DAC. At a median follow up of 4 months (range 2-9) after the first cycle five out of ten patients (50%) are alive and leukemia-free. One patient died of progressive disease and two patients died of infectious complications (pneumonia, colitis) due to DAC-related neutropenia while two patients showed progressive disease. Except of neutropenia no other DAC-induced toxicity was observed including no aggravation of GVHD.

Discussion: A 10-day schedule of DAC with or without DLI is associated with a rapid anti-leukemic effect in a considerable subset of patients with early relapse after allogeneic HSCT including prior failure to azacytidine therapy. Infectious complications due to sustained neutropenia require however careful surveillance of these patients.

Disclosure of Interest: None Declared.

PH-P036

ALLOGENEIC RELATED DONOR STEM CELL TRANSPLANTATION IN AML CR1: ANY RISK FACTOR FOR BETTER SURVIVAL?

S. Güvenç[1,], E. Ozcelik[2], F. Hindilerden[1], N. Hasbal[1], S. Serefhanoglu[2], R. Kucukkaya[1], M. Arat[1]*

[1]Hematology, Istanbul Bilim University, [2]Hematology, Sisli Florence Nightingale Hospital, Istanbul, Turkey

Introduction: Allogeneic stem cell transplantation (allo-SCT) is currently the only curative treatment option for non-M3 AML in first or second remission (CR1, CR2). Cytogenetic risk factors, pretransplant disease status and factors apart from leukemia could affect the success of allo-SCT. It is still uncertain that allo-SCT in CR1 has superior efficacy especially for those patients who have cytogenetically standard risk. The optimization of supportive care in last 2 decades decreased early transplant related mortality substantially.

Materials (or patients) and Methods: In this intent-to treat, single center, retrospective study we analyzed 40 consecutive AML patients who underwent allo-SCT between January 2011 and November 2013 at our centre to determine the role of allo-SCT in CR1 or beyond. Patients were evaluated for overall survival (OS), disease free survival (DFS), early (30 - 100 day) and late (101-365 day) transplant related mortality (TRM). Survival was estimated using the Kaplan–Meier method, and the log-rank test was used to compare survival curves among variables.

Results: Median age was 48 years (19-64 y). Among those patients who had cytogenetic data (28 patient, 70%), 12 (30%) were in high-risk category. Median time from diagnosis to allo-SCT was 6.5 months (2-54 months). Mean follow-up period for living patients was 419 days (105-857). Pretransplant disease status were as follows; 24 patients (60%) in CR1, 12 patients (30%) in CR2 and 4 patients (10%) had active disease. The conditioning regimens for allo-SCT consisted of myeloablative (Bu16-CY120) (*n*=32, 80%) or reduced-intensity regimens (Flu150-Bu8)(*n*=8, 20%). Donors were HLA-matched siblings for 32 patients (80%), unrelated for 7 patients (17.5%) and haploidentical for 1 patient (2.5%). All but 2 patients (95%) received peripheral blood stem cells. Overall, TRM

at day 30 and 100 were 7.5% (*n*=3) and 10% (*n*=4), respectively. Late death (>100 days) was observed in 7 patients (17.5%) and 3 of them were related to allo-SCT. Grade 2-4 aGVHD was seen in 20 (50%) patients and chronic extensive GVHD in 8 patients (20%). Of those 24 patients who underwent allo-SCT in CR1, 18 patients (75%) were still in remission without any sign of disease. Nine of 16 patients (56.3%) who underwent allo-SCT beyond CR1 lost due to relapsed leukemia. Survival analysis revealed superior OS between patients in CR1 (median 901 days) and beyond CR1 (median 202 days) (log-rank, OS *P*=0.003). For disease free survival we have seen the same profile (*P*=0.007). In CR1 patients (*n*=24) univariate analysis revealed no impact of standard-risk cytogenetics, high HCT-CI (≥2), high WBC at dx (>30k), sex (F to M), ABO mismatch and double induction for CR achievement. The only adverse factor for less OS in CR1 was past history of invasive fungal infection pre allo-SCT (*P*=0.038, 901d vs 553d).
Discussion: Allogeneic SCT in AML patients should be undertaken in CR1 with matched sibling donor. Pre-tx invasive fungal infection was the only worst predictive factor for OS in CR1 AML patients cohort.
Disclosure of Interest: None Declared.

PH-P037

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH TREATMENT-RELATED ACUTE MYELOID LEUKEMIA

A. Łojko[1,], A. Czyz[1], M. Matuszak[1], L. Gil[1], A. Nowicki[1], D. Dytfeld[1], M. Komarnicki[1]*
[1]Department of Hematology, University of Med.Sciences, Poznań, Poland

Introduction: The development of treatment-related acute myeloid leukemia (t-AML) is the most serious secondary event that can occur after chemotherapy or radiation therapy. The prognosis in this group of patients is very poor.
Materials (or patients) and methods: Here, we report the outcome of allogeneic hematopoietic stem cell transplantation (alloHSCT) in 13 patients (11 women, 2 men) with t-AML treated in our department between 2004 and 2013. This is 9% of all alloHSCT performed in patients with AML in our department in this period of time. Patients were treated by chemo- or radiotherapy due to the following previous malignancies: breast cancer (*n*=5), ovarian cancer (*n*=1), Hodgkin lymphoma (*n*=4) and non-Hodgkin lymphoma (*n*=3). Patients with treatment-related myelodysplastic syndrome and patients with AML secondary to myelodysplastic syndrome were not included to the analysis. Due to primary malignancies six patients were treated by chemotherapy only, 3 by radiotherapy and 4 by both methods. Three patients were also treated by high dose chemotherapy and autologous HSCT. The median interval between previous chemo- or radiation therapy and diagnosis of t-AML was 4 years (0,5–8 years). Median age of patients at diagnosis of t-AML was 51 years (37-63 years). None of them presented with extramedullary involvement at diagnosis, seven of them presented with chromosomal changes (trisomy 8 *n*=2, inversion 16 *n*=1, complex changes *n*=3). All patients were treated by induction and consolidation chemotherapy due to AML.
Results: Disease status at the time of HSCT was: first complete remission (CR) (*n* = 8); second CR (*n* = 2); and non CR (*n* = 3). Patients received myeloablative (*n*=2) or reduced-intensive (*n*=11) conditioning regimen and peripheral blood stem cells (*n*=12) or cord blood cells (*n*=1) from human leukocyte antigen-matched sibling donors (*n*=5), unrelated donors (*n*=7) or mismatch cord blood (*n*=1). Graft-versus-host disease (GvHD) prophylaxis was performed with a combination of cyclosporine and methotrexate or mycophenolate mofetil, additionally 10 patients received anti-T lymphocyte globulin. Neutrophil engraftment was achieved in all but one patient. Acute graft-versus-host disease (GVHD) grade II-IV occurred in one patient and chronic GVHD was observed in 2 patients. Relapse of disease occurred in three patients (23%), and all these patients died due to relapse. Median time between HSCT and relapse was only 2 months. Only one patient (8%) died due to transplant related reason, multiorgan failure, before neutrophil recovery. One patient died due to brain tumour which was revealed 9 months after HSCT, and one due to reason not related with t-AML and transplantation. After the median follow-up time of 23 months (1-105 months) seven patients (54%) were alive in complete remission of the disease. The one-year overall survival was 58% (95% CI 31-81).
Discussion: In summary, this retrospective analysis indicates that alloHSCT is a curative therapy for some patients with t-AML following primary malignancies, with acceptable toxicity and transplant related mortality. The major cause of death is relapse of the disease.
Disclosure of Interest: None Declared.

PH-P038

CLINICAL IMPLICATION OF TET2 MUTATION WITH NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA IN YOUNGER PATIENTS

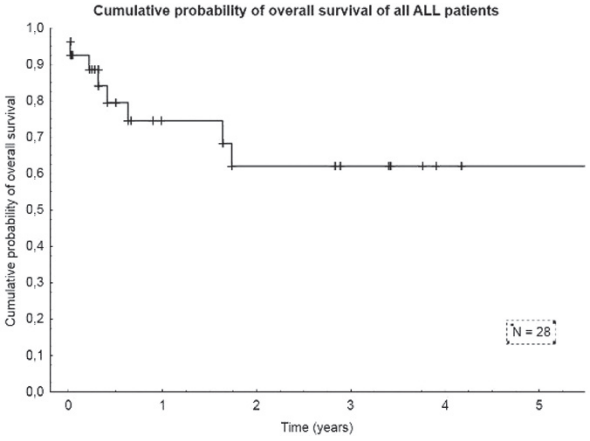
H.-J. Kim[1,], J.-S. Ahn[1], Y.-K. Kim[1], S.-H. Jung[1], D.-H. Yang[1], J.-J. Lee[1], I.-K. Lee[2], N. Y. Kim[2], D. D. H. Kim[3], M. D. Minden[4], C. W. Jung[5], J.-H. Jang[5], H. J. Kim[6], J. H. Moon[7], S. K. Sohn[7], J.-H. Won[8], S.-H. Kim[9], H. Kook[10], N. Kim[11], K. Yoshida[12], S. Ogawa[12]*
[1]Hematology-Oncology, [2]Genomic Research Center for Hematopoietic Diseases, Chonnam National University Hwasun Hospital, Jeollanam-do, Republic Of Korea, [3]Department of Medical Oncology and Hematology, [4]Department of medicine, Princess Margaret Hospital, Toronto, Canada, [5]Division of Hematology-Oncology, Samsung Medical Center, [6]Department of Hematology, The Catholic University of Korea, Seoul, [7]Department of Hematology-Oncology, Kyungpook National University Hospital, Daegu, [8]Department of Hematology-Oncology, SoonChun Hyang University Hospital, Seoul, [9]Department of Hematology-Oncology, Dong-A University College of Medicine, Busan, [10]Department of Pediatrics, Chonnam National University Hwasun Hospital, Jeollanam-do, [11]Department of Bioinformatics, Korean Bioinformation Center, Korea Research Institute of Bioscience and Biotechnology, Univeresity of Science and Technology, Daejeon, Republic Of Korea, [12]Cencer Genomics Project, The University of Tokyo, Tokyo, Japan

Introduction: The tet oncogen family member 2 (*TET2*) is one of the TET family of protein and *TET2* protein is expected to play a role in DNA demethylation. *TET2* mutation has the leukemogenic role and it identified to be mutated in 7% to 23% of acute myeloid leukemia (AML). However, the prognostic significance of *TET2* mutation is controversial in clinical setting.
Materials (or patients) and Methods: Patients were enrolled from three institutions. We performed a *TET2* mutational analysis in patients younger than 65 years of age who had AML with normal karyotype (NK). The Source of the DNA was bone marrow or peripheral blood and mutation analysis for *TET2* (NM_001127208) were performed using Sanger sequencing.
Results: From 1997 to 2012, total 363 younger (<65) patients with NK AML were available the DNA analysis. *TET2* mutation was observed in 47 (12.9%) patients. The 340 patients received the induction chemotherapy and 289 out of 340 patients achieved the complete remission (CR). There was no difference in CR rate according to the status of *TET2* mutation (*P*=0.288). After CR achievement, 146 patients received allogeneic stem cell transplantation (allo-SCT) and 143 patients received the chemotherapy only as a consolidation. The five-year survival in patients received allo-SCT was 55.6% (95% CI: 46.4-64.8) and chemotherapy only group was 37.2% (95% CI: 27.8-46.6) (*P*=0.000).The incidence of *TET2* mutation between two group did not show the statistical difference (*P*=0.530). We analyzed the influence of *TET2* mutation according to the consolidative type (allo-SCT or chemotherapy only). In the chemotherapy only group, the five-year OS of *TET2* wild type was 35.2% (95% CI: 25.3-45.2) and *TET2* mutant was 33.5 % (95% CI : 1.9-68.9) (*P*=0.493). In the allo-SCT group, the five-year OS of *TET2* wild vs mutant was 54.7% (95% CI: 44.5-64.9) and 61.6% (95% CI: 38.9-84.3), respectively (*P*=0.638). Thirty-one patients with *TET2* mutation received allo-SCT (*n*=21) and chemotherapy only (*n*=17) and, the 5-year OS in allo-SCT vs chemo only was 61.6% (95% CI : 38.9-84.3) vs 33.5 % 33.5 % (95% CI : 1.9-68.9) (*P*=0.285).

Discussion: We selected the relatively homogeneous population such as normal karyotype and younger (<65) AML patients. In this population, the incidence of *TET2* mutation was 12.9%. *TET2* mutation did not influence the treatment response such as CR rates and OS according to chemotherapy only or allo-SCT. In *TET2* mutated group, consolidative treatment (allo-SCT vs chemo only) did not show the statistical difference, just demonstrated the favorable tendency for allo-SCT. To confirm the role of allo-SCT in *TET2* mutation, it will be needed clinical trial for large number of patients.
Disclosure of Interest: None Declared.

PH-P039
ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA - TBI BASED MYELOABLATIVE CONDITIONING STILL SAFE AND EFFECTIVE
J. Dybko[1,], D. Urbaniak-Kujda[1], T. Wróbel[1], A. Kaczmar-Dybko[2], K. Kuliczkowski[1]*
[1]Hematology and Bone Marrow Transplantation, Wroclaw Medical University, [2]Radiotherapy, Lower Silesian Oncology Center, Wroclaw, Poland

Introduction: Successful treatment of adult acute lymphoblastic leukemia (ALL) still remains a challenge. Especially treatment of relapsed disease with chemotherapy, even in patients able to pursue consolidation with allogeneic hematopoietic stem cell transplantation (HSCT), has demonstrated limited success. However despite new chemotherapy modalities and tyrosine kinase inhibitors support for Philadelphia positive ALL cases, allogeneic HSCT remains an essential approach on the curative path.
Materials (or patients) and Methods: We retrospectively evaluated 28 patients. All of them have initially treated and then transplanted in our center in recent five years. The group comprised 17 males and 11 females with median age was 31 years (range 18-54). The whole group consisted of 5 T cell derived cases and 23 B cell derived cases with 7 Philadelphia positive cases among them. Other poor prognostic cytogenetic abnormalities were described at initial treatment in 18 cases. In te whole group 16 patients were transplanted from matched unrelated donor and 12 - matched sibling donor. In conditioning all patients received 12 GY fractioned total body irradiation (TBI) and Cyclophosphamide 120 mg/kg b.w. In unrelated setting Thymoglobuline (Genzyme) 4,5 mg/kg b.w. was added. Graft versus host disease and infection prophylaxis were used according to standard protocols.
Results: All patients achieved complete post transplant chimerism. Low stages (I,II) of GvHD were successfully treated in 18 cases. Advanced (III,IV) GvHD stages were diagnosed in 5 patients. No life-threatening infections occurred. Five-year OS of all patients was 65% - 2 patients died due to ALL relapse, 2 - conditioning related toxicity and 4 - advanced stages of GvHD. Five-year PFS was 88%. The average quality of life assessment of all living patients is 5,9 in 7 points scoring system (Burckhardt).



Discussion: Adult acute lymphoblastic leukemia remains a difficult therapeutic problem and requires some novel approaches based on better understanding of minimal residual disease to be successfully cured in all cases. However the results of allogeneic HSCT in our center experience are encouraging. Chemotherapy and TBI based conditioning is generally well tolerated and remains a standard approach especially in younger patients.
Disclosure of Interest: None Declared.

Aplastic Anaemia

PH-P040
UNMANUPLATED HAPLOIDENTICAL HSCT: A FEASIBLE WAY FOR CHILDREN WITH SEVERE APLASTIC ANEMIA
C. Luo[1], Z. Luan[2], C. K. Li[3], Y. Zhu[4], X. Tang[2], V. lee[3], J. Chen[1,] and China Pediatric Bone Marrow Transplant Group*
[1]Hematology Oncology, Shanghai Jiao Tong University School of Medicine, Shanghai Children's Medical Center, Shanghai, [2]Navy General Hospital, Beijing, China, [3]Pediatric Hematology Oncology, Chinese University of Hong Kong, Hong Kong, Hong Kong, [4]West China Second University Hospital of Sichuan University, Chendu, China

Introduction: Survival outcomes from hematopoietic stem cell transplantation (HSCT) in severe aplastic anaemia (SAA) have improved steadily over the past decade but haploidental HSCT remain challenges mainly associated with graft failure and GVHD. So it has been considered in the final choice of treatment status for SAA.
Materials (or patients) and Methods: We retrospectively evaluated the outcome of 27 children with SAA who received unmanuplated haploidentical HSCT in China Pediatric Bone Marrow Transplant Group between July 2002 and Nov 2013. All 27 cases failed to respond to previous immunosuppressive therapy (average 23 months, range from 1 to 72 months) and were heavily transfused before transplantation. 3 patients were even in active infection at the time of haploidentical HSCT because they failed first HSCT.
Results: For the HLA 12/27, 8/27 and 7/27 had 1/6, 2/6 and 3/6 loci mismatch respectively. Among the 27 patients, 25 achieved neutrophil and platelet recovery at a median of 13±1.8 days (range from 10 to 21 days) and 19±9.1 days (range from 7 to 41 days) respectively. Two patients (7.4%) failed to achieve engraftment, both received second same donor HSCT and 1 achieved sustained engraftment. The cumulative incidence was 33.3% for grade II-IV acute GVHD and 27.3% for chronic GVHD. After median 24months (1-138months) follow-up only 3 patients died because of acute GVHD, infection and post transplantation lymphoproliferative disease respectively. The 3 year OS and EFS was 86.9 ± 12.4% and 85.2%± 12.4% for all surviving patients, especially 100% for our 12 patients with 1/6 mismatched HSCT.
Discussion: These excellent outcomes suggest that unmanuplated haploidentical HSCT is a feasible way for children with SAA without HLA-identical sibling donor. It should be considered to be used earlier especially for the patient with only 1/6 mismatched family donor.
Disclosure of Interest: None Declared.

PH-P041
SINGLE CENTRE EXPERIENCE OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) IN HEAVILY PRE-TREATED ADOLESCENT AND YOUNG ADULT (AYA) WITH FANCONI ANEMIA
A. Alhuraiji[1,], M. Aljurf[1], F. Almohareb[1], H. Alzahrani[1], N. Chaudhri[1], F. Alsharif[1], W. Rasheed[1], S. Mohamed[1], G. Aldawsari[1], A. Hanbali[1], T. Elhassan[1], S. Ahmed[1]*
[1]Hematology/HSCT section, King Faisal SPecialist hospital and Research Centre, Riyadh, Saudi Arabia

Introduction: Fanconi anemia (FA) is an inherited disorder that is associated with congenital anomalies, bone marrow failure and an

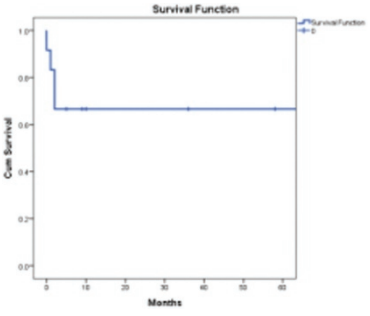
increased risk of cancer. Hematopoietic stem cell transplantation (HSCT) is a modality that is potentially curative for the bone marrow failure that these patients have. Here we report our Centre's experience of HSCT in adolescent and young adult (AYA) patients with FA, a cohort of patients who are older and more likely to be heavily pre-treated than the majority of patients in reported series.

Materials (or patients) and Methods: We carried out a retrospective analysis of patients' data from medical records and electronic systems from 1988-2012. We included patients with confirmed FA based on positive chromosome breakage study who underwent stem cell transplant at our institution aged >14.

Results: A total of 12 patients with FA underwent HSCT. The median age was 17.7 years (range 14-26y) with female predominance of 75%. Patients' baseline characteristics are shown in table 1. Two patients had dysplasia on the bone marrow with abnormal cytogenetic. Prior to HSCT all patients were transfusions dependent; median ferritin level of 10 patients was 3500 ng/l (range 134-6998). Five patients (41%) received prior steroids (*n*=3) and/or androgens (*n*=3). One patient received prior ATG and cyclosporine. Conditioning included TBI in 5 patients (41%). ATG was included in the conditioning regime in 10 patients (83%). Eleven patients (91%) received a graft from an HLA identical donor; stem cell source was bone marrow in 83.3% cases.

The Overall 5 year survival was 67%. Four patients (33%) died, all before day +100 of HSCT without evidence of engraftment. The causes of mortality were transplant related being infection, Acute Respiratory distress syndrome (ARDS) or grade 4 Graft versus host disease (GVHD). At a median follow up of 47 months (range 5-245) for surviving patients (67%), all the latter patients engrafted and remained transfusion independent. Two surviving patients developed aGVHD ≥grade II. One patient developed chronic GVHD.

Age at the time of transplant	**14-26**	**Median 17.77**
Gender	M:F 4:1	
HLA matched		
- · Indentical	11(91.6%)	
- · 1 Antigen mismatch	1(8.3%)	
Stem Cell Harvest		
- · Source	BM 10(83 %) PB 1(8.3%) CB 1(8.3%)	
- · Dose		Median 4.0X10^9/Kg
Conditioning		
- · Cy/TBI +/-ATG	5 (41%)	
- · Cy/ATG +/- Flu +/- Bu	7 (59%)	
GvHD prophylaxis	Cyclosporine A based 91.6%	
- · CSA alone	6 (50%)	
- · CSA + Methotrexate	3 (25%)	
- · Other	3 (25%)	
GvHD grade ≥II		
- · Acute	3(25%)	
- · Chronic	1(8.3%)	



Discussion: Our findings support the feasibility of allogeneic HSCT in older and more heavily pre-treated patients with FA. Patients who engrafted had excellent long-term outcomes. The prevalent risk of early TRM highlights the need for optimization of pre-and peri-transplant supportive care and measures to reduce the risk of graft failure. Further studies of HSCT in AYA and older patients are warranted.

Disclosure of Interest: None Declared.

PH-P042

EFFECT OF STEM CELLS ON ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH IDIOPATHIC APLASTIC ANEMIA

H. Kim [1,] on behalf of The Korean Society of Blood and Marrow Transplantation, K.-H. Lee [2], I. Kim [3], S. K. Sohn [4], S. J. Kim [5], S. H. Kim [6], Y. Park [7], J. H. Choi [8], J. Y. Kwak [9], M. K. Kim [10], S. H. Bae [11], H. J. Shin [12], J. H. Won [13], W. S. Lee [14], S. J. Shin [15], J.-H. Park [1], Y. D. Joo [16]*

[1]Division Of Hematology and Hematological Malignancies, ULSAN UNIVERSITY HOSPITAL, UNIVERSITY OF ULSAN COLLEGE OF MEDICINE, Ulsan, [2]Department of Hematology, ASAN Medical Center, [3]Seoul National University Hospital, Seoul, [4]Kyungpook National University Hospital, Daegu, [5]Samsung Medical Center, Seoul, [6]Dong-A University Medical Center, Busan, [7]Korea University Anam Hospital, [8]Hanyang University Hospital, Seoul, [9]Chunbuk National University Hospital, Jeonju-si, [10]Yeungnam University Medical Center, [11]Daegu Catholic University Hospital, Daegu, [12]Pusan National University Hospital, Busan, [13]Soon Chun Hyang University Hospital, Seoul, [14]Inje University Busan Paik Hospital, Busan, [15]Dongkang Medical Center, Ulsan, [16]Inje University Haeundae Baik Hospital, Busan, Republic Of Korea

Introduction: Stem cell source is the main issue in allogeneic hematopoietic cell transplantation (alloHCT). In these days, there are more and more alternative donors (AD) other than matched sibling donor (MSD) in which bone marrow (BM) was preferred to mobilized peripheral blood (PB) as stem cell source in adult idiopathic aplastic anemia (AA). However PB has some advantages over BM: not requiring general anesthesia; easy accessibility; probability of higher stem cells dose. There needs to answer which stem cell source is preferable in specific donor setting and how much stem cell dose is adequate. Therefore we wanted to investigate the clinical impact of stem cell source on alloHCT in AA according to donor type.

Materials (or patients) and methods: We retrospectively analyzed the effect of stem cells on alloHCT in AA. Mismatched donors (MMD) included haplo-identical family donor, mismatched unrelated donor or partially matched family donor. AD referred either matched unrelated donor (MUD) or MMD. Cord blood stem cells were not included in this analysis.

Results: Total 267 patients were included in this analysis. Donors were MSD in 172, MUD in 38 and (MMD in 58 patients. BM and PB were used as stem cell sources in 194 and 74 patients, respectively. There was no cord blood. BM was associated with low incidence of acute graft versus host disease (GvHD; P<0.001) but not in G3/4 acute GvHD (P=0.427), and this tendency was more dominant in MSD (P=0.011) than in AD (P=0.057). BM, however, had no impact on other transplantation outcomes including chronic GvHD (P=0.673) and primary/secondary graft failure (P=0.774). Higher stem cell dose (CD34+ cells >3*10^6/kg) was also associated with higher incidence of acute GvHD (P=0.039) but not in G3/4 acute GvHD (P=0.805). This tendency was only observed in AD (p=0.094) and not in MSD (P=0.334). Higher stem cell dose had no impact on other transplantation outcomes except for low incidence of extensive chronic GvHD in MSD (P=0.025). Multivariate analysis on overall survival in MSD revealed that only Age at alloHSCT<31 years old (HR 3.234; 95% CI, 1.328-7.877; P=0.010) and prior platelet transfusion less than 86U (HR=2.618; 95% CI 1.017-6.738; P=0.046) were the favorable prognostic factors. On the other hand, multivariate analysis in AD revealed that higher stem cell dose (HR=2.596; 95% CI 1.020-6.609; P=0.045) was the only significant favorable factors on overall. BM stem cell was not

the favorable factor on overall survival (HR 1.998; 95% CI 0.646-6.176; *P*=0.229).
Discussion: In conclusion, peripheral blood stem cells are preferable in AD because higher stem cell dose can be easily achieved for longer overall survival without severe GvHD risk in expense of overall acute GvHD increase. However, BM stem cells are preferred in MSD because of lower incidence of acute GvHD without sacrificing graft failure or overall survival.
Disclosure of Interest: None Declared.

PH-P043

OUTCOME OF HAPLOIDENTICAL PBSC TRANSPLANTATION FOR HIGH RISK APLASTIC ANEMIA WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE IS SIMILAR TO MATCHED SIBLING PBSCT AND DEPENDS ON CHOICE OF DONORS: ADVERSE EFFECT OF NATURAL KILLER CELL ALLOREACTIVITY

S. Rani Jaiswal[1,2,], S. Apte[3], S. Kannan[3], S. Chakrabarti[1,4]*
[1]hemato-oncology And Bmt, Dharamshila Hospital And Research Centre, New Delhi, [2]hemato-oncology And Bmt, Apollo Gleneagles Hospital, Kolkata, [3]hemato-oncology And Bmt, Sahayadri Speciality Hospital, Pune, [4]Hemato-oncology And Bmt, Manashi Chakrabarti Foundation, Kolkata, India

Introduction: Patients with Aplastic Anemia (AA) who are heavily transfused, failed immunosuppressive therapy or have active infection at the time of transplantation have the highest risk of NRM or graft failure. Only 20% of such patients find a Matched Family donor and matched unrelated donors are not available to the majority of those of noncaucasian origin.
Materials (or patients) and Methods: In a pilot study, we carried out haploidentical transplantation (HAPLO) from unmanipulated peripheral blood stem cell (PBSC) graft for 8 patients with high risk AA and compared the outcome with a similar cohort of 16 patients receiving PBSC graft from Matched Sibling Donors (MSD). The MSD group received conditioning with Fludarabine and Cyclophosphamide with Cyclosporine and Methotrexate as GVHD prophylaxis. The HAPLO group received Fludarabine, low dose Cyclophosphamide, Melphalan and ATG with post transplant Cyclophosphamide (PTCY), Cyclosporine, MMF +/- Sirolimus as GVHD prophylaxis.
Results: Both groups were matched for age, gender and disease status; however patients in the HAPLO group were more heavily transfused and transplanted late, whereas MSD group had more active fungal infections at the time of transplant. 7/8 patients in the HAPLO group and all patients in MSD group had sustained engraftment. MSD group received higher CD34 containing graft and had earlier neutrophil engraftment (10 vs 13 days) but similar platelet engraftment (18 days). The incidences of aGVHD and cGVHD were 12.5% and 20% vs 37.5% and 50% in HAPLO and MSD group respectively (*P*=ns), but 3 patients in the HAPLO group experienced severe early alloreactivity not related to GVHD. More patients in the MSD group had invasive fungal infection (*P*=0.03), although the overall incidence of infections were similar in both groups. The 2 years disease free survival (DFS) was 50% (CI 32.3-67.7) in HAPLO group vs 43.8%(CI 31.4 – 56.2) in MSD group (*P* = 0.9). On further analysis of the HAPLO group, the only factor adversely impacting the outcome was NK ligand mismatched donor with high B score (*P*= 0.007). These patients experienced severe and fatal early alloreactivity not witnessed in the other cohorts.
Discussion: In the largest series to date on haploidentical PBSCT with PTCY, we have demonstrated that patients with high risk AA who do not have MSD can undergo unmanipulated PBSC graft with PTCY from haploidentical family donors with similar outcome but should avoid donors with NK ligand mismatch and high B score to prevent early alloreactivity and nonrelapse mortality.
Disclosure of Interest: None Declared.

PH-P044

NON MYELOABLATIVE HAPLOIDENTICAL STEM CELL TRANSPLANTATION WITH POST GRAFT HIGH DOSE CYCLOPHOSPHAMIDE FOR REFRACTORY SAA OR AFTER FAILED UNRELATED OR CORD BLOOD TRANSPLANT

A. Kulasekararaj[1,], V. Potter[2], J. Clay[2], D. Mclornan[2], K. Raj[2], H. De Lavallade[2], M. Kenyon[2], A. Pagliuca[2], G. J. Mufti[1], J. Marsh[1]*
[1]Haematological Medicine, King's College Hospital/King's College London, [2]Haematological medicine, King's College Hospital, London, United Kingdom

Introduction: Alternative donor HSCT for patients with acquired SAA who fail to respond to immunosuppressive therapy and lack a matched sibling or unrelated (UD) donor, using a haploidentical family donor, is a potentially curative option. A haploidentical donor is available for most patients, the cost of the procedure is cheaper than cord HSCT, and time to procure the graft is short. But, published data are limited, mostly restricted to children and report poor outcomes due to poor engraftment and high risk of GVHD. We present a single centre study of patients receiving haploidentical HSCT for SAA.
Materials (or patients) and Methods: Eight patients were transplanted, who either had refractory acquired SAA (*n* = 4) or who failed to engraft following UD (*n* = 3, 2 of whom failed UD HSCT x 2) or cord blood (*n* = 1) HSCT. Two patients had secondary severe marrow aplasia: one following chemotherapy for Hodgkin's disease who failed UD HSCT and one after 2 failed UD HSCTs for MDS. Median age was 32 yr (range 19-57) and median disease duration 46 months (6-91). Family donors were siblings (*n*=5), parents (*n*=2) and children (*n*=1). Conditioning regimen was fludarabine 30 mg/m^2 (days −6 to −2), cyclophosphamide (CY) 14.5 mg/kg (days −6 and −5) and TBI 2 Gy (day −1). Unmanipulated peripheral blood stem cells (PBSC) were infused to maximize cell dose (median CD34+ cell dose 6.2x10^6/kg, range 1.8-8.3. GVHD prophylaxis was CY 50 mg/kg/d (day +3 and +4) to deplete donor allo-reactive T-cells, tacrolimus 1mg/day for 9 months and taper between 9-12 months, and MMF 15 mg/kg until day 35. Median follow of survivors was 14.8 months (7.2-44.4). Median (and range) Karnofsky and HCT-CI scores were 80% (50-90) and 3 (0-5), respectively.
Results: Six patients had sustained neutrophil engraftment; median time to neutrophil engraftment 18.5 days (range 16-23) and 5 sustained platelet engraftment 26 days (range 21-27). Full donor chimerism in unfractionated cells, CD3 and CD15 lineages was achieved and maintained at last follow up. Two patients who failed to engraft died on days +60 and +137 from sepsis. Both had multiple HLA antibodies directed against the donor, which persisted at high level (MFI) despite treatment with rituximab and plasma exchanges pre-transplant. Two patients developed CMV viraemia, and there was no case of EBV post-transplant lymphoproliferative disorder. One developed Guillan Barre syndrome. There was only one acute GVHD (Gd II skin) and no chronic GVHD. Median follow up was 12.2 months (3.2-40.4). The conditioning regimen was well tolerated and with no haemorrhagic cystitis. One patient is currently 30 weeks pregnant at day+ 470 day post-transplant.
Discussion: We show in a small cohort that non-myeloablative haploidentical HSCT with post-graft high dose CY and PBSCs is a feasible option. Engraftment occurred after failed prior MUD or cord HSCT. Our patients had a high HCT-CI and poor performance status, but had minimal toxicity from the transplant procedure. The lack of GVHD (only one case of acute Gd II skin GVHD) despite using PBSC is of interest and warrants further exploration in larger studies. High levels of donor specific anti-HLA allo-antibodies explain the non-engraftment in two patients and warrants careful screening of family donors, which if present, likely preclude them from the procedure.
Disclosure of Interest: None Declared.

PH-P045

DEFINING PARTIAL REMISSION AFTER IMMUNOSUPPRESSION THERAPY FOR SAA

A. Rovó[1,], A. Tichelli[1], H. Schrezenmeier[2], G. Socié[3], A. Bacigalupo[4], B. Höchsmann[2], R. Peffault de la Tour[3], J. Passweg[1], C. Dufour[5], J. Marsh[6] and on behalf of the Severe Aplastic Anemia Working Party (SAAWP) of the European Group for Blood and Bone Marrow Transplantation (EBMT)*

[1]University Hospital Basel, Basel, Switzerland, [2]University Hospital Ulm, Ulm, Germany, [3]Hospital Saint Louis, Paris, France, [4]Ospedalo San Martino, Genova, [5]G Gaslini Childrens' Hospital, Genova, Italy, [6]King's College Hospital, London, United Kingdom

Introduction: Immunosuppression (IS) with ATG and cyclosporine is the treatment of choice for severe aplastic anemia (SAA) patients not eligible for hematopoietic stem cell transplantation. Severity degree criteria at diagnosis are well defined; in contrast the definition of remission after IS is not totally satisfactory. For the definition of complete remission (CR) there are several criteria worldwide, however with minor impact on the clinical outcome. Partial remission (PR) includes all patients after IS who do not meet criteria for SAA any longer and are independent of transfusions. We hypothesize that partial remission status is more heterogeneous and therefore a subclassification would be more informative for patients' outcome.

Materials (or patients) and Methods: To confirm our hypothesis we used the database from the randomized EBMT-G-CSF study (Tichelli *et al*, Blood 2011). For the analysis we included all patients in PR (according to NHI criteria). We defined good PR all patients fulfilling simultaneously hemoglobin (Hb) ≥80 g/L and platelets (Plt) ≥ 30 G/L. Patients with at least one of these blood values below the cut-off criteria were considered as poor PR. Neutrophils were not included in this analysis since all patients in PR had values above 0.5 G/L. We compared event free survival (EFS: loss of response, secondary myelodysplastic syndrome, death) and overall survival (OS) at six years of both groups considering platelet counts, hemoglobin and the type of PR (good versus poor) at day 90 and 180. From 205 patients of G-CSF study, at day 90 and 180, 45 and 54 patients respectively were in PR and evaluable.

Results: Patients with Plt ≥ 30 G/L at day 90 showed significant better EFS at 6 years (62%) than those below (37%; *P*=0.045). There was no difference between patients with higher or lower Hb (*P*=0.906). When compared good versus poor PR, there was no difference for EFS (*P*=0.245) neither for OS (*P*=0.365). Patients with Plt ≥ 30 G/L at day 180 showed significant better EFS at six years (64%) than those below (20%; *P*=0.008). Patients with Plt above 30 G/L showed also a better OS (91%) than those with lower values (53%; *P*=0.011). There was no difference for patients with higher or lower Hb (*P*=0.232). When considered good versus poor PR, there was a trend for better OS in favor of good PR (63% versus 44%; *P*=0.081) whereas EFS showed not difference (90% versus 67%; *P*=0.10).

Discussion: Platelet values at day 90 and 180 in PR patients seem to be the main prognostic factor for EFS. In this analysis Plt values on day 180 was the only prognostic factor for OS. Hemoglobin does not seem to be relevant; however the low number of patients included in this analysis might limit the interpretation of these results. The subclassification of patients in *good and poor partial remission* seems to be meaningful for the outcome. Platelets appear to be the most relevant prognostic factor.

Disclosure of Interest: None Declared.

PH-P046

DONOR TYPE APLASIA (DTA) OCCURRING 12-44 MONTHS AFTER MATCHED SIBLING OR UNRELATED DONOR BONE MARROW TRANSPLANTATION IN CHILDREN WITH SEVERE APLASTIC ANAEMIA

A. Shaw[1,], J. R Passweg[2], J. de la Fuente[3], R. Bajwa[4], J. Stein[5], M. Bradbury[6], M. Morgan[7], M. Cummins[8], J. M. Cornish[8], J. P. Moppett[8], C. Graham Steward[8]*

[1]Medical School, Bristol University, Bristol, United Kingdom, [2]Hematology Division, Hôpitaux Universitaires de Genève, Geneva, Switzerland, [3]Division of Paediatrics, Imperial College Healthcare NHS Trust, London, United Kingdom, [4]Department of Hematology/Oncology/BMT, Nationwide Children's Hospital, Columbus, United States, [5]Hemato-Oncology Department, Schneider Children's Medical Center of Israel, Petach Tikvah, Israel, [6]Paediatric Haematology, Addenbrookes Hospital, Cambridge, [7]Paedatric Haematology/Oncology, Southampton University Hospitals NHS Trust, Southampton, [8]Bone Marrow Transplant Unit, Royal Hospital for Children, Bristol, United Kingdom

Introduction: There have been sporadic reports of the development of delayed disease recurrence following bone marrow transplantation (BMT) for severe aplastic anaemia (SAA) despite sustained majority or full donor chimerism. This is termed "donor-type aplasia" (DTA). Unpublished Japanese registry data from the period 1980-2010 reported DTA in 5.7% of 660 patients aged <20 years following family/unrelated donor transplants but a surprisingly high 10-year overall survival at 87%.[1] The study identified statistically significant correlations with use of fludarabine in conditioning therapy (*P* < 0.0001), low infused total nucleated cell (TNC) dose (≤3 x10^8/kg, *P*=0.008) and use of immune suppressive therapy (IST, *P*=0.04) before transplant. Replacement of conventional cyclophosphamide (Cy) 200 mg/kg conditioning therapy with Cy 100 mg/kg plus fludarabine resulted in a 14% incidence of DTA compared to 0% in the Cy 200 group (*P*=0.04).

Materials (or patients) and Methods: We describe another 7 children from five institutions from Europe, Israel and the USA who developed DTA in the period 2001-2010. They had presented with SAA/VSAA and proceeded to transplant aged 5.8-15.8 years. Only one received upfront IST; this patient went on to receive 10/10 high resolution matched unrelated donor (MUD) BMT 25 months after presentation. The remaining six patients all had matched siblings (MSD) and underwent transplantation within 1-5 months of presentation.

Conditioning for MSD comprised Cy 200mg/kg total dose (6 patients) plus ATG (various formulations and doses, 3pts) or Alemtuzumab (0.9-1 mg/kg total dose, 3pts) and for MUD BMT Cy 120mg/kg, fludarabine 150mg/m^2 plus Alemtuzumab 0.9 mg/kg. GVHD prophylaxis comprised ciclosporin/methotrexate in four MSD BMT and ciclosporin alone in the remainder. Infused TNC doses averaged 4.48 x 10^8/kg (range 2.1-8.4).

Results: DTA developed at 12-44 months post-BMT. Most recent white blood cell chimerism prior to developing DTA was 61, 72, 80, 90, 91, 93 and 100%. Parvovirus was a likely aetiological factor in three patients: in two DTA developed soon after primary parvovirus infections and a third patient went from 40% marrow cellularity to severely hypocellularity soon after secondary reactivation. Two patients developed Aspergillus infections requiring partial lung and kidney resections respectively as a consequence of their recurrent pancytopenia.

One patient received two donor leukocyte infusions to combat mixed chimerism and persisting transfusion dependence but without benefit. Two patients received CD34-selected stem cell top-ups, again without sustained response. Two patients are too early following development of DTA to present outcome data. The remaining five patients underwent a second BMT, all from their original donors. Three are completely well with good counts at 8-124 months post-second transplant. One patient developed further DTA and required 5/6 matched cord blood transplantation and another developed atypical haemolytic uraemic syndrome and persistent thrombocytopenia.

Discussion: In contrast to Japanese experience, DTA was skewed to patients who had received sibling BMT (6/7 cases) and only one

had received fludarabine during conditioning therapy (for MUD BMT). Parvovirus appeared to be a strong candidate in causing DTA in three patients. All patients are alive but response to second transplant was variable.
Disclosure of Interest: None Declared.

PH-P047

A COMPARISON BETWEEN PERIPHERAL BLOOD STEM CELL TRANSPLANTATION AND BONE MARROW TRANSPLANTATION AS PROGENITOR CELL SOURCE IN SEVERE APLASTIC ANEMIA

A. Ghavamzadeh[1,], K. Alimoghaddm[1], M. Jalili[1], A. Jalali[1], L. Sharifi Aliabadi[1], A. A. Hamidieh[1], S. A. Mousavi[1], B. Bahar[1], M. Vaezi[1], M. Jahani[1]*
[1]Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of Medidal Sciences, Tehran, Iran, Republic Of Islamic

Introduction: Severe Aplastic Anemia (SAA) is a rare and lethal disease which could be cured by hematopoietic stem cell transplantation (HSCT). *There is still controversy over* the use of bone marrow as the recommended source for HSCT. Here, we report the results obtained in our center by comparing patients who received peripheral blood stem cell transplantation (PBSCT) with those who underwent bone marrow transplantation (BMT).
Materials (or patients) and Methods: A total of 185 patients diagnosed with SAA received allo-HSCT from full-matched HLA-identical sibling donors between 1991 and 2013. 40 patients (median age: 17.5 years, 1-32) underwent bone marrow transplantation and 145 patients (median age: 24, 2-50) received PBSCT. According to our protocol, all patients received the conditioning regimen containing cyclosphamide with or without ATG and cyclosporine and methotrexate for graft-versus -host disease (GvHD) prophylaxis.
Results: The median follow-up was 81.5 (54-231) months for the BMT group versus 49 (4-169) months for the PBSCT group. Thirty (75%) patients in the BMT group and 115 (79.3%) in the PBSCT group remained alive in the end of the study. The 5-year disease-free survival was 57.5% and 76.9% in the BMT and PBSCT groups, respectively (*P*-value=0.008, CI). The 5-year overall survival was 72.5% and 78.3% in the BMT and PBSCT groups, respectively (*P*-value=0.357, CI). Eleven (27.5%) patients in the BMT group and 8 (5.5%) patients in the PBSCT group relapsed after transplantation (*P*<0.001). The median days for WBC engraftment was 17.5 (9-56) days in the BMT group and 11 (7-43) days in the PBSCT group (*P*<0.001). The median days for platelet engraftment was 21.5 (10-67) days in the BMT group and 16 (9-63) days in the PBSCT group (*P*=0.023). There was no difference in the incidence of acute GvHD between the two groups. The cumulative incidence of chronic GvHD for those who survived more than 100 days was 25.7% in the BMT group, compared to 51% in the PBSCT group (*P*=0.014).
Discussion: Results of the current study indicated that there was no difference in overall survival, but disease-free survival was significantly better in the PBSCT group due to less rejection.
Compared to BMT group, engraftment was faster in the PBSCT group and hospitalization was significantly shorter. The incidence of chronic GvHD was higher in the PBSCT group, but most of them were limited. Although bone marrow is considered as the preferred source for transplantation in other studies, according to our results, more attention should be given to the use of PBSC for transplantation of patients with SAA due to its less rejection and limited cGvHD.
Disclosure of Interest: None Declared.

PH-P048

FEASIBILITY AND OPTIMAL SCHEDULE OF USING ECULIZUMAB IN PATIENTS WITH HEMOLYTIC PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (HPNH) WITH SEVERE APLASTIC ANEMIA (SAA) PRIOR TO HAEMOPOIETIC STEM CELL TRANSPLANT (HSCT)

A. Kulasekararaj[1,], V. Potter[2], S. Gandhi[1], D. Mclornan[1], H. delavallade[2], R. Kavita[1], N. Benson-Quarm[3], J. Large[3], M. Kenyon[2], A. Pagliuca[1], G. Mufti[2], J. Marsh[2]*
[1]King's College Hospital, [2]Kings College Hospital, [3]Kings Collge Hospital, London, United Kingdom

Introduction: Eculizumab, humanized monoclonal antibody directed against complement component C5, has changed the treatment paradigm of patients with haemolytic paroxysmal nocturnal haemoglobinuria (hPNH). HSCT should be the preferred option for PNH patients on eculizumab who develop overt bone marrow failure (BMF) during their clinical course or presenting concomitantly with SAA and PNH. The optimal use of eculizumab in the peri-transplant setting to prevent haemolytic/thrombotic complications and also the ideal conditioning regimen to eradicate the PNH clone is yet undetermined.
Materials (or patients) and Methods: We analyzed 7 patients with PNH and SAA who underwent HSCT at our Centre for their BMF. Of these 5 patients were on eculizumab for a median of 8 months (range 4-12) for PNH prior to worsening of their aplasia necessitating regular blood product (red cells and platelet) support. Two patients were initiated eculizumab during the pre-transplant period for their coexisting haemolytic PNH and SAA, whilst donor search was undertaken.The median age 32 years (range 18-56 yrs) and median disease duration was 48 months (range 4-252 months). All patients had evidence of haemolysis, elevated LDH and a large granulocyte clone (median 95%, range 57-100).The conditioning received was a fludarabine/BU-based RIC HSCT, of these 4 received T-cell depletion using ATG (anti-thymocyte globulin) and 1 with alemtuzumab (100% CD3+ cells co-expressed CD52). Two patients received a haploidentical transplant with post-transplantation cyclophosphamide. The source of stem cells in all 7 was GCSF mobilized peripheral blood stem cells.Eculizumab was continued until the transplant and the last dose was given on the start of the conditioning protocol.
Results: Pre-transplant 3 patients successfully underwent embryo (*n*=1) and ova (*n*=2) cryopreservation with ovarian hyper stimulation protocol (supraphysiological doses of estrogen) with eculizumab used prophylactically to cover thrombotic complication, as they were severely thrombocytopenic and were unable to receive low molecular weight heparin.
Neutrophil and platelet engraftment occurred at 16 and 21 days respectively. No increased infectious complications was observed. The PNH clone was undetectable on D+14 and none was seen after engraftment with normalization of LDH. No clinical evidence of venoocclusive disease (VOD) was seen. Acute GVHD (grade 2) and Chronic GvHD (skin and gut) were observed in one patient each. Two patients failed to engraft, of which one was successfully rescued with a haploidentical HSCT although the PNH clone disappeared following the conditioning used in the first transplant for both patients. Full donor chimerism in unfractionated, CD3 and CD15 lineages was achieved at D100 post HSCT.
Discussion: This data demonstrated the feasibility of using eculizumab in patients with PNH and SAA, during the peri-transplant setting. The use of myeloablation in conditioning facilitates the eradication of the PNH clone. The successful use of eculizumab in preventing the thrombotic risks associated with ovarian hyper stimulation protocol is also evident. Larger studies and uniform strategies are needed to evaluate the optimal use of anti-complement therapy for SAA/PNH patients undergoing HSCT.
Disclosure of Interest: None Declared.

PH-P049

HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM AN ALTERNATIVE DONOR FOR CHILDHOOD APLASTIC ANEMIA: HLA HAPLOIDENTICAL FAMILY DONOR VS HLA MISMATCHED UNRELATED DONOR

Y. Takahashi[1,], Y. Sekiya[1], N. Kawashima[1], A. Narita[1], M. Irie[1], H. Muramatsu[1], A. Hama[1], S. Kojima[1]*

[1]Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan

Introduction: Treating patients with severe aplastic anemia (SAA) who fail to respond to immunosuppressive therapy (IST) and do not have an HLA-matched donor is challenging. Moreover, patients with SAA who develop infection because of neutropenia or graft failure after hematopoietic stem cell transplantation (HSCT) require urgent HSCT. HLA haploidentical family donor could be considered as one of alternative donor sources because it's 1) easy to find the donor in relatives, 2) fast to engraft and 3) possible to use donor cell derived cell therapies such as virus specific cytotoxic T lymphocytes (CTLs) and mesenchymal stromal cells (MSCs). We analyzed 25 children with aplastic anemia (AA) who underwent HSCT from HLA mismatched unrelated donor (mismatched UR-HSCT) on schedule or HLA haploidentical donor (haplo-HSCT) for an urgent need of SCT.

Materials (or patients) and Methods: Nineteen children with SAA conducted mismatched UR-HSCT and six children with SAA received urgent haplo-HSCT between 2000 and 2013 in Nagoya University hospital. The conditioning regimen in mismatched UR-HSCT consisted of cyclophosphamide (200 mg/kg), antithymocyte globulin (ATG, 10 mg/kg), and total body irradiation (TBI, 5 Gy). In haplo-HSCT, patients received a conditioning treatment consisting of fludarabine 30 mg/m^2 i.v. x 4 days, melphalan 70 mg/m^2 i.v. x 2 day and TBI 5Gy in two fractions. BMT was conducted on day0 and G-CSF mobilized peripheral blood stem cells (PBSC) were infused on day 6 to boost numbers of stem cells in haplo-HSCT while ATG were given at 10mg/kg prior to BMT and at 5mg/kg one day before PBSCT. All 25 patients received GVHD prophylaxis consisted of tacrolimus and short term MTX.

Results: The reasons of haplo-HSCT were very severe aplastic anemia with a neutrophil count of zero cells/mm^3 with infections in two patients, graft failure after the first SCT in three patients and no suitable unrelated donor in one. The median age was 9 (3-15) years old in mismatched UR-HSCT and 10 (5-15) years old in haplo-HSCT. The engraftment was achieved in 17 out of 19 (89.5%) patients of mismatched UR-HSCT and the two rejected patients were rescued by the second urgent haplo-HSCT. One of the patients received donor bone marrow derived MSCs infusion on day 0 in combination of haplo-HSCT to enhance the engraftment. All the patients of haplo-HSCT achieved engraftment. Acute GVHD grade II or more and chronic GVHD were seen in 3 and 4 out of 19 mismatched UR-HSCT respectively and 3 and 3 out of 6 haplo-HSCT respectively. CMV reactivations were observed in 15 UR-HSCT and 6 haplo-HSCT and one patient developed ganciclovir/foscavir resistant CMV infection and rescued by CMV specific CTLs therapy. EBV reactivations were observed in 4 UR-HSCT and in 3 haplo-HSCT and the patients were treated by rituximab. One patient developed CD20 negative EBV associated lymphoproliferative disease and rescued by EBV specific CTLs therapy. All 25 patients are alive with the median follow-up period of 7 years (from 6 month to 11 years and 8 months).

Discussion: HLA mismatched UR-HSCT is a useful option for children with SAA and unmanipulated haploidentical SCT with the option of cell therapies such as virus specific CTLs and MSCs was a feasible salvage therapy for children with SAA who don't have HLA matched related donor and need urgent HSCT.

Disclosure of Interest: None Declared.

PH-P050

TRANSPLANTATION FROM MATCHED UNRELATED DONORS IN PEDIATRIC SEVERE APLASTIC ANEMIA: EXPERIENCE WITH TCR ALPHA/BETA AND CD19 DEPLETION AS GRAFT PROCESSING METHOD

M. Maschan[1,1,], L. Shelikhova[2], D. Balashov[1], E. Kurnikova[3], E. Boyakova[1], V. Kalinina[4], Y. Skvortsova[1], E. Gutovskaya[1], V. Tsetlina[1], R. Khismatullina[1], P. Trakhtman[1], G. Novichkova[5], A. Maschan[1]*

[1]Hematopoietic Stem Cell Transplantation, Federal Center For Pediatric Hematology, [2]Hematopoietic Stem Cell Transplantation, Federal Center For Pediatric Hematology, [3]Transfusiology, [4]Molecular Biology, [5]Hematology, Federal Center For Pediatric Hematology, Moscow, Russian Federation

Introduction: Hematopoietic stem cell transplantation from unrelated donors remains the only curative option for severe aplastic anemia patients refractory to ATG/CsA immunosuppression (IST). Although the results of MUD transplantation in SAA have improved significantly with molecular typing, fludarabine in conditioning and improved GVHD control, especially in campath-based protocols, GVHD and infections remain a serious problem, associated with significant morbidity and mortality. We investigated the role of new method of graft processing - TCR alpha/beta depletion as a way to improve the results of MUD transplants in SAA.

Materials (or patients) and Methods: Ten patients with SAA were treated since November 2012 till August 2013. Median age at HSCT was 14(3-22) years, 6 male/4 female. 5/5 pts were refractory/relapsed after at least two courses of ATG/CsA, 2 pts had concurrent severe hemolytic PNH. Time interval from diagnosis to transplant was 4,3(1-10) years. Donors were unrelated volunteers, 10/10 matched. Six pairs were gender mismatched. CMV status was D+/R+ in 2, D-/R+ in 8. Preparative regimen included cyclophosphamide 100 mg/kg, fludarabine 150mg/kg, ATG(horse, ATGAM) 100mg/kg and 6Gy thoraco-abdominal irradiation. Two patients recieved alemtuzumab instead of ATG because of anaphylaxis. Post-transplant GVHD prophylaxis included Tacro till day 60 and Mtx on days +1,+3,+6. PBSC grafts were depleted of TCRalpha/beta cells and CD19 cells with CliniMACS device as recommended by the manufacturer. Patients recieved a median of 8,6(6,8-13,5)x10^6 CD34 per kg, 3(1-30) x10^4 TCRalpha/beta per kg.

Results: All patients engrafted with a median of 14 days for WBC and 13 days for platelets. In one patient secondary graft failure developed. He was salvaged later with a second unrelated graft. Acute grade 2 skin GVHD developed in 1 patient. No case of grade 3-4 aGVHD was observed. Five (50%) patiens had CMV reactivation requiring therapy, median time to CMV clearance was 2 (1-3) weeks. Prolonged mixed chimerism in T-cells did not compromise graft function. One patient developed moderate steroid-sensitive pneumopathy of uncertain etiology. With this exception no sign of chronic GvHD was noted. With a median follow up of 8 (3-14) months all patients are alive, 8 of them off any IST.

Discussion: In our experience the described combination of conditioning regimen and graft manipulation method provide a safe way (at least in the short term) of unrelated hematopoietic stem cell transplantation in severe aplastic anemia.

Disclosure of Interest: M. Maschan Conflict with: Lecturing for Miltenyi Biotec, L. Shelikhova: None Declared, D. Balashov: None Declared, E. Kurnikova: None Declared, E. Boyakova: None Declared, V. Kalinina: None Declared, Y. Skvortsova: None Declared, E. Gutovskaya: None Declared, V. Tsetlina: None Declared, R. Khismatullina: None Declared, P. Trakhtman: None Declared, G. Novichkova: None Declared, A. Maschan: None Declared.

PH-P051

VIRUS SPECIFIC CYTOTOXIC T LYMPHOCYTE SUB-POPULATION STUDY

W. Yingying[1], V. Decot[1,2], M. D. C. Bittencourt[3], L. Clement[4], J.-f. Stoltz[1,2], D. Bensoussan[1,2,5,]*

[1]Unite therapie cellulaire de Banque de tissu et sang placentaire a CHU Brbois NANCY, [2]UMR 7365 and FR 3209 CNRS-INSERM, [3]Laboratoire d'Immunologie et Plateforme Nancytomique, [4]Unité de Transplantation Médullaire Allogénique, [5]Faculté de Pharmacie, Département de Microbiologie-Immunologie, Nancy, France

Introduction: Viral infections are the major cause of morbidity and mortality for the patients following haematopoietic stem cell transplant (HSCT). Absence of efficacy of anti-viral drug is often correlated to absence of anti-virus specific immunity. Moreover, anti-viral drugs sometime cause important side effects. An alternative therapy is specific immunotherapy by virus specific T cell (CTL: Cytotoxic T Lymphocytes). It has proved effective in clinical studies to control virus infections and to contribute to virus-specific immunity reconstitution. However we aimed to identify the T sub-populations in CTLs after IFNg immunomagnetic selection. Thanks to the recent discovery of stem cell like memory T cell (T_{SCM}), which is a long life memory subpopulation, with a high proliferative potential and an enhanced capacity for self-renew. We also identified this sub-population in CTLs.
Materials (or patients) and Methods: Freshly selected virus-specific T cells (EBV-CTL *n*=1 ; ADV-CTL *n*=2 ; CMV-CTL *n*=2) were stained by the LIVE/DEAD AQUA fluorescent-reactive, anti-CD4, anti-CD8, anti-CD3, anti-CD45RA, anti-CD27, anti-CD197 (CCR7), anti-CD95, anti-IFN-g. Expression of CD27, CCR7, CD45RA and CD95 was analysed on $CD3^+CD4^+IFN\text{-}g^+$ and $CD3^+CD8^+IFN\text{-}g^+$ gated T cells. Naïve T cells (T_N) were defined as $CD45RA^+CCR7^+CD95^-$; Stem cell like memory T cells (T_{SCM}) as $CD45RA^+CCR7^+CD95^+$; Central memory T cells (T_{CM}) as $CD45RA^-CCR7^+$; Effector memory T cells (T_{EM}) as $CD45RA^-CCR7^-$ and Effector T cell (T_{EFF}) as $CD45RA^+CCR7^-$. Flow cytometric acquisition was performed on 500 000 events per sample on a Navios cytometer (Beckman Coulter). Kaluza software (v1.2) was used for analysis (Beckman Coulter).
Results: Within all the virus specific CTLs (ADV-CTL, EBV-CTL and CMV-CTL), all the T cell sub-populations (T_N, T_{SCM}, T_{CM}, T_{EM}, T_{EFF}) were observed among $CD3^+CD4^+IFN\text{-}g^+$ and $CD3^+CD8^+IFN\text{-}g^+$ T cells. TEM was the most important in $CD3^+CD4^+IFN\text{-}g^+$ and $CD3^+CD8^+IFN\text{-}g^+$ T cells (>80%). However, stem cell like memory T cell compartment (T_{SCM}) was also identified in $CD3^+CD4^+IFN\text{-}g^+$ (0.028-0.45%) and $CD3^+CD8^+IFN\text{-}g^+$ (0.0-1.08%) except in ADV-CTL. T_N was present mainly in $CD3^+CD4^+IFN\text{-}g^+$ T cells for EBV- CTL, but mainly in $CD3^+CD8^+IFN\text{-}g^+$ for ADV-CTL and CMV-CTL.
Discussion: In anti-virus CTLs selected by IFN-g based immunomagnetic technology, all the T cell sub-populations described by then are present. Although the number of isolated CTL available for infusion to patients is low, the presence of Tscm allows for a high *in vivo* expansion potential on antigen stimulation.
Disclosure of Interest: None Declared.

PH-P052

IMMUNOAFFINITY SELECTION OF VIRUS ANTIGEN SPECIFIC T-CELLS WITH A NEW FULLY AUTOMATED DEVICE: COMPARISON BETWEEN CLINIMACS AND PRODIGY

S. Klöß[1], R. Esser[1], M. Marburger[1], S. Tischer[2], C. Priesner[1], K. Aleksandrova[1], B. Maecker-Kolhoff[3], H.-G. Heuft[2], L. Goudeva[2], R. Blasczyk[2], U. Köhl[1], B. Eiz-Vesper[2], L. Arseniev[1,]*

[1]Institute for Celltherapeutics, [2]Institute for Transfusion Medicine, [3]Clinic for Paediatric Haematology and Oncology, Medizinische Hochschule Hannover, Hannover, Germany

Introduction: The infusion of selected virus-specific T-cells represents an option for the treatment of drug resistant CMV, EBV or other viral infections post transplantation. The re-stimulation for IFNγ secretion and selection of these cells can be performed via the CliniMACS® Cytokine Capture System® (CCS, Miltenyi Biotec GmbH, Germany). Recently a novel device of the same manufacturer, the CliniMACS Prodigy® (Prodigy) was made available, promising a fully automated, GMP-compliant and highly efficient process. Within an application for a GMP-manufacturing licence the performance of this machine in comparison with the routinely used CliniMACS® Plus instrument (Plus) has been evaluated.
Materials (or patients) and Methods: Lymphapheresis (≥3×10^9 WBC, >10% T-cells, viability >98%) were collected from 3 healthy CMV positive individuals after obtaining a written inform consent. Starting with 1×10^9 WBC (refer to table for exception) three selections of CMV-antigen specific T-cells were carried out with the Prodigy and Plus simultaneously. The MACS® GMP PepTivator® HCMVpp65 (Miltenyi Biotec GmbH, Germany) was applied for re-stimulation. The starting and target cell fractions were analysed using up to 9 colour single platform flow cytometry for viable CD3+ IFNγ secreting cells, their subsets, and contaminating lymphocytes.
Results: The concomitant selection results for the target fractions are shown in the table.

Parameter	1st run		2nd run		3rd run	
	Prodigy	Plus	Prodigy*	Plus	Prodigy	Plus
$CD3^+/IFN\gamma^+/7AAD^-$ cells $\times10^4$ (target T-cells)	377	142	7.5	5.4	75.8	115
Ratio $CD4^+$ vs. $CD8^+$ within $CD3^+/IFN\gamma^+$ cells	1 : 4.8	1 : 5.1	1 : 8.4	1 : 2.7	7.8 : 1	4.8 : 1
$CD3^+/IFN\gamma^-/7AAD^-$ cells $\times10^4$ (contaminating T-cells)	106	33	1.2	23	3.7	67
% $CD45^+/7AAD^-$ (overall leucocytes' viability)	52	41	37	19	48	63

*0.58 $\times10^9$ instead of 1×10^9 WBC from the original lymphapheresis were available for this process.

The volume of the final target fraction was 7-9.6 ml with the Prodigy and 40-43 ml with the Plus, respectively. The hands-on duration including quality control analyses was substantially shorter when using the fully automated system (<7 vs. >12 h with the Plus). No microbial contamination was found.
Discussion: The immunoaffinity selection of antigen specific cells cannot be assigned as a routine procedure. However the CliniMACS® Plus instrument is used for several years in clinical scale preparation thus may be the standard when new approaches have to be evaluated. Both machines seem to provide cell fractions with similar characteristics. In each procedure the minimum (specified in advance) of 1×10^4 target cells was isolated and the contaminating T-cells were sufficiently low to avoid a high risk of GvHD. The proportions of dead cells were relatively high in all instances, which seem to be expected in such preparations with low cell numbers. Neglecting some initial technical difficulties the CliniMACS Prodigy® will be an alternative especially taking into account the significantly reduced hands-on time.
Disclosure of Interest: None Declared.

PH-P053

DUAL SPECIFIC CYTOTOXIC POTENTIAL AND MEMORY PHENOTYPE OF IL-15-ACTIVATED CYTOKINE-INDUCED KILLER CELLS TARGETING VIRUS INFECTION AND LEUKEMIA IN PEDIATRICS

V. Pfirrmann[1,], E. Rettinger[1], A. Quaiser[1], S. Oelsner[1,2], J. Cinatl[3], T. Klingebiel[1], P. Bader[1]*

[1]Department Stem Cell Transplantation and Immunology, JW Goethe University Frankfurt, Hospital for Children and Adolescents, [2]Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, [3]Institute for Experimental Cancer Research in Pediatrics, JW Goethe University Frankfurt, Frankfurt/Main, Germany

Introduction: Viral reactivation or *de novo* infection with human pathogenic Adenovirus (AdV) and Cytomegalovirus (CMV) are

frequent complications after allogeneic stem cell transplantation. In addition relapse of the underlying disease is the other major cause of treatment failure. Screening for minimal residual disease (MRD) allows identification of impending relapse and pre-emptive immunotherapy such as the application of donor lymphocyte infusions (DLI) or the transfer of cytokine induced killer (CIK) cells may in principle prevent relapse in a group of high risk pediatric patients.
Materials (or patients) and Methods: CIK cells are generated from peripheral blood mononuclear cells (PBMC) of CMV- or AdV-seropositive healthy donors according to the standard protocol. Besides the standard stimulations, IL-15 is supplemented and viral CMV- or AdV-antigen in the form of a peptide pool is presented in the medium to increase the frequency of existing virus-specific cytotoxic T-lymphocytes (CTL) identified by flow cytometry stained with specific MHC-Multimers. The cell-mediated cytotoxicity is evaluated *in vitro* targeting leukemia cell line and primary viral infected cells.
Results: Besides the main population, containing $CD3^+CD56^-$cells, T-NK cells ($CD3^+CD56^+$) an essential subpopulation is expanding which consist mainly of a terminal differentiated activated $CD8^+$ TEMRA ($CD45RA^+CD62L^-$) or TEM ($CD45RO^+CD62L^-$) phenotype, respectively (d0 32.2±14.03x10^6 – d15 1485.14±1153.71x10^6, mean of total cell numbers, *n*=7) . The incubation with viral antigen leads to an up to 11.0 fold donor-dependent increase regarding CMV-specific $CD8^+$ cells. The simultaneous peptide stimulation during the culture period has no negative influence on the anti-tumor effect directed to the M4 AML subtype cell line THP-1 *in vitro* ($CIK_{antigen\ pos}$ 48.25±25.17% AML lysis, $CIK_{antigen\ neg}$ 53.50±11.36% AML lysis; mean of *n*=4; E:T ratio 40:1. In subsequent *in vitro* experiments the enhanced cytotoxic capacity of antigen-stimulated CIK cells targeting viral antigen-loaded T2 cell line is shown ($CIK_{antigen\ pos}$ 28.2±23.40% T2 lysis, $CIK_{antigen\ neg}$ 5.0±3.67% T2 lysis; mean of *n*=5; E:T ratio 5:1). In follow-up *in vitro* experiments donor-mismatched fibroblasts are infected with CMV and first results may indicate to an increased lysis of the infected fibroblasts by $CIK_{antigen\ pos}$ cells compared to $CIK_{antigen\ neg}$ cells. Uninfected fibroblasts representing healthy recipient tissue and therefore function as a control for Graft-versus-Host disease (GvHD) are not killed at all.
Discussion: The generation of dual specific cytotoxic CIK cells may be an improved immunotherapy after stem cell transplantation inducing cytotoxicity against leukemia cells and might help to clear specifically virus reactivation. Beyond that the minimal alloreactive potential and therefore the low risk of inducing GvHD disease, of the CIK cells is already shown in murine and human *in vivo* settings.
Disclosure of Interest: None Declared.

PH-P054
GENETICALLY MODIFIED CYTOKINE-INDUCED KILLER (CIK) CELLS FOR TARGETED CANCER THERAPY

S. Oelsner[1,2,], E. Rettinger[1], V. Pfirrmann[1], M. E. Friede[2], W. S. Wels[2], P. Bader[1]*
[1]Stem Cell Transplantation and Immunology, Goethe University Frankfurt, Hospital for Children and Adolescents, [2]Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt am Main, Germany

Introduction: Pre-emptive immunotherapy based on minimal residual disease (MRD) status with donor lymphocyte infusions (DLI) using cytokine-induced killer (CIK) cells may be beneficial to prevent relapse without causing graft-versus-host-disease (GvHD). While CIK cells have shown potent *in vivo* activity against various cancer types such as lymphomas or colorectal cancer, their cytotoxicity against B-ALL, characterized by the expression of CD19, has been limited. Hence, retargeting of CIK cells using chimeric antigen receptors (CARs) to facilitate selective target cell recognition and enhance specific cytotoxicity represents a promising approach.
Materials (or patients) and Methods: CIK cells were generated following an optimized protocol by *ex vivo* expansion of donor-derived peripheral blood mononuclear cells (PBMC) through the addition of interferon (IFN)-γ, anti-CD3 antibody, IL-2 and IL-15. Cells were transduced using CAR encoding VSV-G pseudotyped lentiviral vectors. CARs comprise an extracellular scFv antibody fragment as an antigen-binding domain, linked via a flexible hinge region and a transmembrane domain to an intracellular signaling moiety such as CD3 zeta chain (first generation CAR), or zeta chain fused to a costimulatory protein domain such as CD28 (second generation CAR). Transduction efficiency and cytotoxicity were determined using flow cytometric methods.
Results: We established an optimized protocol for transduction of CIK cells with CD19-specific first and second generation lentiviral CAR constructs, and characterized cells for expression of an EGFP marker gene and CAR surface expression. Effects of exposure to lentiviral vector particles on the development of CIK cell subpopulations and CAR expression were monitored over four weeks of continuous culture. Thereby transduction did neither affect the relative proportion of $CD3^+CD56^-$, $CD3^+CD56^+$ and $CD3^-CD56^+$ CIK cells, nor their activity against CD19-negative targets. To investigate functionality of CD19-specific CARs and the contribution of CAR-mediated recognition of CD19 to cytotoxicity, we generated a CD19-expressing variant of MDA-MB453 breast carcinoma cells by retroviral transduction. While retargeted CIK cells had no significant effect on parental MDA-MB453 cells (specific lysis of 3.75% ± 4.05), they efficiently lysed MDA-MB453/CD19 cells (specific lysis of 47.8% ± 0.4) in the same assay. In subsequent *in vitro* cytotoxicity assays we could further demonstrate potent and selective cytotoxicity of retargeted CIK cells towards established cancer cell lines endogenously expressing CD19 and primary pre-B-ALL blasts. Thereby coincubation of cells for 14 hours at different E/T ratios significantly increased specific lysis of target cells.
Discussion: Our results demonstrate potent and specific cytotoxicity of CIK cells expressing CD19-specific lentiviral CAR constructs against otherwise CIK-resistant established cancer cells and primary pre-B-ALL blasts. Ongoing work now aims at characterization of retargeted CIK cells in suitable *in vivo* models in NOD/SCID common γ chain knockout (NSG) mice.
Disclosure of Interest: None Declared.

PH-P055
TREATMENT OF CHRONIC GRAFT-VERSUS-HOST DISEASE WITH DECIDUAL STROMAL CELLS AND TRACING WITH 111INDIUM RADIOLABELLING

T. Erkers[1,], H. Kaipe[1], S. Nava[1], B. Gustafsson[2], B. Jansson[2], R. Axelsson[2], O. Ringdén[1]*
[1]Division of Therapeutic Immunology, Center for Allogeneic Stem Cell Transplantation, [2]Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden

Introduction: Decidual stromal cells (DSCs) isolated from fetal membranes of term placentas, are easily expanded and highly immunosuppressive *in vitro*. DSCs express mesenchymal stromal cell (MSC) markers, and have a high expression of T-cell inhibitory markers and integrins. In the present study, we introduce DSCs as a cellular therapy for cGvHD.
Materials (or patients) and Methods: Three patients (1 (ALL), 2 (AML), and 3 (KML)) with severe extensive cGvHD were treated with DSCs (1-2.8 x 10^6 cells/kg). Patient 1 and 2 received two infusions and patient 3 received one dose. One third of DSCs administered to patient 1 and 2 were labelled with ^{111}Indium and the *in vivo*-distribution was tracked for 48h. Blood samples were obtained before and up to 4-10 weeks after the first infusion. Samples were analysed by flow cytometry and luminex.
Results: All patients had cGVHD of skin, liver and obstructive bronchiolitis. Patient 2 had malabsorption. Following DSC-treatment, patient 1 showed no effect on liver or skin. Esophageal varices disappeared and banding has not been needed since infusion of DSCs. Patient 2 had liver enzymes normalized and albumin stabilized, and a transient improvement of skin could be seen. Spirometry showed improvement after bronchial dilatation. DSC-treatment showed no effects on patient 3. Patients 1 and 2

are regarded as partial responders (PR) and patient 3 as a non-responder (NR).
Patients receiving ^{111}In-DSCs showed the same distribution pattern of the isotope over time. The isotope was initially located in the lungs, followed by dissemination to liver and spleen.
The flow cytometry and luminex data are presented as the median frequency of data from all time points for each patient. Patient 3 had high frequencies of HLA-DR$^+$ cells within the CD3$^+$CD4$^+$ cell population (Th) (median 72.9%, range 72.7-73.3%). The corresponding proportions in patients 1 and 2 were 21.5% (17.6-21.9) and 36.5% (25.8-50-8), respectively. Among CD3$^+$CD8$^+$ cells (Tc), the frequency of HLA-DR-expression was 33.6% (30.9-37.5), 60.5% (56.7-68.1) and 80.6% (70.8-83.8) for patient 1, 2, and 3, respectively.
The percentage of Th-cells with a naïve (CD45RA$^+$CCR7$^+$) phenotype was 4.8% (3.6-6.3) in patient 3, but 24.4% (4.3-24.4) and 25.1% (11.2-26.3) in patient 1 and 2, respectively. The proportion of terminally differentiated (CD45RA$^-$CCR7$^+$) Th-cells was 2.3% (2.1-2.6), 7.4% (2.4-8.7) and 12.7% (10.9-23.2) in patients 1, 2, and 3, respectively.
The frequency of Tregs (CD4$^+$CD25^{high}CD127$^{low/-}$) was 11.5% (8.63-15.9) for patient 3, whereas they were 6.4% (4.8-6.5) and 3.3% (2.5-4.8) for patient 1 and 2, respectively. Patient 3 had the highest proportion Th-cells with a Th17 (CD45RA$^-$CXCR3$^-$CCR4$^+$CCR6$^+$), Th1/Th17 (CD45RA$^-$CXCR3$^+$CCR4$^-$CCR6$^+$) and Th2 phenotype (CD45RA$^-$CCR4$^+$CXCR3$^-$CCR6$^-$). Patient 3 also had the highest median plasma concentrations of IL-17, IL-4 and IFN-γ.
Discussion: DSCs induced PR in two out of three patients. The distribution of DSCs do not differ from what has been seen with MSCs, despite that DSCs express higher levels of integrins known to be important for homing to damaged tissue. The data suggests that patient 3 (NR) have a more activated/exhausted immune system. If this affects the DSC-treatment is difficult to determine in such a small study. However, luminex and flow cytometry data correlate regarding plasma concentrations of cytokines and presence of specific T cell subsets in peripheral blood.
Disclosure of Interest: None Declared.

PH-P056
STANDARDIZATION OF *EX VIVO* EXPANSION OF GAMMA/DELTA T CELLS FOR IMMUNOTHERAPY OF HEMATOPOIETIC MALIGNANCIES
L. Lamb[1,], H. Kim[1], J. Bowersock[1], S. Mineishi[1]*
[1]Medicine, University of Alabama at Birmingham, Birmingham, United States

Introduction: αOur previous studies have shown that TCR-γδ T cells homeostatically reconstitute in large numbers in approximately 25% of patients receiving haploidentical marrow grafts depleted of αβ T cells (αβ TCD). These γδ T cells exhibit potent anti-leukemia activity, and patients who recover from BMT with increased γδ T cells show 9-year leukemia-free survival (71% vs. 20% with normal γδ T cell recovery; P <0.0001) and without increased incidence or severity of GvHD (P = 0.96). Until recently, the lack of suitable reagents for αβ TCD or γδ T cell manufacturing and high risk of haploidentical transplantation slowed efforts to build upon these initial findings. In this study, we present preliminary findings from our laboratory of two cGMP-compliant graft-engineering strategies for human clinical-scale γδ T cell-enriched donor innate lymphocyte immunotherapy (DILI).

[PH-P056]

Materials (or patients) and Methods: A total of three products were examined. All products were cultured in our GMP facility with ISO-7 classified air filtration and exchange. Leukapheresis products were obtained from three volunteer donors, purified by density-gradient centrifugation, divided, and cultured in 50%RPMI/50% Clicks supplemented with 10% HS, 2mM l-glutamine, 2mM Zoledronic Acid, and 100μ/mL IL2 either in standardized T150 cell culture flasks or in a polycarbonate bioreactor chamber (CentriCult®; Miltenyi Biotec, Bergisch Gladbach, DE). Cells were kept at a density of 1 x 10^6/ml and the culture supplemented with IL-2 50u/mL every other day until cell harvest at day +14 at which time the product was depleted of αβ T cells. Cells from both methods were evaluated for lymphocyte subsets, sterility and potency against standardized human leukemia cell lines.
Results: Results from DILI manufacturing are detailed in the table below. All products produced γδ T cell expansions ranging from 74 to 200 fold. The Prodigy® CentriCult® bioreactor chamber showed no significant impediments for use for this manufacturing protocol. Post-manufacturing release testing showed minimal residual αβ T cells with predominant composition of γδ T cells and a smaller NK population. Expanded γδ T cells expressed activation-associated T cell antigens and were cytotoxic against AML lines K562, KG1a, HL-60, AML193; the myeloma cell line U266/TIB-196; and glioblastoma lines U251MG and U87MG. Culture products tested negative for gram stain, 14-day anaerobic and aerobic bacterial cultures, endotoxin, and mycoplasma with the exception of product 1 manufactured in flasks that grew out *S. aureus* during culture, likely due to manipulation required for multiple flasks.
Discussion: These results affirm that clinical–scale manufacturing of cytotoxic *ex vivo* expanded/activated γδ T cells in a Donor Innate Lymphocyte Infusion product can be accomplished in a cGMP compliant manner. Manufacturing with the Miltenyi Prodigy® is feasible, potentially allowing a simplified protocol with less potential for microbiologic contamination, thereby expanding the use of DILI therapy for prophylaxis or treatment of resistant disease.
Disclosure of Interest: None Declared.

Experiment	Source	αβ dose*	γδ dose*	NK dose*	Fold γδ Cells	Viability
1	Standard	No TCD	6.58x10^6/kg	1.64x10^6/kg	204	91.3
2a	Standard	<1x 10^5/kg	4.08x10^8/kg	2.57x10^5/kg	80	82.5
2b	Prodigy	<1x 10^5/kg	1.49x10^6/kg	1.54x10^5/kg	74	77.5
3a	Standard	<1x 10^5/kg	7.07x10^6/kg	1.31x10^6/kg	102	75.0
3b	Prodigy	<1x 10^5/kg	3.74x10^7/kg	1.29x10^7/kg	200	90.0

*based on 70kg,

PH-P057
SELECTIVE DEPLETION OF RECIPIENT-ALLOREACTIVE T-CELLS WHILE RETAINING VIRAL-SPECIFIC AND MEMORY T-CELLS ENABLES SAFE AND EFFICACIOUS HAPLO-IDENTICAL HSCT
J. Velthuis[1,], L. De Jong[1], R. Sidi Boumédine [2], M.-P. Giard[2], D.-C. Roy[2], M. Ruediger[1]*
[1]Kiadis Pharma, Amsterdam-Duivendrecht, Netherlands, [2]Hôpital Maisonneuve-Rosemont, Montreal, Canada

Introduction: Haplo-identical HSCT may resolve the lack of sufficient suitable completely HLA-matched donors for treatment of end-stage blood-cancer patients with a HSCT. Yet, current techniques do not allow for such procedure as presence of T-cells will cause severe GvHD and absence of T-cells (i.e. naked HSCT) will lead to occurrence of opportunistic infections.
Materials (or patients) and Methods: Kiadis Pharma is developing ATIR, a T-cell immunotherapy based on a donor lymphocyte preparation selectively depleted of host alloreactive T-cells, through use of photo-dynamic therapy. In a dose-finding phase I/II clinical study (CR-GVH-001), the product was shown to enable safe and efficacious haplo-identical HSCT. Currently, a subsequent phase II clinical study (CR-AIR-007) is ongoing.
Results: Using recently developed analytical methods, the ATIR product from both studies was/is extensively characterized

showing that only recipient-alloreactive T-cells were selectively depleted from the product while retaining reactivity against unrelated 3rd party antigens and general potent T-cells. Additionally, ATIR was shown to have retained viral-specific T-cells, preserved the presence of memory and naïve T-cells and showed responsiveness to pathogens. Thereby, ATIR will provide mature immune cells that offer immune protection without eliciting severe GvHD.
Discussion: The *in vitro* characterization data are supported by clinical data showing absence of TRM during 5-year follow-up (CR-GVH-001) over a broad dose range and no occurrence of severe GvHD/infections in the ongoing clinical study (CR-AIR-007). Together, these data show that using ATIR as an adjunctive medication, haplo-identical HSCT can be safe and efficacious.
Disclosure of Interest: None Declared.

PH-P058

EX VIVO ACTIVATED AUTOLOGOUS NK CELLS EFFICIENTLY LYSE TUMOR CELLS FROM METASTATIC COLORECTAL CARCINOMA PATIENTS: A PERSPECTIVE FOR IMMUNOTHERAPEUTIC APPROACHES

F. Ferulli[1], I. Turin[1], S. Brugantelli[1], S. Delfanti[1], M. Tanzi[1], O. Luinetti[1], M. Paulli[2], M. Maestri[1], R. Maccario[1], E. Montini[1], E. Todisco[3], D. Pende[4], P. Pedrazzoli[1], D. Montagna[2,]*
[1]Fondazione IRCCS Pol. San Matteo, [2]Fondazione IRCCS Pol. San Matteo, Università di Pavia, Pavia, [3]Istituto Clinico Humanitas, Rozzano (MI), [4]IRCCS AOU San Martino-IST, Genova, Italy

Introduction: Over the last decade the development of new treatments for advanced disease have improved survival rates for metastatic colorectal (mCRC) patients, but therapies are still associated with a poor prognosis and significant morbidity. The functional behavior of natural killer (NK) cells makes them appealing potential effectors for cancer immunotherapy, however in mCRC patients only anecdotal cases have been reported so far both in the autologous and allogeneic setting. In this study we evaluated the capacity of autologous NK cells, freshly or after cytokine activation, to lyse mCRC cells and the expression of ligands for adhesion and triggering NK receptors involved in CRC recognition and killing to clarify the mechanisms involved in tumor susceptibility or resistance to NK cells.
Materials (or patients) and Methods: After obtaining informed signed consent, 25 mCRC patients who underwent surgery to remove tumor or metastases have been enrolled to date. mCRC cells were isolated, *in vitro* expanded (after pathologic confirmation of neoplastic origin) and analyzed for the expression of HLA class I and ligands for NK triggering receptors. NK cells were purified and analyzed for the presence of receptors involved in NK-mediated cytotoxicity. The expression of NK receptors and levels of cytotoxic activity against patients mCRC cells were also evaluated after overnight (ON) activation of NK cells with IL-2 and/or IL-15.
Results: Tumor cells were successfully expanded from 21 of 25 samples. Results of experiments performed in 10 patients documented a substantial inability of patients freshly NK cells to lyse mCRC cells (<8% lysis at E:T ratio of 20:1). ON cytokine activation resulted in greatly increased NK cytotoxic potential in all patients evaluated (IL-2: mean 28%; range:10-71; and IL-15: mean 40%; range 16-76 at E:T ratio of 20:1). NKp30 and NKp46 were variably expressed by patient NK cells and could be up-regulated after ON activation, while mCRC cells expressed different levels of most NK ligands. Additional days of NK cell activation was able to further enhance their lytic capability. Preliminary experiments, suggest that mCRC are susceptible to anti-EGFR-induced ADCC mediated by NK cells regardless KRAS mutation status.
Discussion: Our data support the ongoing design of an immunotherapy approach with autologous NK cells for poor prognosis mCRC patients.
Disclosure of Interest: None Declared.

PH-P059

EXPERIMENTAL AND CLINICAL STUDIES USING DECIDUAL STROMAL CELLS FROM FETAL MEMBRANE LAYERS FOR IMMUNE MODULATION AND GRAFT-VERSUS-HOST DISEASE

B. Sadeghi[1,], T. Erkers[1], Y. Heshmati[2], I. Magalhaes[1], S. Nava[1], P. Mollden[1], J. Walfridsson[2], H. Kaipe[1], O. Ringdén[1]*
[1]Laboratory Medicine, Therapeutic Immunology, [2]Department. of Medicine, KAROLINSKA INSTITUTET, Stockholm, Sweden

Introduction: We introduced mesenchymal stem cells (MSCs) for treatment of acute graft-versus-host disease (GVHD). The placenta and fetal membranes protects the fetus from the mother's immune system. Decidual stromal cells (DSCs) are easy to expand and give better immunosuppression in mixed lymphocyte culture (MLC) than other stromal cells. The DSCs were positive for CD29, CD44, CD73, CD90, CD105 and CD49D, but were negative for hematopoietic markers. DSCs are of maternal origin. Indoleamine2,3-deoxygenase, prostaglandin E2, PD-L1 and interferon-g participate in the immunosuppression by DSCs, as shown in blocking experiments in MLC. DSCs promote CD4+, CD25+, FoxP3+ regulatory T-cell expansion in a contact-dependent manner. We report our experience.
Materials (or patients) and Methods: In vitro, in MLC and *in vivo* to study graft-versus-host disease (GVHD), we used a major histocompatibility mismatched mouse model, Balb/C mice as recipients and C57BL/6 as donors. Conditioning was busulfan and cyclophosphamide or 8 Gy of total body irradiation. Escalating doses of human DCSs, 1 x 10^5–10^6, were infused to recipient mice at day +1, +3, +5, +7 and +21 after hematopoietic stem cell transplantation (HSCT). Weight, mortality and histopathology were determined. DSCs were transduced with immunosuppressive genes, IL-10, prostaglandin E2 receptor (EP2), IDO and PDL-1 using a lenti virus vector.
Eight patients with steroid-refractory grade III-IV acute GVHD were given 0.9-2.8 x 10^6 DSCs/kg at 15 infusions. Median age was 57 years.
Results: In mice using Balb/c as responder and C57BL/6 as stimulatory cells in MLC, human DSCs induces a dose-dependent immunosuppression. In a xeno MLC setting, the proliferation of mouse (Balb/c) splenocyte, stimulated with human lymphocytes, were inhibited by human DSCs. In Balb/c mice conditioned and transplanted with BM and splenocytes from C57BL/6 donor (Mismatched GVHD model), human DSCs could attenuate the severity of GVHD and treatment on day +3 seemed the optimal infusion time. Human DSCs were transduced with lentivirus + GFP vector bearing IDO, prostaglandin E2 receptor (EP2), IL-10, IFNg and PDL-1. Transduced cells also inhibited mice MLC. The best effect was seen with PDL-1 transduced DSCs.
In the patients there was no toxicity from infusion of DSCs. Two patients had complete response and four had a partial response. Melena stopped in three patients. Three patients survive from 1½ years to 3 years.
Discussion: MSC therapy has been widely used. Some patients respond while others don't. A randomized trial in the US using industrial MSCs, showed no difference in overall response versus placebo. Experiments in mice models for GVHD suggest that MSCs need be licensed with interferon-g, nitric oxide or transduced with IL-10 to show an effect. DSCs may be a valid alternative to bone marrow-derived MSCs, because of better expansion potential and maybe better immunosuppressive effects. We have shown that human DSCs in a xenomodel prevent development of GVHD in mice, but survival was not significantly improved. PDL-1 transduced DSCs seem to give the best effect. Clinically, DSCs were found to be effective with three out of eight patients being long-term survivors, as opposed to around 10% as expected in patients with steroid-refractory acute GVHD.
In conclusion, DSCs may be successfully used for immune modulation and GVHD.
Disclosure of Interest: None Declared.

PH-P060

GENERATION OF TUMOR-SPECIFIC CYTOTOXIC T-LYMPHOCYTES FROM PERIPHERAL BLOOD OF COLORECTAL CANCER PATIENTS FOR ADOPTIVE T-CELL TRANSFER

S. Carluccio[1,], S. Delbue[1], L. Signorini[1], S. Dallari[1], F. Elia[2], A. Galli[3], A. Della Valle[3], P. Ferrante[1], M. Bregni[4]*

[1]University of Milan, Milano, [2]Fondazione Ettore Sansavini, Iugo, Ravenna, [3]Istituto Clinico Città Studi, Milano, [4]Ospedale di Circolo di Busto Arsizio, Busto Arsizio, Varese, Italy

Introduction: Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide. More than 1 million people are diagnosed with CRC each year, and a staggering 0.5 million died of the disease in the same time period.

Adoptive T-cell transfer (ACT) refers to an immunotherapeutic approach in which anti-tumor T lymphocytes, usually the tumor infiltrating lymphocytes (TIL), are identified, grown *ex vivo* and then re-infused into the cancer patients. This strategy has emerged as an effective treatment for patients with metastatic melanoma and other solid tumors, while at present, both active and adoptive immunotherapy do not play an important role in the treatment of advanced CRC.

Aim: In order to develop an ACT protocol for CRC treatment, an experimental approach that does not require neither the definition of molecular defined tumor antigens, nor the availability of TIL was designed. It was based on the *in vitro* stimulation of patient's CD8+-enriched T-cells from peripheral blood mononuclear cells (PBMCs) with dendritic cells (DCs), pulsed with apoptotic tumor cells as a source of tumor antigens, to generate autologous CTLs with strong anti-tumor activity.

Materials (or patients) and Methods: 78 CRC patients were enrolled. Tumor biopsies were obtained at surgery, together with 100 ml of heparinized peripheral blood (PB). Tumors were mechanically dissociated to a single-cell suspension and cultured to obtain a tumor cell line from each patient. DCs were generated from previously separated PBMCs, using a magnetic positive selection of CD14+ monocytes, cultured in presence of recombinant human Interleukin-4 (rh IL-4) and recombinant human Granulocyte-Macrophage Colony-Stimulating Factor (rh GM-CSF) for 6-7 days. Anti-tumor CTLs were elicited in co/micro-cultures using DCs as antigen-presenting cells, autologous apoptotic tumor cells as source of antigens and T CD8+ lymphocytes enriched effectors, in presence of weakly irradiated T CD4+ lymphocytes and PBMCs, Interleukin 7 (IL-7) and Interleukin 12 (IL-12), with weekly stimulation. The immune monitoring of the different cell populations was performed by flow cytometry analysis. CTLs Interferon-γ (IFN-γ) secretion was assessed by ELISpot assay, to evaluate their activation in response to autologous tumor.

Results: Primary tumor cell lines were obtained from 20 out of 78 patients (25,6%). DCs were generated from 26 patients, and among them, 6 had the corresponding tumor cell line. This was the reason why co/micro-cultures were set up for 6 patients. ELISpot results showed that strong and significant IFN-γ secretion was detected at the third, fourth and fifth stimulations for one patient and at the second for another patient, whereas for three patients a weak secretion was detected during the second and third stimulations. T-cells from one patient did not react to the stimulations.

Discussion: Despite the gut intestinal flora had adversely affected the establishment of primary tumor cell lines, an important success rate of 25,6% was obtained. In addition, although our immunological study must be performed on an increased number of CRC patients, our results suggested that the generation of tumor-specific CTLs could be useful for supporting an ACT approach in CRC.

Disclosure of Interest: None Declared.

PH-P061

HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOLLOWING MYELOABLATIVE CONDITIONING REGIMENS IN HEMATOLOGIC DISEASES WITH G-CSF MOBILIZED PERIPHERAL BLOOD STEM CELLS GRAFTS WITHOUT T CELL DEPLETION: A SINGLE CENTER REPORT OF 38 CASES

K. Miao[1,], R. Lu[2], J. Li[1], S. Qian[1]*

[1]First Affiliated Hospital of Nanjing Medical University, Nanjing, China, [2]Hematology, First Affiliated Hospital of Nanjing Medical University, Nanjing, China

Introduction: Because many Chinese patients with hematologic diseases who need allogeneic hematopoietic stem cell transplantation (allo-HSCT) lack a human leukocyte antigen (HLA)-matched donor, haploidentical HSCT has provided an alternative option.

Materials (or patients) and Methods: Here, we report the results of Haplo-HSCT with granulocyte colony stimulating factor (G-CSF) mobilized peripheral blood stem cells as the grafts without T-cell depletion in thirty-eight patients.

Results: Thirty-eight patients with the median age of 30.5 years (ranging from 13 to 53 years) were enrolled in this study, and 29 cases were in high risk status. The patients received myeloablative preconditioning with or without total body irradiation and acute graft-versus-host disease (GVHD) prophylaxis consisting of basiliximab, cyclosporine A, methotrexate, mycophenolate mofetil and a rabbit anti-thymocyte globulin. Thirty five patients attained successful neutrophil and platelet recovery. The median time for neutrophil recovery was 16 days (ranging from 10 to 23 days) and the median time for platelet recovery was 19 days (ranging from 10 to 66 days). During the follow-up at a median time of 33.1 weeks (ranging from 1.1 to 412.6 weeks), 11 (28.9%) patients developed aGVHD grade I – II, 7 (18.4%) patients developed aGVHD grade III – IV. The incidence of cGVHD was 27.6%. Nine (23.7%) patients died within the first 100 days after transplantation. The cumulative survival proportions at one year and two years were 52.51±8.57% and 43.76 ± 9.11%. The disease-free survival rate longer than 3 years was 40.34±8.71%.

Discussion: The results suggest that G-CSF-primed merely peripheral blood stem cell grafts without *in vitro* T cell depletion are an appropriate stem cell source for Haplo-HSCT, which may be an important HSCT strategy for patients without HLA full-matched donors.

Disclosure of Interest: None Declared.

PH-P062

FAST PRODUCTION OF HUMAN PLATELET LYSATE BY USE OF ULTRASOUND FOR THE *EX-VIVO* EXPANSION OF BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

M. Bernardi[1,2,], E. Albiero[1,2], A. Alghisi[3], K. Chieregato[1,2], C. Lievore[3], D. Madeo[2], F. Rodeghiero[1,2], G. Astori[1]*

[1]Advanced Cellular Therapy Laboratory, Department of cellular therapy and hematology, San Bortolo Hospital, [2]Research Laboratories, Hematology Project Foundation, [3]Immune Transfusion Service, Department of cell therapy and hematology, San Bortolo Hospital, Vicenza, Italy

Introduction: in addition to their use in regenerative medicine, mesenchymal stromal cells (MSC) are used in the prophylaxis and treatment of Graft Versus Host Disease due to their strong immune-modulatory properties. Moreover, these cells have been shown to prevent graft failure and promote engraftment in hematopoietic stem cell transplantation (HSCT).

MSC can be isolated from bone marrow, adipose tissue, cord blood or umbilical cord blood but for obtaining a sufficient number of cells for clinical application, MSCs need to be *ex-vivo* expanded. Expansion is possible when fetal bovine serum (FBS), which promotes cell growth and proliferation is added to basal media but its use is discouraged by regulatory authorities, due the risk of zoonoses and immune reactions. Human platelet lysate (Hu-PL), containing several bioactive molecules and growth factors, has been proposed as a valid substitute of FBS. Growth factors release

is commonly achieved after one or more overnight freezing/thawing cycles of platelet rich plasma; however the process is time consuming and difficult to standardize.
We have developed a fast method to obtain PL from PRP by using ultrasound. Efficiency was tested by expanding bone marrow (BM)-MSC in the obtained Hu-PL.
Materials (or patients) and methods: sonication was performed by treating PRP bags in an ultrasonic bath at a frequency of 20 kHz for 30 minutes at room temperature. To evaluate platelet lysis we measured PDGF-AB release by ELISA. Efficiency was tested by measuring cumulative population doubling time (cPD), differentiation capacity and immunogenic properties of BM-MSC expanded in D-MEM+10%Hu-PL and in D-MEM+10%FBS.
Results: 74% of PDGF-AB was released after treatment. Hu-PL significantly enhanced BM-MSC proliferation rate compared to FBS. The cPD of cells growth in Hu-PL at 10%, 7.5% and 5% was significantly better when compared to cells expanded in 10% FBS. BM-MSC expressed MSC markers and were able to differentiate into adipogenic, osteogenic and chondrogenic lineages. Immunosuppressive activity of BM-MSC expanded in Hu-PL was maintained when co-cultured with T-cells.
Discussion: we conclude that Hu-PL can be quickly produced by sonication; moreover, Hu-PL reduce the cell PD time compared to FBS maintaining both cell differentiation potential and immunomodulatory capacities.
Disclosure of Interest: None Declared.

PH-P063

ABSENCE OF MICRONUCLEUS FORMATION IN CHO-K1 CELLS CULTIVATED IN PLATELET LYSATE ENRICHED MEDIUM PRODUCED BY PLATELET RICH PLASMA SONICATION

M. Bernardi[1,2,], V. Adami[3], E. Albiero[1,2], D. Madeo[1], G. Astori[2], F. Rodeghiero[1,2]*
[1]Research Laboratories, Hematology Project Foundation, [2]Advanced Cellular Therapy Laboratory, Department of cellular therapy and hematology, San Bortolo Hospital, Vicenza, [3]High Throughput Screening core facility,CIBIO, University of Trento, Trento, Italy

Introduction: Human platelet lysate (Hu-PL) represents an effective substitute of fetal bovine serum (FBS) for mesenchymal stromal cells (MSC) expansion. Compared to FBS, Hu-PL favors MSC proliferation significantly shortening the population doubling time and avoiding the risks related to the use of animal derivatives. Growth factors contained in the platelet granules are commonly released upon disruption following one or more freezing/thawing (F/T) cycles. As an alternative to the F/T method, we have recently described the use of ultrasounds for the production of Hu-PL starting from platelet rich plasma (PRP). Since Hu-PL significantly increases the growth rate of MSC compared to FBS and given the possibility of free radicals formation during the process of PRP sonication, we have investigated both the MSC chromosomal stability during expansion by karyotype analysis and Hu-PL safety applying the cytokinesis-block micronucleus assay (CBMA).
Materials (or patients) and Methods: Hu-PL was produced by sonication of the PRP bags for 30 minutes at 20 kHz. MSC have been isolated from bone marrow (BM) samples and expanded in D-MEM+10% Hu-PL. Karyotyping by G-banding was carried out at the end of passage 6. For CBMA, the Chinese hamster ovary cell line (CHO-k1) lacking DNA repair mechanism was exposed to increasing concentrations of Hu-PL from 0.1% to 30% in three independent experiments and using different Hu-PL batches. For the positive control, micronucleation was induced by exposing the CHO-k1 to Mitomycin-C. After 24h, cytokinesis was blocked by Cytochalasin B. Cells were fluorescent stained with Hoechst and micronuclei were automatically detected using an high content imaging system (Operetta) analyzing at least 2000 binuclear cells/well.
Results: In our experiments growth proliferation induced by the use of Hu-PL did not lead to micronuclei formation on CHO-K1 cells compared to negative control ($P<0.01$). Moreover, karyotype did not reveal genomic alterations on BM-MSC expanded in 10% Hu-PL.
Discussion: micronuclei formation is considered a biomarker of chromosomal damage, genome instability and cancer risk. Our results suggest that MSC can be safely expanded in Hu-PL produced by sonication.
Disclosure of Interest: None Declared.

PH-P064

AUTOMATED WASHING OF AUTOLOGOUS HEMATOPOIETIC STEM CELL GRAFTS AFTER THAWING DOES NOT IMPAIR ENGRAFTMENT

B. Calmels[1,], A. Drezet[2], C. Huynh[1], A. Autret[3], A.-M. Stoppa[4], R. Bouabdallah[4], D. Coso[4], C. Malenfant[1], C. Lemarié[1], C. Chabannon[1]*
[1]centre De Therapie Cellulaire, Institut Paoli-calmettes, [2]UMR1068, INSERM, [3]Unité Biostatistiques - Département de la Recherche Clinique et de l'innovation, [4]Département D'onco-hématologie, Institut Paoli-calmettes, Marseille, France

Introduction: Autologous hematopoietic stem cell transplantation (AHSCT) is still widely used for treatment of patients with hematologic diseases. Cryopreservation and storage are mandatory, allowing administration of high dose chemotherapy. Reinfusion of grafts thawed at the bedside might be associated with side effects attributed to cryoprotectant or cell lysis products. In order to remove such byproducts, biomedical devices like Cytomate or Sepax have been developed for washing out thawed grafts. However, washing of thawed grafts remains controversial since CD34+ progenitor cell loss is unavoidable. This is opposed to bedside thawing, where cell loss is supposed to be lower, while unknown owing to the absence of quality control. There are, to our knowledge, no reports comparing these 2 procedures for their relevant clinical endpoints, i.e. hematopoietic reconstitution in the autologous setting. In order to determine whether cell loss associated with thawing and washing has a detrimental effect on engraftment, we retrospectively compared two cohorts of matched patients, receiving either washed or unwashed autologous grafts at a single institution.
Materials (or patients) and Methods: Between Jan. 2000 and Dec. 2004, 1,360 AHSCT were performed: we selected 364 of them for which a) grafts were thawed at the bedside and b) hematopoietic reconstitution raw data were available in the electronic data management system. We selected 218 AHSCT that fall within 4 diagnosis groups for which intensive chemotherapy regimens were homogeneous (Table 1); among this group, we retained the 65 AHSCT for whom cryopreserved CD34+ cell doses were within the therapeutic range of 2.0 to $5.0x10^6$/kg. We then sought to compare this group to a matched cohort of AHSCT washed before infusion: among the 1,570 AHSCT performed between Jan. 2005 and Dec. 2010 we selected 580 AHSCT for which: a) hematopoietic reconstitution raw data were available in the electronic data management system, b) patients received the same 4 intensive regimen than the unwashed group and c) grafts were homogeneously processed (dry thawing followed by washing on CytoMate); among this cohort, the 260 AHSCT with cryopreserved CD34+ cell doses between 2.0 and $5.0x10^6$ $CD34^+$/kg were defined as the washed group.
Results: In order to set up two comparable groups of AHSCT, we used a case-match design with one-to-two matching. Each of the 65 unwashed AHSCT was matched with 2 washed AHSCT issued from the 260 potential matches, using 4 baseline variables: sex, age at treatment, diagnosis and total cryopreserved CD34+ cell dose. The case-match analysis exhibits, as expected, no significant difference in terms of age, sex ratio, diagnosis and CD34+ cryopreserved cell dose (Table 1). Median time to reach $0.5x10^9$/L circulating neutrophils is comparable between the two groups, with 12.4 and 12.5 days in the unwashed versus washed groups, respectively.

Table 1 : Patients, autografts and clinical characteristics

	Unwashed (n=65)	Washed (n=130)	p-value
Sex (M/F)	30/35	52/78	0.41
Age (median [range])	55.3 [17-71]	56.7 [23-71]	0.74
Diagnosis / High dose chemotherapy (n [%])			
Plasma cell disorders / Melphalan	48 [73.8]	97 [74.6]	0.15
Lymphoma / BEAM	8 [12.3]	23 [17.7]	
Acute leukemia / BU-MEL	5 [7.7]	2 [1.5]	
Solid tumors / CY-MEL	4 [6.2]	8 [6.2]	
Number of CD34+ cells cryopreserved (10^6/kg)	3.7	3.8	0.29
Days to neutrophils >0.5G/L (median ± sd [range])	12.4 ± 1.4 [10-15]	12.5 ± 1.6 [8-17]	0.67

Discussion: Our approach allowed us to compare 2 matched cohorts issued from 2,930 AHSCT performed over 10 years at a single institution. This accurate matching leads us to conclude that washed autologous grafts do not compromise hematopoietic reconstitution as compared to bedside thawing. Moreover, using automated devices such as CytoMate or Sepax allow for better standardization, improved stability of thawed cell products as well as precise determination of infused $CD34^+$ cells, and might thus be preferred over bedside thawing.
Disclosure of Interest: None Declared.

PH-P065

HTERT/SURVIVIN MRNA-LOADED DENDRITIC CELL VACCINATION COMBINED WITH *EX-VIVO* EXPANDED T CELL TRANSFER IN STAGE IV MELANOMA PATIENTS SHOW A LONGER OVERALL SURVIVAL IN PATIENTS WITH SUSTAINED IMMUNE RESPONSES AGAINST HTERT

I. Bigalke[1,], S. Brinchmann-H. Torhaug[2], M. Lundby[1], C. Mollatt[1], E. Marit Inderberg Suso[1], A. Kolstad[3], G. Gaudernack[4], A. Marie Rasmussen[4], S. Aamdal[2], G. Kvalheim[1]*
[1]Department of Cellular Therapy, [2]Department of Clinical Cancer Research, [3]Department of Oncology, [4]Department of Immunology, Oslo University Hospital, Oslo, Norway

Introduction: Up to now immune responses following therapy of malignant melanoma with dendritic cells (DCs) appear to be only transient. Therefore the combination of DCs with antibodies, T cells and/or low-dose chemotherapy as possibilities to prolong the immunological effect and improve the clinical outcome is now under investigation.
We conducted a clinical phase I/II trial to investigate the combination of autologous, tumor antigen specific mRNA loaded DCs with expanded autologous T cells in melanoma stage IV patients.
Materials (or patients) and Methods: DCs were generated by enriching monocytes using the Elutra cell separator and differentiating them to immature DCs (iDCs) with GM-CSF and IL-4. iDCs were harvested on day 5 electroporated with hTERT and Survivin mRNA and matured by adding maturation cocktail as described by Jonuleit *et al*. After 24 hours the mature DCs were frozen in aliquots.
Blood samples were taken before start of DC vaccination and at defined time points during vaccination. Patients who showed immune responses against hTERT and/or Survivin after initial treatment with DCs were offered additional treatment with *ex-vivo* expanded T cells.
A second leukapheresis was performed and T cells were enriched with the Elutra cell separator. After depletion of Tregs using CD25 Dynabeads, T cells were expanded with CD3/CD28 Dynabeads for 10 days in a WAVE bioreactor. Patients were non-myeloablative conditioned with Fludarabine and Cyclophosphamide and after removing of CD3/CD28 beads $3x10^{10}$ T cells were transfused fresh. DC vaccination was continued the day after T cell transfer.
Results: Three patients have been treated with this DC/T cell combination. All of them showed immune responses against hTERT peptides either already before start of DC vaccination or 3-5 months after the first vaccination. Only patient three showed an immune response against Survivin peptides before DC-vaccination, which disappeared with start of vaccination. Patients one and two had a sustained response against hTERT peptides after treatment with T cells. In patient one this response dropped 20 months after beginning of DC-vaccination correlating with progression of the disease. In patient two the response dropped 29 months after start of DC-vaccination and was combined with an up-coming immune response against Survivin and a progression of disease from week 31 on. In patient three the response against hTERT peptides vanished at the time of T cell transfer and was combined with a strong response against Survivin peptides. The disease progressed after 11 months.
Patients included in this study who did not receive additional T cells had a progression free survival (PFS) of 3 to 13 months with a median of 7 months. Patients who did not mount any immune response had a PFS of 3 to 10 months while patients who showed an immune response before start of vaccination or developed an immune response at a later time point against hTERT alone had a PFS of 8 to 13 months.
Discussion: These data indicate that immune responses can be prolonged by additional T cell transfer. Prolonged responses against hTERT seem to result in longer PFS. The role of responses against hTERT and Survivin and their impact on clinical outcome in melanoma patients has to be further investigated.
Disclosure of Interest: None Declared.

PH-P066

DECIDUAL STROMAL CELL THERAPY MAY INDUCE MULTISPECIFIC ANTI-HLA ANTIBODIES IN EPIDERMOLYSIS BULLOSA PATIENTS, BUT NOT IN ALLOGENEIC SCT PATIENTS

H. Kaipe[1,], L.-M. Carlson[1], T. Erkers[1], S. Nava[1], P. Molldén[1], M. Alheim[2], O. Ringdén[1]*
[1]Karolinska Institutet, [2]Karolinska University Hospital, Stockholm, Sweden

Introduction: Junctional epidermolysis bullosa Herlitz (JEB-H) is caused by a congenital deficiency in laminin-5, leading to life-threatening skin and mucosal fragility. There is currently no established cure. Fetal membranes and cells from term placentas have wound healing capacities, and we have previously used decidual stromal cells (DSCs) from fetal membranes to treat acute GVHD. The immunogenicity of allogeneic stromal cells *in vivo* is poorly characterized.
Materials (or patients) and Methods: Amniotic membranes (AM) from a term placenta were applied to severe wounds of an 11 months old JEB-H patient. The patient was treated with five infusions of allogeneic DSCs (2×10^6/kg) within a three-month period. The patient had received two units of blood, three and one month prior to the first treatment with AM. Serum samples from the JEB-H patient and from SCT patients treated with DSCs for acute GVHD (n=8), chronic GVHD (n=3) and haemorrhagic cystitis (n=5) were analyzed by flow cytometric crossmatching (FCXM). The number of DSC doses ranged from 1-5 infusions, derived from 1-3 placental donors, and the patients were followed for at least four weeks.

Samples that were positive in FCXM were analyzed by Luminex to determine specificity of anti-HLA antibodies. Peripheral blood mononuclear cells (PBMCs) from the JEB-H patient were stimulated with DSCs and the proliferative response was measured by ^{3}H-thymidine incorporation. The expression of laminin-5 on DSCs was confirmed by fluorescence microscopy.
Results: One week after the AMs were applied improvement in healing was observed over the elbows. The patient was given DSCs two weeks after the application of AMs. Already after three days, lesions over the groins were improved. Subsequently, there were improvements in the face and the fingertips. Healing processes were seen in the middle of the blisters, as opposed to at the margin which may occur spontaneously in JEB-H. After three weeks, the wound over the left ear was completely healed and the face had further improved. A second dose of DSCs was given and the elbows were improved. After three more infusions of DSCs the improvements were transient. The patient survived 23.5 months, as compared to average five months.
After four infusions of DSCs from three different donors, the JEB-H patient had developed anti-HLA antibodies specific to 38 HLA class I antigens. PBMCs from the patient had a higher proliferative response to DSCs than to third-party PBMCs (median stimulation index (SI) 6.3 (range 4.3-8.2) and 1.9 (range 1.7-2.7), respectively), which contrasts to the pattern observed in PBMCs from healthy donors (median SI 2.8 versus 6.8). Two out of 16 SCT patients showed a positive FCXM test. One of these had multispecific HLA-antibodies before DSC infusion. The other patient showed a positive FCXM test four weeks after DSC infusion, but had no anti-HLA antibodies as confirmed by Luminex analysis.
Discussion: AM and DSC-infusions may improve the healing process of blisters in JEB-H patients, but the response appears to be transient. DSCs may induce multispecific anti-HLA class I antibodies in patients with a competent immune system. None of the SCT patients had developed anti-HLA antibodies after DSC infusion, indicating that the risk of alloimmunization by DSCs is low in immunocompromised patients.
Disclosure of Interest: None Declared.

PH-P067

THE CYTOTOXICITY OF CYTOKINE INDUCED KILLER CELLS IS FULLY RETAINED BY THE SORTED CD56+ CELL FRACTION AND SEEMS TO BE INDEPENDENT BY LYTIC DEGRANULATION

K. Chieregato[1,2,], C. Zanon[1], S. Castegnaro[1], F. Rodeghiero[1,2], G. Astori[1]*
[1]Department of Cellular Therapy and Hematology, San Bortolo Hospital, [2]Hematology Project Foundation, Vicenza, Italy

Introduction: Cytokine induced killer cells (CIK) comprise two main fractions: the first, $CD56^+$, represented by NK-T cells ($CD3^+CD56^+$) and NK cells ($CD3^-CD56^+$), the second, $CD56^-$, characterized by T lymphocytes ($CD3^+CD56^-$). The $CD56^-$ fraction is thought to be responsible of CIK alloreactivity, whereas the anti-tumor effect seems to be mainly restricted to the CD56+ fraction. Here, we investigated the $CD56^+$ alloreactivity and cytotoxicity by evaluating the cytokine secretion profile and lytic degranulation.
Materials (or patients) and methods: CIK (*n*=6) were obtained by stimulating PB-MNC with IFN-gamma (day 0), IL-2, anti-CD3 monoclonal-antibody (day 1) and IL-2 every 3 days. At day 21, $CD56^+$ and $CD56^-$ fractions were immunomagnetically sorted. Cell alloreactivity of the CIK bulk and the sorted populations was tested by mixed lymphocyte reaction (MLR), cytotoxicity against K562 cells by calcein-release assay. Cell degranulation was quantified by CD107a expression and Th1 (IFN-gamma, TNF-alfa, IL2) and Th2 (IL4, IL6, IL10) cytokines were then quantified by ELISA in the supernatant after MLR and cytotoxic assay.
Results: The CIK alloreactivity was mainly restricted to the CIK bulk and to the CD56– subset as confirmed by the Th1/Th2 cytokines released in the supernatant. The CIK cytotoxicity was fully retained by the CD56+ cell subset, and appeared to be independent by lytic degranulation as confirmed by the absence of differences in the expression of CD107a on NK, NK-T and by the cytokines released in the supernatant. This suggests that the CIK cytotoxicity could be mediated by an alternative mechanism probably dependent by FAS-L or CD3 pathways.
Discussion: In a clinical setting, the immunomagnetic purging of the CD56- cell fraction by the CIK bulk could limit the risk of Graft Versus Host Disease maintaining the antitumor effect restricted to the CD56+ cell subset.
Disclosure of Interest: None Declared.

PH-P068

LYMPHODEPLETION FOLLOWED BY SUICIDE-GENE-TRANSDUCED DONOR LYMPHOCYTE INFUSION: A STRATEGY TO SAFELY ENHANCE THE GRAFT-VERSUS-TUMOR EFFECT

S. Maury[1,], M. Rosenzwajg[2], R. Redjoul[1], A. Marcais[3], A. Xhaard[4], L. Cabanne[1], C. Bernard[2], F. Suarez[3], R. Peffaultdelatour[4], L. Gregoire[1], K. Debbache[1], J.-I. Beaumont[1], N. Azar[2], M. Cherai[2], D. Bories[1], J. Cohen[1], C. Cordonnier[1], F. Lemoine[2], D. Klatzmann[2]*
[1]Henri Mondor University Hospital, Creteil, [2]Pitié Biotherapy, [3]Necker Hospital, [4]St Louis Hospital, Paris, France

Introduction: Donor lymphocyte infusions (DLI) can produce lasting remissions in patients with relapsed chronic myeloid leukemia (CML), but are less effective in non-CML diseases. Chemotherapy-induced lymphodepletion prior to DLI, achieved with cyclophosphamide (Cy) and fludarabine (Flu), has been shown to enhance activation of donor lymphocytes and cause significantly more acute graft-versus-host disease (GVHD) than DLI alone. To safely balance the toxic versus beneficial effects of activated donor lymphocytes, we combined lymphodepleting chemotherapy with the infusion of donor T cells engineered to carry a suicide gene to treat patients with aggressive hematologic malignancies.
Materials (or patients) and Methods: Donor T cells were transduced with a retroviral vector expressing the Herpes simplex thymidine kinase (TK) suicide gene and the human Thy-1 selection marker and expanded in culture for 15 to 19 days prior to their infusion. In this phase I/II trial, the safety and efficacy of Cy/Flu/DLI-TK was studied between February and December 2011 in 10 adults with relapsed multiple myeloma (*n*=5) or myelodysplasic syndrome/acute leukemia (*n*=5). One had previously failed to respond to at least one standard DLI while others had not received any DLI before inclusion. All were free of immunosuppressive therapy at inclusion. Patients were infused with a mean cell-dose of 5 (range: 1-10) $x10^6$ $CD3^+$ cells/kg of which 98% were TK^+ cells (range: 97%>99%). DLI products contained a mean ratio of 0.6% (range: 0.1–1.6) $CD4^+FoxP3^+Helios^+$ regulatory T-cells. This trial was registered at www.clinicaltrials.gov as NCT 01086735.
Results: The Cy/Flu regimen was profoundly lymphodepleting and led to a mean 3-day period of neutropenia below 500 PMN/µL. Three patients developed acute GVHD following TK^+ cell infusion. One patient had a grade I cutaneous GVHD resolving with local steroids. In another patient with grade III cutaneous and digestive involvement, intra-venous administration of ganciclovir (GCV) led to the complete and lasting resolution of symptoms, correlated with clearance of TK^+ cells in peripheral blood. A third patient had a grade III liver GVHD. Due to only partially controlled leukemia at the time of DLI, GCV treatment was postponed for one week after GVHD onset in order to support a putative GVL effect. Treatment with GCV led to the rapid disappearance of TK^+ cells in peripheral blood and liver but without clinical improvement after 2 weeks, so that an additional immunosuppressive therapy should be instituted. Six patients, including one of the 2 treated with GCV, experienced relapse/progression of their malignancy and received additional anti-cancer therapy. With a median follow-up of 22 months after Cy/Flu/DLI-TK, 6 patients are alive, 5 of them being disease-free. In patients not treated with GCV, TK+ cells could still be detected in peripheral blood till at least 12 months after their infusion.
Discussion: Lymphodepletion followed by suicide-gene-transduced T-cell infusion may help to safely enhance the immune activity of DLI, which could be further improved by regulatory T-cell depletion (Maury *et al*, Science Translat Medicine 2010).
Disclosure of Interest: None Declared.

PH-P069

HUMAN MESENCHYMAL STROMAL CELLS, IN THE PRESENCE OF GAMMA INTERFERON, INHIBIT JURKAT T CELL PROLIFERATION

V. Kindler[1,]*
[1]Hematology, Geneva University Hospital, Geneva 4, Switzerland

Introduction: Mesenchymal stromal cells (MSC) amplified *in vitro* are able to inhibit steroid-resistant graft-versus-host disease (GVHD) occurring in some patents reconstituted with allogenic hematopoietic stem cells. However, it is not established whether the potent regulatory effect of MSC is restricted to alloreative T cells or whether it may also modulate residual disease relapse. We explored this issue *in vitro* using Jurkat T cells (Jurkat) as a model for residual disease.
Materials (or patients) and Methods: MSC were purified from femoral head remains after informed consent of patients undertaking hip surgery, and amplified in the presence of platelet lysate. Amplified MSC were plated in 24-well plates and Jurkat were seeded either directly on MSC or were deposited in transwells above the MSC. Cultures were performed for 48 hours in RPMI medium with 10% FCS, with or without 250 µ/ml γ-interferon (IFN). Cell cycle status of Jurkat, as well as L-tryptophan (Tryp) and kynurenine (Kyn) titers of supernatants were determined. The sensitivity of Jurkat toward Tryp depletion was assessed using Tryp-free RPMI and graded concentrations of the amino acid.
Results: When incubated with MSC and IFN, apoptotic and G-phase of Jurkat were respectively increased by 6.3-fold and 1.3-fold, and SG2M phase represented 0.5-fold the value observed in cocultures with MSC in the absence of IFN (mean of 5 experiments). IFN alone, in absence of MSC had no effect on Jurkat cell cycle. MSC-induced inhibition was effective both in regular cultures and when Jurkat were seeded in transwells preventing cell contacts. Jurkat proliferation inhibition was associated with a decrease of Tryp and an increase of Kyn titers in the culture supernatants. Complementation experiments using Tryp-free RPMI confirmed that Jurkat required Tryp for proliferation and showed that Tryp concentrations <3 µM altered cell cycle. Kyn did not mediate MSC inhibition as the complementation of Tryp-replete cultures with Kyn did not modify the cell cycle status.
Discussion: This study shows that MSC regulatory effect extends to malignant cells. MSC-induced Jurkat inhibition was direct, required exogenous IFN and was mediated via a cell contact-independent mechanism that involved Typ catabolism. These observations suggest that MSC infused to a patient to fight the GVHD may, in addition of controlling the GVHD, inhibit residual disease progression as long as leukemic cells are sensitive to Tryp depletion. This may be the case for B lymphocyte or T lymphocyte derived malignancies, whose normal counterparts are highly sensitive to Tryp depletion. By contrast, if the residual disease is resistant to low Tryp titers, repetitive infusions of MSC should be avoided because they may also inhibit T cells specific for the residual disease and favor in this way its relapse.
Altogether these data suggest that cells derived from the mesenchymal lineage exhibit a control upon tumor cells that has been largely underrated so far. It may well be that MSC, in the presence of IFN (known to inhibit tumor growth in many instances) may eradicate a significant portion of Tryp-sensitive tumors without the help of adaptive immunity. However once facing a Tryp depletion-resistant tumor, MSC activation via IFN may lead to tumor escape. Further investigations addressing this issue are required before establishing the innocuousness of repetitive infusions of MSC in leukemic patients.
Disclosure of Interest: None Declared.

PH-P070

SAFE AND REPRODUCIBLE PRODUCTION OF FUNCTIONAL DENDRITIC CELLS FOR IMMUNOTHERAPY IN GLIOBLASTOMA PATIENTS

S. Nava[1,], D. Lisini[1], S. Pogliani[1], M. Dossena[2], S. Pellegatta[3], G. Finocchiaro[3], E. Agostino Parati[1,2], S. Frigerio[1]*
[1]Cell Therapy Production Unit, [2]Laboratory of Cellular Neurobiology, [3]Unit of Molecular Neuro-Oncology, IRCCS Neurological Institute C.Besta Foundation, Milano, Italy

Introduction: In the last few years cell therapy based on dendritic cells (DC) pulsed with tumor lysate become a promising approach in addition with conventional therapy for the treatment of glioblastoma (GB)-affected patients. The success of this approach strongly depends on the possibility to generate routinely large amounts of high quality, functional mature DC (mDC) in compliance with Good Manufacturing Practices (GMP). Moreover, a high level of standardization is required to ensure maximum reproducibility of the mDC production process.
Materials (or patients) and Methods: In Cell Therapy Production Unit (UPTC) of Neurological Institute C. Besta Foundation a protocol for mDC production under GMP conditions was validated for treatment of GB-patients. From 2010 to 2012 37 lots of mDC were produced. The aim of this study was to evaluate retrospectively: a) sterility, absence of mycoplasma, endotoxin and adventitious viruses, b) feasibility of producing a large number of autologous mDC, c) quality of mDC, in terms of viability, maturation status and potency. Compendial methods, according to European Pharmacopoeia were used to test sterility, mycoplasma, endotoxin and adventitious viruses. Non compendial methods were applied to evaluate viability, phenotype and potency. In particular viability was assessed by trypan blue exclusion test, phenotype by cytofluorimetric analysis of the typical mDC markers CD80, CD83, CD86, HLA-DR and potency by Mixed Lymphocyte Reaction (MLR).
Results: 37/37 lots of mature mDC were sterile, endotoxin levels were <2.86 UI/ml (cut off value) and in all lots mycoplasma and adventitious viruses were absent. With respect to the number of mature mDC obtained, 35/37 lots were conform for the total number of cells for ensure the full treatment of patients; in 2/37 lots the final number of mDC was insufficient to ensure all the planned vaccinations. All mDC lots showed very high viability after thawing (Mean: 93,82%, SD:3,20%). Phenotype evaluation of mDC showed a marked up-regulation of the typical DC markers in comparison with immature DC. Moreover the values among the lots were very constant: CD80 (Mean: 79,5%, SD:5,5%), CD83 (Mean: 53,7%, SD:16,2%), CD86 (Mean: 94,5%, SD:2,1%), HLA-DR(Mean: 98,5%, SD:1,4%). MLR test for the evaluation of the potency of the mDC demonstrated that all lots were functional, with respect to their ability in the induction of lymphocytes response.
Discussion: These results demonstrate that our protocol for DC production is highly reproducible and permits routinely to generate large amounts of safe and functional mDC for *in vivo* use in immunotherapy approaches.
Disclosure of Interest: None Declared.

PH-P071

IN VITRO EXPANSION AND FUNCTIONAL NORMALIZATION OF T CELLS FROM CLL PATIENTS USING BLINATUMOMAB: A GMP-COMPLIANT PROTOCOL FOR ADOPTIVE TRANSFER

J. Golay[1,], A. D'Amico[1], G. Borleri[1], M. C. Finazzi[1], G. Quaresmini[1], D. Nagorsen[2], M. Introna[1], A. Rambaldi[1]*
[1]Hematology, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy, [2]Global Clinical Development, Amgen, Thousand Oaks, United States

Introduction: Treatment of CLL often results in severe life threatening immunosuppression and these patients may benefit from adoptive therapy with normal T lymphocytes.
Materials (or patients) and Methods: T cells were expanded from CLL patients peripheral blood by stimulation with bispecific antibody blinatumomab (CD3xCD19, Amgen) in serum-free medium

containing rhIL-2. The expanded populations were characterized by flow cytometry.
Results: We performed 18 expansions starting from a mean of 10 ml peripheral blood from untreated CLL patients (range 2-30 ml) that contained a mean of 9.3% T cells. This method allowed reproducible expansion in about 22 days of mean $410x10^6$ $CD3^+$ T cells (range 71 to $2184x10^6$) with a mean 224 fold expansion (range 4.4-1326). Final products were depleted of CLL cells from days 7-14 onwards (mean 0% at day 18-25), the only significant contaminant being NK cells (mean 18.5%). Only in one case, a significant percentage of CLL B cells could be observed at the end of culture (day 24), but this was due to the particularly high percentage neoplastic cells in the starting population in this patient (98%), resulting in relatively late depletion of these cells, which took place between days 14 and 21, and therefore remained detectable at day 24 (3.8% CLL B cells at day 24). Despite the very low percentage of starting T cells in this specific patient (1.2%), $152x10^6$ T cells could be obtained, with a 42 fold expansion.
The resulting blinatumomab expanded T cells (BET) contained both $CD4^+$ and $CD8^+$ cells in varying proportion. Only in one case the final product was composed predominantly of $CD4^+$ cells (95%). Expanded T cells were polyclonal, as shown by TCR Vβ expression by flow cytometry. Indeed CMV specific clones, detected by CMV peptide ($pp65_{495-503}$)-loaded HLA-A*0201 tetramer, were also expanded using this method. Final T cells were composed predominantly of the effector and central memory subsets. Th1 were slightly prevalent over Th2 cells (mean 20% and 10%, respectively), whereas Th17 and Treg were less than 1%. We also observed that the CD272 and CD279 synapse inhibitors diminished in BET compared to the starting CLL T populations (from 73% to 19% and 61% to 18%, respectively). These data suggest that stimulation and expansion with blinatumomab and rhIL-2 has normalized expression of these regulators on CLL T cells. Indeed BET were highly cytotoxic against $CD19^+$ targets cell lines or primary CLL cells, with 70-90% lysis at a 3:1 effector target ratio in presence of blinatumomab.
Finally BET were compared to Xcellerated cells expanded using anti-CD3/CD28 Dynabeads and rh-IL-2. The 2 protocols showed equivalent efficiency and comparable cell composition at the end of culture.
Discussion: We conclude that the use of blinatumomab and rhIL-2 provides a reproducible, simple and GMP-compliant protocol, allowing expansion of large numbers of autologous polyclonal T cells from relatively small volumes of peripheral blood from CLL patients and efficient simultaneous depletion of CLL cells. This procedure is an attractive option for adoptive therapy for these patients after immunosuppressive treatments.
Disclosure of Interest: J. Golay: None Declared, A. D'Amico: None Declared, G. Borleri: None Declared, M. C. Finazzi: None Declared, G. Quaresmini: None Declared, D. Nagorsen Conflict with: Amgen, M. Introna: None Declared, A. Rambaldi: None Declared.

PH-P072
PHENOTYPING AND FUNCTIONAL COMPARISON OF CMV-CTLS *IN-VITRO* EXPANDED WITH CONVENTIONAL AND SMART DENDRITIC CELLS

P. Reddy Varanasi[1,2,3,], S. Borchers[1,2], A. Daenthanasanmak[1], G. A. Salguero Lopez[1], L. Hambach[1,2], B. Eiz-Vesper[4,5], R. Stripecke[1], C. Falk[2,3], M. Messerle[3,6], U. Köhl[2,5], A. Ganser[1,2], E. Mischak-Weissinger[1,2,3]*
[1]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, [2]Integrated Research and Treatment Centre Transplantation – IFB-Tx, [3]German Centre for Infection Research-DZIF, [4]Institute of Transfusion Medicine, [5]Institute for Cellular Therapeutics, [6]Institute of Virology, Medical School Hannover, Hannover, Germany

Introduction: Cytomegalovirus (CMV) plays an important role in the morbidity and mortality of patients after allogeneic hematopoietic stem cell transplantation (HSCT). Cellular therapies such as adoptive transfer of CMV specific cytotoxic T lymphocytes (CMV-CTLs) are currently applied to control therapy refractory CMV reactivation. However *in-vitro* expanded CMV CTL can lose their proliferative potential and their capacity to persist in the patient after adoptive transfer. A better understanding of the phenotype of *in vitro* expanded CMV-CTLs may help to further improve current CTL expansion techniques.
Aims: Here we studied the impact of conventional CD14+ derived dendritic cells (convDCs) and self-differentiated myeloid-derived antigen presenting-cells (smartDCs) on the CMV CTL phenotype and the functional activity. The analysis comprised exhaustion and senescence markers and cytokine release of the expanded CMV-CTLs.
Materials (or patients) and Methods: The convDCs were generated by culturing CD14+ cells in the presence of recombinant human IL-4 and GM-CSF for 5 days. Self-differentiated myeloid-derived antigen presenting-cells (smartDCs) were generated by transduction of CD14+ cells with a lentivirus encoding GM-CSF, IL-4 and the CMVpp65 protein and subsequent *in-vitro* culture for 7 days. Both convDCs and smartDCs were matured on day 5 (IL-1b, IL-6, TNF a and PGE_2) and cultured for 38-48 hours. Peptide loaded convDCs and endogenously expressing CMVpp65 smart DCs were used to stimulate CMV-CTLs for 7 days. The expanded T-cells cells were analysed based on the surface marker expression by a multicolour flow cytometry panel. Exhaustion was monitored by PD-1 (programmed death-1) and anti-Tim3 (T cell immunoglobulin domain and mucin domain 3) expression. Senescence was studied by CD57 expression.
Results: In the cohort of three CMV seropositive donors (n=3) we have observed that memory distribution (CCR7+CD45RA–, CCR7-CD45RA-) of the expanded T-cells remained the same in CD8 T cells and CMV-CTLs regardless of the use of convDCs or smartDCs for stimulation. CD8 T-cells stimulated with convDCs loaded with CMV pp65 peptide pool and smartDCs endogenously expressing CMV pp65 showed significant increases of Tim3, PD1 in comparison to unstimulated CD8 T-cells (Tim3: P<0.0001, PD1: P<0.01). Similarly we found increase of Tim3 on the CMV tetramer positive subpopulation after stimulation with convDCs loaded with CMV pp65 peptide pool and smartDCs (P<0.05). Finally, we found a significant increase of CD57 on CD8 T cells stimulated with convDCs loaded with CMV pp65 peptide pool compared to unstimulated T-cells but not in CD8 T-cells stimulated with smartDCs endogenously expressing CMVpp65 (CD8-CD57: P<0.05). Interestingly, no increase of CD57 was found for the CMV tetramer positive subpopulation after stimulation with convDCs loaded with CMV pp65 peptide pool and smartDCs.
Discussion: Our preliminary *in-vitro* data show an increase in the exhaustion markers (Tim3, PD1) on both CD8 T cells and CMV-CTLs stimulated with both conv DCs and smart DCs and CD57 on CD8 T cells stimulated with conv DCs. Further evaluation of these markers in CMV-CTL might help to improve adoptive transfer by defining a subpopulation leading to efficient and prolonged control of viral reactivation.
Disclosure of Interest: None Declared.

PH-P073
INTERIM REPORT OF A CLINICAL STUDY WITH HAPLOIDENTICAL NK-CELLS AGAINST HIGH RISK MDS AND AML

A. T. Björklund[1,], M. Carlsten[1], L. Liu[2], M. Shaffer[3], S. Cooley[4], J. S. Miller[4], E. Watz[3], B. Wahlin[5], M. Palma[6], L. Hansson[6], P. Ljungman[7], E. Hellström-Lindberg[7], H.-G. Ljunggren[2], K.-J. Malmberg[2]*
[1]Hematology Center, Karolinska University Hospital, [2]Karolinska University Hospital, Center for Infectious Medicine, [3]Karolinska University Hospital, Clinical Immunology and Transfusion medicine, Huddinge, Sweden, [4]University of Minnesota, Masonic Cancer Center, Minneapolis, United States, [5]Karolinska University Hospital, Hematologiskt centrum Karolinska Huddinge, Huddinge, [6]Hematologiskt centrum Karolinska Huddinge, Solna, [7]Hematologiskt centrum Karolinska Huddinge, Huddinge, Sweden

Introduction: Here we report from an ongoing phase I/II study of HLA-haploidentical NK cell therapy to patients with high-risk myelodysplastic syndrome (MDS) and acute myeloid leukemia

(AML). The preparative regimen consisted of intermediate doses of Cyklophosphamide (Cy), Fludarabin (Flu) and titrated doses of total lymphoid irradiation (TLI). The trial design excluded systemic IL-2 treatment to avoid expansion of regulatory T cells.
Materials (or patients) and Methods: The first 6 patients was treated with Cy/Flu and TLI followed by infusion of short-term (16hours) IL-2 activated NK cells. Four patients had high risk MDS or MDS-AML and two had relapsed primary AML.
Results: The treatment was well tolerated and no severe non-infectious toxicity has been noted in the patients. Three of the patients had positive microchimerism, with detectable NK cells of donor origin at day 7, but none of the patients reached the primary endpoint (>100 donor NK cells/µl at day 14). Nevertheless, all patients displayed reduced tumor burden 1 month after therapy. Interestingly, two patients achieved complete remission that lasted at least 3 months and one of these has proceeded to allogeneic stem cell transplantation. Two additional patients had partial remission with stable blast cell counts for 3 and 6 months in the absence of additional therapy, respectively. Two patients with minor response and progressive disease died in infections within three months of therapy.
Discussion: Although the long-term efficacy needs to be evaluated, the results suggest that NK cell therapy may induce remission in patients with chemo-refractory disease and provide a bridge to allogeneic stem cell transplantation.
Disclosure of Interest: None Declared.

PH-P074

IMPACT OF CD8-DEPLETED DONOR-LYMPHOCYTE INFUSIONS AFTER T-CELL DEPLETED ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION–LONG TERM FOLLOW UP DATA

E. Maria Wagner[1,], D. Wehler[1], A. Naomi Lay[1], T. Schmitt[1], K. Kolbe[1], M. Theobald[1], W. Herr[2], R. Georg Meyer[1]*
[1]Haematology, Oncology and Pneumology, University Medical Center, Mainz, [2]Haematology and Internal Oncology, University Hospital, Regensburg, Germany

Introduction: We applied prophylactic CD8-depleted ($CD8^{depl}$) donor lymphocyte infusions (DLI) in the setting of T-cell depleted allogeneic hematopoietic stem cell transplantation (HSCT) in a phase I/II trial. T-cell depletion was carried out by the use of high-dose Alemtuzumab (100mg or 60mg for unrelated or sibling donor transplantation, respectively). We have demonstrated the feasibility of this approach after having treated 23 patients in this protocol (Meyer *et al.* Blood 2007).
Materials (or patients) and Methods: From 2004 to 2011, 134 patients with different hematologic diseases were included and followed for a median observation time of 2.6 years after transplantation (range, 1.5-9 years). Median age was 56 years (range, 19-71). Stem cell source was peripheral blood from either matched siblings (n=23), matched unrelated (n=62), or donors with single HLA mismatches (unrelated n=48, related n=1). Tapering of cyclosporine A was started in the 6th week after transplantation. Subsequently, $CD8^{depl}$ DLI were administered prophylactically in escalating doses starting with $1x10^6$ CD4 T cells/kg bodyweight.
Results: 53 patients received at least one dose of DLI. Among 81 patients who did not qualify for DLI, 60 patients had primary mild GVHD. Following DLI, acute GVHD was the major reason for withholding subsequent DLI-doses (66%), mainly because of acute GVHD ≤ 2°. Extensive chronic GVHD was diagnosed in 9.4% of the patients. Overall survival after 1 and 3 years was 60% and 43%, respectively. Survival significantly differed between the DLI and non DLI group after 3 years (63.8% vs. 30.1%, P=0.002). The inferior outcome of the non-DLI group was very similar when only those patients who did not receive DLI despite the absence of GVHD were considered (28.6%). The largest patient cohort in our trial were patients with AML and MDS (n=21 with and n=33 without DLI). In these patients, the survival benefit for the DLI group after 3 years was significant (75.8% vs 28.9%, P=0.0012). Interestingly, the relapse rate did not differ between DLI and non-DLI patients. The presence of GVHD at any time was associated with a reduced relapse rate (57.9% vs. 31.4%, P=0.0015), independent of DLI-application. However, GVHD had no impact on overall survival. Decreasing donor T-cell chimerism (TCC) was found in 34 patients who subsequently received DLI and 13 who did not. Following $CD8^{depl}$ DLI, 31 patients (88%) converted to full donor. In contrast, only 2 of the patients with decreasing TCC in the non-DLI group (15.3%) converted spontaneously. All patients with mixed TCC relapsed later on.
Discussion: In summary, we observed that the application of prophylactic $CD8^{depl}$ DLI was associated with a survival benefit and the re-establishment of full donor T-cell chimerism in the context of T-cell depletion. Still, this non-randomized trial is not sufficient to demonstrate any causality. In AML/MDS-patients the improved survival seems to be associated with lower treatment-related mortality rather than a reduced relapse-rate. Our data strongly ask for randomized trials comparing prophylactic application of $CD8^{depl}$ DLI vs. no DLI as well as $CD8^{depl}$ vs. non-manipulated DLI in a preemptive setting.
Disclosure of Interest: None Declared.

PH-P075

DONOR LYMPHOCYTE INFUSION FOR AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A RETROSPECTIVE ANALYSIS OF 57 PATIENTS.

A. Sperotto[1,], D. Damiani[1], A. michelutti[1], F. Patriarca[1], M. Cavallin[1], M. Battista[1], F. Zanini[1], A. Geromin[1], M. Cerno[1], R. Fanin[1]*
[1]Division of Hematology, Udine, Italy

Introduction: The impact of donor lymphocyte infusion (DLI) on its outcome is well known in patients with chronic myeloid leukaemia but still limited in patients with other haematological disease relapsed after allogeneic stem cell transplant (SCT).
Aim. To analyze the efficacy of DLI either in patients relapsed after SCT and as pre-emptive purpose.
Materials (or patients) and Methods: Fifty – seven high – risk haematological were included in the retrospective analysis: 20 (35.0%) acute myeloid leukaemia (AML); 17 (30.0%) lymphomas; 8 acute lymphoblastic leukaemia (ALL); 8 multiple myeloma (MM); 4 chronic myeloid leukaemia (CML). Forty-four (77.0%) patients had relapsed or a progressed disease at first DLI; 6 (5.5%) had molecular relapse; 3 (5.5%) had a chronic phase CML, 2 (3.5%) patients were treated for mixed chimerism and 2 (3.5%) as a pre-emptive program. A dose escalated program of DLI was performed with an initial DLI CD3+ cell dose per kilogram of recipient body weight equal to $0.5x10^7$/kg for sibling transplant and to $0.5x10^6$/kg for unrelated transplant.
Results: A median of 3 (1–9) DLI was performed. Twenty-two (38.5%) patients developed acute graft – versus – host disease: grade III in 7 (12.0%) patients. No DLI related deaths were observed.
With a median follow- up of 12 months (2–144), 30 (52.5%) patients are alive: 24 (428.0%) in complete remission. All the patients treated for molecular relapse acute leukaemia or for chronic phase CML (9 - 15.5%) are alive in molecular remission. Fourteen (32.0%) of the 44 patients treated for relapsed/progressed disease are alive in complete remission. Full donor chimerism was documented in the two patients treated for mixed chimerism. One – year overall and progression – free survival are 72.0% and 70.0% respectively.
Discussion: a lot of open questions still remain regarding DLI, but our results support the use of DLI for relapsed/progressed patients, with a low toxicity. Moreover, as expected, high – rate of complete remissions were observed in early relapses, confirming the relevance of molecular monitoring after transplant. For high – risk patients, DLI should be considered as pre-emptive program after transplant.
Disclosure of Interest: None Declared.

PH-P076

INFLUENCE OF KIR GENOTYPE ON SURVIVAL IN THE SETTING OF DUAL STEM CELL TRANSPLANTATION

M. Bastos-Oreiro[1,2,], M. Kwon[1,2], A. Navarro[1], C. Martinez Laperche[1,2], E. Buces[1,2], A. Perez Corral[1,2], C. Pascual[1,2], J. Gayoso[1,2], P. Balsalobre[1,2], D. Serrano[1,2], C. Muñoz[1,2], I. Buño[1,2], J. Anguita[1,2], J. L. Diez-Martín[1,2]*

[1]Hematology, Hospital General Universitario Gregorio Marañón, [2]Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain

Introduction: Little consistent data on the beneficial effect of NK cells has been reported in patients receiving umbilical cord blood (CB) transplantation. Our aim is to evaluate the influence of donor and recipient KIR genes content, in the setting of haplo-cord SCT (stem cell transplant) consisting of single CB in combination with CD34+ cells from a third party HLA-mismatched donor.

Materials (or patients) and Methods: 31 consecutive patients with haematological malignancies who received haplo-cord SCT between 2004 and 2012, were included (Table 1). The presence or absence of 15 KIR genes was determined by KIR genotype, analyzed by PCR (KIR Typing, Miltenyi Biotec) on genomic DNA (Maxwell 16 Blood DNA Kit, Promega) from peripheral blood samples of patients and CB units. Genotypes for the centromeric (Cen) and telomeric (Tel) parts of the KIR locus were assigned according to the presence or absence of one or more B haplotype KIR genes (Cooley *et al*, Blood 2010).

Results: Demographic data and KIR genotype characteristics of patients and CB units are listed in Table 1. Patients with A/A genotype presented worse disease free survival (DFS) compared with patients with B/x genotype (40% vs 79%, *P*= 0,016). We didn't find a protective effect for relapse with donor B/x genotype, even in those patients with A/A genotype who received CB units with Bx genotype (68 vs 72%, *P*= 0.8). Patients with haplotypes with Cen A/B motifs showed better DFS compared with patients with A/A motifs (88% vs 50%, *P*= 0.015). Also, patients who received UCB with haplotypes with Cen A/B motifs showed better DFS than CB with A/A motifs (53% vs 92%; *P*= 0.015). We didn't find differences between the Tel gene content. Mismatch of inhibitors KIR receptors (iKIR) gene content between CB and patients had no impact on the DFS and overall survival (OS), compared with no mismatch pairs. Patients receiving CB units with +KIR2DS1, or +KIR3DS1, didn't show a benefit in survival compared with patient transplanted with CB negative for those genes. However if we focus on AML, positive patients showed better results compared with the negative ones, despite the p value was no statistically significant (DFS: 71% vs. 50%, *P*=0.9 and 75% vs. 40%, *P*= 0.1 and OS: 100% vs. 66%., *P*= 0.1 and 100% vs. 60%, *P*= 0.07).

Discussion: Our results suggest that in the setting of haplo-cord SCT, patients with B/x genotypes have superior survival, compared with patients with A/A genotypes. However, contrary to data published in others SCT modalities, we didn't find a protective effect of CB B/x genotype, +KIR2DS1 or +KIR3DS1 CB, or iKIR mismatch between CB and patients. +KIR2DS1 and +KIR3DS1 CB in AML patients showed a tendency to better survival.

Disclosure of Interest: None Declared.

PH-P077

HYPOMETHYLATING AGENT AZACITIDINE INDUCES FOXP3 NEGATIVE HLA-G EXPRESSING IMMUNOREGULATORY T CELLS

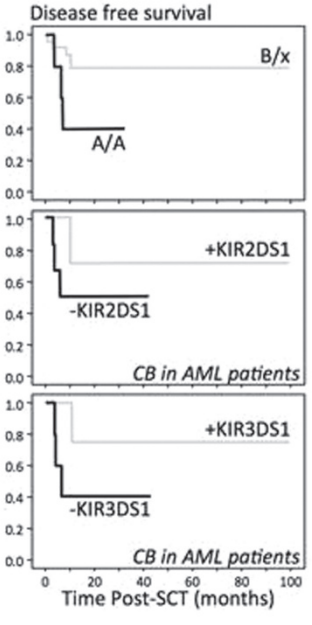
P. Stamou[1,], A. Vittoraki[1], D. Kokkinou[1], A.-L. De Lastic[1], D. Marioli[1], V. Goudogianni[1], E. Theofani[1], P. Zikos[2], A. Spyridonidis[1]*

[1]Bone Marrow Transplantation Unit, University Hospital of Patras, [2]Hematology Division, General Peripheral Hospital of Patras "Agios Andreas", Patras, Greece

Introduction: Major obstacles in using FOXP3$^+$ T regulatory cells as T-cell based immunotherapy against GVHD after allogeneic hematopoietic cell transplantation are their low numbers in the circulation and the lack of specific cell surface markers for efficient purification. DNA methylation has been considered to play a role in the regulation of T-cell effector function and cytokine gene expression, indicating a promising role of hypomethylating agents for immunomodulation. Recently it was shown that *in-vitro* treatment of conventional T-cells with hypomethylating agent azacitidine (aza) induced FoxP3 expression and converted CD4$^+$CD25$^-$ cells into immunosuppressive T-cells the suppressor function of which is independent of FOXP3 expression (Blood 2010;116:129-139), suggesting that aza induced suppressor function depends on the modification of other hypomethylated genes. Human leukocyte antigen-G (HLA-G) is a non-classical HLA class I molecule, shown to exert immunoregulatory functions, the

[PH-P076]

Number of patients		31
Median age, years (range)		43 (22-62)
Sex, n (%)	Male	17 (73)
	Female	14 (27)
Diagnosis, n (%)	AML	13 (42)
	ALL	9 (29)
	MPSc	3 (10)
	HL	1 (3.5)
	NHL	2 (6)
	MDS	2 (6)
	AA	1 (3.5)
Response pre-transplant n (%)	1CR	16 (51)
	2CR	4 (13)
	AD	11 (36)
Conditioning intensity n (%)	RIC	2 (66)
	MAC	29 (34)
Cord Haplotype n (%)	A-A	7 (22.5)
	B-x	24 (77.5)
	Cen B-B	1 (3.5)
	Tel B-B	5 (16)
Patient haplotype n (%)	A-A	5 (16)
	B-x	26 (84)
Allograft positive for KIR2DS1 n (%)		15 (47)
Allograft positive for KIR3DS1 n (%)		18 (58)



expression of which is epigenetically regulated. We investigated whether hypomethylating agent aza can induce HLA-G$^+$ immunoregulatory T cells.
Materials (or patients) and Methods: Negative selected CD3$^+$ T cells from peripheral blood of healthy individuals were stimulated with anti-CD3$^+$ plus anti-CD28$^+$ coated beads and then treated for 72 hours with aza (0.5-15 mM) in the presence of 50U/ml inteleukin-2 (IL-2). Phenotypical characterization of *in-vitro* aza treated cells was performed with flow cytomentry. Aza-induced HLA-G$^+$ T cells, were irradiated and then used as third party cells in CFSE based suppression assay, in which anti-CD3/CD28 beads were used as stimulators. For the analysis of the *in-vivo* effect of aza on HLA-G expression, peripheral blood mononuclear cells (PBMC) of patients with myelodysplastic syndrome (MDS) were isolated at baseline and after Vidaza treatment and were analyzed by FACS for HLA-G expression.
Results: *In-vitro* treatment of CD3$^+$ T cells with aza increases the percentage of HLA-G$^+$ cells. The optimum aza concentration for maximum HLA-G induction with the lowest toxicity in CD3 T cells at protein and mRNA level, was determined at 5 mM (HLA-G$^+$:6.88±3.9%, *P*=0.0022). Maximum HLA-G induction was observed on CD4$^+$CD25$^+$ population (*n*=2, 9.21±5.5%). Aza treatment of FACS-sorted CD4$^+$CD25^{neg}HLA-G^{neg} cells induced CD4^{low}CD25$^+$HLA-G positive cells, revealing that aza induced HLA-G$^+$ cells are not the result of a selectively expanded proexisting HLA-G$^+$ population. Strikingly aza induced CD4^{low}CD25$^+$HLA-G$^+$ are FoxP3 negative. *In-vitro* Aza induced HLA-G$^+$ cells display HLA-G dependent suppressive function. The % of CD4$^+$HLA-G$^+$ and CD8$^+$HLA-G$^+$ peripheral blood lymphocytes of healthy donors was 1.56±0.5 and 3.03±1.2 respectively while in MDS-Vidaza treated patients was 4.25±0.7 and 2.28±0.3 respectively. Although preliminary data do not show a significant increase in % and absolute number of CD4 and CD8 lymphocytes of MDS-Vidaza treated patients, CD4$^+$CD25^{high}HLA-G$^+$ cells showed a 5 fold increase on day 15 post Vidaza treatment.
Discussion: In conclusion, we generated a CD4^{low}FoxP3^{neg} immunoregulatory population, which expresses extracellulary HLA-G and therefore can be easily isolated for adaptive T-regulatory therapies. On the other hand, our results indicate that the use of aza post-transplant as a mean of reduction of disease relapse risk is questionable since aza may impair the GvL effect.
Disclosure of Interest: None Declared.

PH-P078
DIFFERENTIAL COSTIMULATORY REQUIREMENTS OF HUMAN MEMORY T-CELL SUBSETS: IMPLICATIONS FOR ADOPTIVE IMMUNOTHERAPY OF CANCER AND INFECTIONS
V. Volpin[1,], N. Cieri[1,2], C. Bonini[1]*
[1]Experimental Hematology Unit, Division of Regenerative Medicine, Gene Therapy and Stem Cells, San Raffaele Scientific Institute, [2]Università Vita-Salute San Raffaele, Milan, Italy

Introduction: Costimulation plays a critical role for T-cell activation. Recent studies have highlighted that costimulatory signals are required not only for efficient naïve T-cell priming but also for secondary recall responses. Nevertheless the costimulatory requirements of memory responses have been much less studied than those of primary responses, especially in humans. Nonetheless, the study of the costimulatory requirements of memory T cells is of high relevance considering that memory T cells play a critical role in several diseases, including infections, cancer and autoimmunity. Here, we evaluated the ability of the costimulatory molecules of the CD28-family (CD28 and ICOS) and of the TNFR-family (4-1BB, OX40, GITR, CD27, CD30, HVEM) to *ex vivo* expand human memory T-cell subsets, namely central memory (T_{CM}), effector memory (T_{EM}) and the recently identified memory stem T cells (T_{SCM}), for immunotherapeutic purposes.
Materials (or patients) and Methods: To evaluate the effects of costimulatory signals on memory T cells, we activated purified memory T-cell subsets with coated anti-CD3 antibody and soluble agonistic antibodies directed against costimulatory molecules. Retroviral transduction was performed to formally prove the proliferation and postmitotic status of analyzed cells, and low doses (5ng/ml) of homeostatic cytokines (IL-7 and IL-15) were added to cultures. The efficacy of costimulatory molecules was measured in terms of T-cell expansion and preservation of the original surface phenotype.
Results: In resting conditions, we observed that the large majority (more than 90%) of memory T cells were positive for CD28, CD27 and HVEM costimulatory molecules, while expression of ICOS, 4-1BB, OX40, GITR, and CD30 was up-regulated upon TCR triggering. Interestingly, we found that each human memory T-cell subset best responded to distinct costimulatory signals: CD28 best supported the expansion of T_{SCM} coupled to the preservation of original phenotype, while CD27 best expanded T_{CM} and T_{EM} lymphocytes. In addition, CD27 and CD28 imprinted distinct functional programs, fostering gIFN and IL-2 production respectively in all subsets analyzed.
We next assessed IL-7 Receptor alpha (CD127) expression, a marker of T-cell fitness. Among T_{SCM} cells, CD28-costimulation preserved CD127 expression on CD4+ T cells significantly better than 4-1BB-, CD27- and CD30-mediated costimulation.
Discussion: Altogether, our data suggest that tailoring the costimulatory signals to the T cell subset to be expanded could improve the *ex vivo* manipulation protocols necessary for adoptive immunotherapy of cancer and infectious diseases.
Disclosure of Interest: None Declared.

PH-P079
LARGE-SCALE MYELOID DENDRITIC CELLS GENERATION FROM UMBILICAL CD34+ CELLS USING G-REX BIOREACTORS
E. Gounari[1], A. Papadopoulou[1], N. Zogas[1], N. Psatha[1], G. Karponi[1], F. Zervou[1], D. Bougiouklis[1], A. Xagorari[1], M. Yiangou[2], A. Anagnostopoulos[1], E. Yannaki[1], P. Kaloyannidis[1,]*
[1]Gene and Cell Therapy Center, Hematology Department, BMT Unit, G.Papanicolaou Hospital, [2]School of Biology, Department of Genetics, Development and molecular biology, Aristotle University, Thessaloniki, Greece

Introduction: Dendritic cells (DCs) have the unique ability to prime naïve T-cells, thus representing a fundamental tool in cancer therapy and especially in vaccine- or antitumor specific T cell–based immunotherapy. However, the laborious and costly methods to generate and expand DCs from the peripheral blood (PB) or monocytes, limit their potential for broad clinical applications. In addition, although DCs can be produced from cord blood (CB)-derived CD34+ cells, the final DC yields are low to be clinically translated. New methods and sources are needed in order to obtain sufficient numbers of DCs (10^6 –10^8 DCs).
Materials (or patients) and Methods: In this study we sought to investigate the potential of large scale generation of DCs from CB-derived CD34+ cells, using the G-Rex10 bioreactors (Wilson Wolf Corporation) instead of the standard culture dishes or flasks. We additionally asked for the optimal culture conditions and tested the capability of produced DCs to induce Th1 responses upon stimulation with a mixture of Toll Like Receptor ligands (TLR-Ls). Non transplantable CB units were used after a signed informed consent from the parents. CD34+cells were enriched to more than 90% purity from CB units (*n*=5) by an immunomagnetic separation method. The CD34+ cells were cultured in the presence of AB-blood group serum (ABS) and culture media supplemented with SCF (50ng/ml) and GM-CSF (100ng/ml) for 2, 3, 4, or 5 weeks (wks) and with IL-4 (50ng/ml) plus GM-CSF (100ng/ml) for 1 additional wk. The cells were initially plated in a 6-well plate (10^5 cells/well) and once the cells expanded up to $5x10^6$, half were transferred into G-Rex10 bioreactor and half were cultured in "conventional" culture plates in the presence of the above mentioned conditioned medium. In culture plates the cells were splited at confluency while in G-Rex10 every 3 days.
Results: The highest median absolute number of myeloid DCs (CD33+/CD11+) in the bioreactor was obtained by 5-wks culture over the 3-, 4-, and 6-wks culture ($1.5x10^9$ vs $0.017x10^9$ vs $0.8x10^9$ vs $1.2x10^9$, respectively). The number of myeloid DCs that were conventionally cultured under the same conditions did not exceed

a median number of $10x10^6$ cells. To evaluate the impact of the serum origin in DCs expansion, we cultured CB-derived CD34+ cells into G-Rex10 bioreactor, in the presence of either autologous CB serum (ACBS) or ABS. At the end of the culture, we identified $1.5x10^9$ DCs with myeloid characteristics in the ABS culture while only $0.011x10^9$ in the ACBS culture ($P<0.05$). To address whether DCs expanded from CB-derived CD34+ cells have the ability to produce Th1 responses, we stimulated DCs with a mixture of TLR-L 3 (Poly I:C: 20µg/ml) and TLR-L 7/8 (R848: 4µg/ml) for 48 hours and measured the levels of IL-12p70, TNF-α, IL-6 and IL-10 by ELISA. Increased levels of IL-12p70, TNF-α and IL-6 were detected, while the IL-10 levels were low to undetectable, indicating that the produced DCs with myeloid features have a strong potentiality for Th1 responses.
Discussion: Overall, we report for first time, over a 10^4 fold myeloid DCs expansion from CB-derived CD34+cells, by using the new generation G-Rex10 bioreactors and describe optimal culture conditions. This large scale myeloid DC generation could significantly contribute to the clinical applicability of DCs in cancer immunotherapy.
Disclosure of Interest: None Declared.

PH-P080
THIRD GENERATION AUTOLOGOUS MYELOID-DERIVED DENDRITIC CELLS DEVELOPED FROM THE PATIENTS WITH CMML AND NON-CML SUBTYPES OF MDS DEMONSTRATE PHENOTYPIC PROPERTIES OF MATURE FUNCTIONAL DC
K. Anderson[1,], I. Dybedal[2], I. Bigalke[1], D. Schendel[3], L. Tjeldhorn[1], O. Chowdhury[4], P. Woll[4], S. E. Jacobsen[4], G. K. Kvalheim[1]*
[1]Department of Cellular Therapy, The Radium Hospital, Oslo University Hospital, [2]Department of Hematology, Rikshospitalet, Oslo University Hospital, Oslo, Norway, [3]Deutsches Forschungszentum für Gesundheit und Umwelt (GmbH), Helmholtz Zentrum München, Neuherberg, Germany, [4]Haematopoietic Stem Cell Biology Laboratory, WIMM, Oxford University, Oxford, United Kingdom

Introduction: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell diseases characterized by inefficient haematopoiesis with frequent progression to acute myeloid leukaemia (AML). Chronic myelomonocytic leukaemia (CMML) is a clonal haematopoietic malignancy characterised by the features of both myeloproliferative neoplasm and MDS, such as monocytosis, variable degree of leukocytosis, anaemia, thrombocytopenia and dysplastic changes in different haematopoietic lineages, and has poor prognosis with median survival of barely 1 to 3 years. Both MDS and CMML are disorders of elderly and, with the exception of allogeneic bone marrow transplantation (ASCT), have no curative treatment options. Because of the older age, only a minority of CMML patients are eligible for ASCT. In recent years, active immunotherapy (AIT) using dendritic cell (DC) vaccines transfected with various leukaemia-associates antigens has become an exciting concept for treating these patients. Moreover, recent reports have indicated effectiveness of combining chemotherapy with AIT that leads to a longer overall survival of cancer patients.
Materials (or patients) and Methods: The purpose of this study was, first of all, to find out whether myeloid-derived DCs generated from MDS patients acquire phenotypic and functional characteristics of mature DCs. Secondly we were interested in how many of investigated patients could be potential candidates for DC vaccine treatment taking in account their clinical characteristics and general condition. Importantly, monocytes of MDS patients are often a part of malignant clone.
In our experiments we used PB MNCs from 10 CMML patients, 5 non-CMML patients and 5 healthy controls. Median age of CMML vs. non-CMML patients was 69 and 68, respectively. Mean absolute monocyte count was $4,0x10^9$/l and $1,0x10^9$/l for CMML vs. non-CMML patients. Mean haemoglobin was 13 vs. 12 g/dl for CMML and non-CMML patients. Four CMML patients had abnormal karyotype by conventional cytogenetics (i.e. +8) or mutational analysis (RUNX1, JAK2), while 3/5 non-CMML patients had various cytogenetic abnormalities. Two CMML patients were previously treated with either hydroxyurea or azacitidin, while all 5 non-CMML patients were under active treatment at the time of sampling. All patients had ECOG status 0-1.
Results: Mature DCs were developed using a novel production protocol consisting of two days of culturing peripheral blood monocytes in the presence GM-CSF and IL-4 followed by one day of maturation using GM-CSF, IL-4, TNF, INF-gamma, PGE_2, IL-1beta and R848. Our results show that we were able to generate mature functional DCs, assessed by phenotype, Signal-3 and/or migration assays, in 9/10 CMML cases and all non-CMML patients. There were insignificant variations in down-regulation of CD14 and expression level of CD80, CD86, CD40, CD83, CD274, CCR7 and HLA-DR for CMML and MDS patients compared to normal controls.
Discussion: Taken together, the results of our study show that all but one patient in the CMML group and all patients in non-CMML group could be relevant candidates for vaccination within a clinical protocol using DCs transfected with WT1 and PRAME mRNA (protocol under preparation).
Kristina Anderson and Ingunn Dybedal equally contributed to the study.
Disclosure of Interest: None Declared.

PH-P081
COMPARISON OF IMMUNOMODULATORY ACTIVITIES OF EXOSOME-ENRICHED FRACTIONS
L. Kordelas[1,], K. de Miroschedji[2], S. Radtke[2], A.-K. Ludwig[2], P. A. Horn[2], D. W. Beelen[1], B. Giebel[2]*
[1]Department of Bone Marrow Transplantation, [2]Institute for Transfusion Medicine, University Hospital Essen, Essen, Germany

Introduction: More than 300 NIH registered clinical trials applied mesenchymal stem cells (MSCs) to treat patients with a variety of different diseases e.g. myocardial infarction, stroke and graft-versus-host disease (GvHD). Initially, MSCs were thought to replace lost cells in damaged tissues. Despite controversial reports regarding the outcome of MSC treatments, they rather seem to exert their beneficial effects by the secretion of immunosuppressive factors. In this context small extracellular vesicles (80-140 nm), exosomes, were identified to mediate the immunosuppressive effects of MSCs. Indeed, by treating a steroid-refractory GvHD patient with MSC-exosomes containing high concentrations of anti-inflammatory cytokines (TGF-b1, IL-10, HLA-G), we gained evidence that they can effectively suppress GvHD symptoms. Assuming that MSC-exosomes of different donors differ in their immunosuppressive potential, we now have compared the impact of different MSC-exosome preparations on the proliferation behavior of stimulated T cells.
Materials (or patients) and Methods: Carboxyfluorescein succinimidyl ester (CFSE)-labeled PBMCs were stimulated with phytohaemagglutinin (PHA) in the presence of exosomes enriched from supernatants of different donor-derived MSCs. After 5 days, the CFSE intensity–as a marker for lymphocyte proliferation – was assessed by flow cytometry and analyzed for CD3-positive cells.
Results: CFSE-intensity–as an indicator for lymphocyte proliferation – of CFSE-labeled PBMCs which were stimulated with PHA in the presence of exosomes enriched from supernatants of different donor-derived MSCs varies from 0.2% to 72%. Controls of CFSE-labeled PBMCs without exosomes show a CFSE-intensity of 0.3% without and 61.7% after stimulation with PHA (s. Figure 1).
Discussion: Our results confirm the assumption that MSC-exosomes of different donors differ in their capability to modulate the proliferation stimulating impact of PHA on CD3 positive human T cells. Since in most clinical applications, especially in the treatment of steroid refractory GvHD patients, a strong immunosuppressive effect of MSC-exosomes is desired, we are currently searching for surrogate markers allowing the fast identification of MSCs whose exosomes exert strong immunosuppressive impact and which can effectively be used in the clinical setting.
Disclosure of Interest: None Declared.

PH-P082

CHARACTERIZATION OF THE *IN VITRO* IMMUNOMODULATORY PROPERTIES OF MICROVESICLES ISOLATED FROM MESENCHYMAL STROMAL CELLS

M. E. Bernardo[1,], A. Conforti[2], M. Scarsella[2], E. Giorda[2], B. Simone[2], N. Starc[2], G. Li Plra[2], A. Proia[2], R. Carsetti[2], F. Locatelli[2]*
[1]Department of Onco-hematology, IRCCS Bambino Gesù Children's Hospital, [2]Bambino Gesù Children's Hospital, Rome, Italy

Introduction: Mesenchymal stromal cells (MSCs) are multipotent cells that exert immunomodulatory effects; however, the mechanisms underlying these effects have not been completely clarified. Aim of this study was to compare *in vitro* the immunomodulatory properties of MSCs with those of microvesicles (MVs) released in supernatants from the same MSCs.
Materials (or patients) and Methods: MSCs were generated from bone marrow of 12 healthy donors (HDs) and MVs were isolated from their supernatant by serial ultracentrifugation. Both MSCs and MVs were characterized by flow cytometry and co-cultured with peripheral blood mononuclear cells (PBMCs) of 12 HDs and stimulated *in vitro* with both PHA and CpG to induce T and B cell proliferation, respectively. Growth factors and cytokines were quantified in the supernatants by ELISA.
Results: MVs were identified as 1mm particles positive for CMFDA, CD107 and CD13 (suggesting a mechanism of membrane budding from MSCs). MSCs inhibited PHA-induced T-cell proliferation up to 80% (SD ±8.16; *P*=0.0001 as compared with condition PBMCs/PHA) and 60% (SD ±20.86; *P*=0.0001) with MSC:PBMC ratios 1:2 and 1:10 respectively, whereas MVs reduced it up to 30% (SD ±39.32; *P*=0.01 as compared with condition PBMCs/PHA; MV dilution of 1:2 in co-culture final volume). MSCs reduced CpG induced B-cell proliferation up to 70% (SD ± 2.82; *P*=0.002 as compared with condition PBMCs+CpG) and plasma cell activation up to 50% (SD ±10.59; *P*=0.001; MSC:PBMC ratio 1:10), whereas MV-induced inhibition was up to 60% (SD ±9.95; *P*=0.02 as compared with condition PBMCs+CpG) and 30% (SD ±13.53; *P*=0.02), respectively (same diluting conditions). In both T- and B-cell cultures, MSC co-culture induced an increase of IL-6, IL-10, TGFb and a decrease of IL-2 and IFN, whereas in MV co-culture no differences were revealed in cytokines levels.
Discussion: Our data indicate that MSC-derived MVs display a lower immunomodulatory effect *in vitro*, as compared to their cellular counterpart. Caution should be employed when considering the potential use of MV in substitution of MSCs in therapeutic approaches, especially when aiming at treating immune-mediated disorders.
Disclosure of Interest: None Declared.

PH-P083

ESTABLISHMENT OF A GMP-COMPLIANT MESENCHYMAL STROMAL CELL BANK FROM THE POOL OF BONE MARROW OF EIGHT THIRD-PARTY HEALTHY DONORS

Z. Kuci[1,], H. Boenig[2], M. Bunos[3], H. Kreyenberg[4], M. Skific[5], K. Michel[1], G. Lucchini[1], T. Klingebiel[1], P. Bader[1], S. Kuci[1]*
[1]Hematology/Oncology, University Children's Hospital, Frankfurt am Main, [2]Cell Processing, Institute of Transfusion Medicine and Immunohematology, Franfkurt am Main, [3]Cell Processing, Institute of Transfusion Medicine and Immunohematology, [4]Hematology/Oncology, University Childern's Hospital, Frankfurt am Main, Germany, [5]Division of Cellular Therapy, University Hospital Center, Clinical Dept. of Transfusiology and Transplantation Biology, Zagreb, Croatia

Introduction: Mesenchymal stromal cells (MSCs) represent a heterogeneous cell population concerning their proliferative, differentiation and allosuppressive potential. This is a donor-dependent property, which leads to the controversial clinical data. To avoid this great inter-donor variability, we established a GMP-compliant, serum-free MSC-Bank from the pool of bone marrow mononuclear cells (BM-MNCs) of eight allogeneic healthy donors.
Materials (or patients) and Methods: **Phase I:** Pivotal experiments. In this phase we performed pivotal experiments, which led to the development of standard operating protocols of the MSC-Bank. We tested the BM-MNCs of 4 donors either individually or after pooling them for: clonogenicity, efficiency to give rise to MSCs, proliferative and allosuppressive potential. **Phase II:** BM-sample collection and generation of MSC-Bank. Written informed consent from eight healthy bone marrow donors was obtained for collection of up to 230 ml bone marrow. The blood samples were tested for markers of transfusion transmitted diseases (TTD). BM-MNCs were isolated in GMP-facility and frozen separately in cryobags. After thawing, BM-MNCs from 8 donors were washed, pooled and cultured in DMEM supplemented with 5% platelet lysate (PL) until generated MSCs reached 80% confluence. After harvesting, the MSCs were frozen in 209 cryovials $1.5x10^6$ MSCs, which represent the MSC-bank. **Phase III:** Validation of MSC-bank. Three randomly-chosen MSC-cryopreserved vials were thawed, analyzed for their viability and expanded for 12-14 days in DMEM+10% PL until the end of second passage (end product). To test the quality of frozen end products, three different bags containing expanded MSCs were thawed and tested for their viability, sterility, phenotype, proliferation/senescence, differentiation capacity, and their allosuppressive potential in mixed lymphocyte reaction (MLR).
Results: The major message of phase I experiments was that MSCs generated from the pool of BM-MNCs of 4 donors were more allosuppressive in MLR than MSCs from each donor individually. All donors for MSC-Bank were negative for markers of TTD. From pooled BM-MNCs of eight donors we generated $320x10^6$ MSCs which were frozen in 209 cryovials. To generate end products for validation of MSC-bank three randomly-chosen MSC-cryovials were thawed and expanded until the end product (P2), obtaining ~ $432x10^6$ MSCs/vial (about 8.5 population doublings-PD). End products demonstrated a typical MSC-phenotype and revealed the first signs of senescence starting at P4 and ceased their proliferation at P10 or P11 (68 days). All three end products differentiated in osteoblasts and adipocytes. Surprisingly, in MLR the allosuppressive effect of MSC-end product generated from the pool of 8 BM-MNCs was significantly higher ($P<0.04$) than the mean effect of MSCs from 8 donors individually as well as the pool of MSCs from 8 donors. So far, we expanded 13 MSC-cryovials of MSC-bank and obtained $6,5x10^9$ MSCs. Until now, 36 MSC-end products were used in 15 patients for the treatment of aGvHD with no adverse effects.
Discussion: To our knowledge, this MSC-bank represents the first validated xeno-free bank generated from the pool of 8 BM-donors. As these MSCs are from the same source, their use may ensure more consistency and reproducibility of their effect and therefore may be advantageous for clinical studies.
Disclosure of Interest: None Declared.

PH-P084

DASATINIB FOR THE MODULATION OF M1/M2-MACROPHAGE DIFFERENTIATION

M. Braun[1], F. Fritzsch[1], M. L Reß[1], Y.-E. Yoo[1], P. G Schlegel[1], M. Wölfl[1,]*
[1]University Children's Hospital, Würzburg, Germany

Introduction: Dasatinib is a dual bcr/abl/src-kinase inhibitor used for the treatment of bcr/abl+ leukemias. Originally it was designed as immunosuppressant and has been shown to inhibit effector T-cell activation.
We recently identified a previously unknown stimulatory effect of dasatinib on maturing human dendritic cells, leading to enhanced IL-12 production in response to TLR4-agonists (Wölfl *et al*., Blood 2013). Furthermore dasatinib also had strong stimulatory effects on other cells of myeloid origin.
Materials (or patients) and Methods: Here studied the effect of dasatinib on the differentiation of M1/M2-macrophages, using a cytokine-defined *in vitro* system. The differentiation in M1 and M2 macrophages is important, as these subtypes display either inflammatory (M1) characteristics versus leukemia/lymphoma enhancing capacity in M2-macrophages.
Human CD14+ monocytes were differentiated to macrophages, using GM-CSF (M1), M-CSF(M0), M-CSF and IL-4(M2a) or M-CSF

and IL10(M2c) and evaluated phenotypically and functionally in response to an LPS-stimulus.
Results: When dasatinib was present at the time of LPS-mediated activation, increased IL-12 production was observed in M1-macrophages. More importantly a shift of M2a-macrophages towards higher inflammatory activity was achieved. However, incubation with IL-10 lead to terminal differentiation of macrophages with an M2c-type function, regardless of dasatinib-treatment.
Discussion: Differentiation to the unfavorable M2-macrophage phenotype may be counteracted by treatment with src-kinase inhibitors during the differentiation process. However, once terminally differentiated, M2c-macrophages remain unaltered by dasatinib. These findings lay the basis for modulating the microenvironment using src-kinase-inhibitors to direct the innate immune response towards inflammatory, anti-tumor activity.
Disclosure of Interest: None Declared.

Chronic Leukaemia

PH-P085
REDUCED-INTENSITY TRANSPLANTATION IN PATIENTS OLDER THAN 60 YEARS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: SIMILAR RESULTS WITH RELATED OR UNRELATED DONOR, EARLIER INDICATION FOR TRANSPLANTATION COULD IMPROVE RESULTS – SINGLE CENTRE EXPERIENCE

M. Karas[1,], K. Steinerova[1], V. Vozobulova[1], D. Lysak[1], S. Vokurka[1], P. Jindra[1]*
[1]Hematology and Oncology Department, Charles University Hospital Pilsen, Pilsen, Czech Republic

Introduction: Reduced-intensity allogeneic stem cell transplantation (RIT) can improve treatment results among patients (pts) with high-risk or advanced chronic lymphocytic leukemia (CLL). However, most of pts with CLL are (pts) older than 60 years and published data concerning RIT outcome of this group of elderly pts are limited. To evaluate the potential role of RIT in pts older than 60 years with CLL and the role of use of unrelated donor we retrospectively analysed the results of those pts transplanted in our centre during last 10 years.
Materials (or patients) and Methods: From 9/2003 to 9/2013 33 consecutive pts with median of age 63 years (range: 60-70 years) with high-risk or advanced CLL (17p-/p53 mutation, chemoresistant CLL, relapse ≤ 24 moths, ≥ 3 treatment lines) underwent RIT (33% HLA identical related, 45% HLA matched unrelated, 22% HLA mismatched unrelated). Source of stem cells was peripheral blood and the median of infused CD 34+ cells was $5.1x10^6$/kg (range: $1.2\text{-}12.2x10^6$/kg). The conditioning regimen consisted of fludarabine and melphalan (55%) or fludarabine and cyclophosphamide (45%). Cyclosporine and methotrexate were administered as GVHD prophylaxis. Pts undergoing unrelated RIT had comparable prognostic variables as pts undergoing related RIT except of younger age of donors (P=0.0001) in unrelated RIT.
Results: 91% of patients engrafted. None of 6 pts in CR before RIT progressed at day +30 after RIT and among 27 pts beyond CR before RIT 89% of them achieved at least PR at day +30 after RIT. 18 pts (54%) developed acute GVHD (3 pts grade III-IV) and among 26 evaluable pts 15 (58%) of them developed chronic GVHD (5 limited, 10 extensive). With median follow-up 43 moths (range 3-110 months) 8 pts (24%) are alive in CR. 9 pts (27%) relapsed or progressed with median time to progression 3 months (range 1-30 months) and died with median time after progression 23 months (range 1-52 months). 16 pts (49%) died due to NRM (94% infections). NRM till day +100 after RIT was 15%. The estimated probabilities of 3-years PFS and OS are 27% and 36%. There were no statistical difference between results of related and unrelated RIT.
Discussion: In spite of relatively small number of evaluated pts and retrospective type of analysis our data show that RIT even in use of unrelated donor in pts over 60 years of age (usually heavily pretreated and with serious comorbidities) achieves in about 30% of them long lasting disease control of high-risk or advanced CLL. On the other hand, characteristics of our patient cohort suggest a later indication for RIT in elderly pts with CLL. Later indication for RIT means higher pretreatment and selection of aggressive CLL clones, which probably also leads to higher risk of mortality and relapse incidence after RIT. The role in later indication for RIT could play the fact that concerns about risks of transplant mortality in older pts led to efforts to control the disease using standard chemotherapy, even in the presence of known adverse prognostic factors of CLL. Earlier indication for RIT could improve transplant results in elderly pts with prognostically unfavourable leukemia.
Disclosure of Interest: None Declared.

PH-P086
THE OUTCOME OF REDUCED-INTENSITY TRANSPLANTATION IN ELDERLY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA IN COMPARISON WITH ELDERLY PATIENTS TRANSPLANTED FOR ACUTE MYELOID LEUKEMIA: IS THERE DIFFERENCE IN TIMELINESS OF INDICATION FOR TRANSPLANTATION?

M. Karas[1,], K. Steinerova[1], A. Jungova[1], D. Lysak[1], S. Vokurka[1], V. Vozobulova[1], P. Jindra[1]*
[1]Hematology and Oncology Department, Charles University Hospital Pilsen, Pilsen, Czech Republic

Introduction: Reduced-intensity allogeneic stem cell transplantation (RIT) could improve treatment results in patients (pts) older than 60 years with high-risk hematologic malignancies. Acute myeloid leukemia in elderly pts has poor prognosis and despite of higher risk of transplant mortality indication for RIT is usually performed early after diagnosis of AML. Different situation may be among elderly pts with CLL, which is considered as a chronic disease, and concerns about the risks of transplant mortality can often outweigh known risk factors of CLL, which may lead to delay of indication for RIT. To evaluate the potential role of primary diagnosis on outcome of RIT in pts older than 60 years, we retrospectively analysed and compared the results of RIT in elderly pts transplanted for CLL with elderly pts undergoing RIT for AML in our centre.
Materials (or patients) and Methods: From 1/2003 to 9/2013 33 consecutive pts older than 60 years (CLL group) usually with high-risk or advanced CLL and 50 consecutive pts older than 60 years (AML group) with usually high-risk AML underwent RIT. There were no difference in prognostic variables between both groups concerning age, PS, HCT-CI, type of donor, number of infused CD34+ cells, type of GVHD prophylaxis. There was only one difference in the type of conditioning regimen (CLL group – 51% FLU/MEL, 49% FLU/CYI; AML group – 100% FLU/MEL).
Results: CLL group: with median follow-up 43 moths (range 3-110 months) 8 pts (24%) are alive in CR. 9 pts (27%) relapsed or progressed with median time to progression 3 months (range 1-30 months) and died with median time after progression 23 months (range 1-52 months). 16 pts (49%) died due to NRM. NRM till day +100 and +365 after RIT were 15% and 33%. The estimated probabilities of 3-years PFS and OS are 27% and 36%. AML group: with median follow-up 39 moths (range 5-105 months) 21 pts (42%) are alive and 20 pts (40%) are in CR. 12 pts (24%) relapsed or progressed with median time to progression 5 months (range 3-49 months) and 11 of them died with median time after relapse 2 months (range 0-10 months). 18 pts (36%) died due to NRM. NRM till day +100 and +365 after RIT were 8% and 20%. The estimated probabilities of 3-years PFS and OS are 48% and 53%. Comparison of these two groups showed a statistically significantly better PFS (P=0,04) and a trend toward better OS in the group of patients transplanted for AML.
Discussion: our data show better RIT outcome in elderly pts transplanted for high-risk AML in comparison with pts transplanted for high-risk or advanced CLL even though AML is generally considered to be more unfavorable disease in comparison with CLL. Potential explanation for these "surprising" results could be later

indication for RIT in CLL pts. Typically chronic course of CLL can lead to extension of non-transplant treatment before indication for RIT, which leads to selection of aggressive malignant cells with higher risk of relapse and heavily pretreated pts at high-risk of NRM. Whether it is a problem of our center or it is a general trend can only be verified by analysis of data from other centers.
Disclosure of Interest: None Declared.

PH-P087

OUTCOMES OF CORD BLOOD TRANSPLANTATION USING REDUCED INTENSITY CONDITIONING REGIMEN FOR CHRONIC LYMPHOCITYC LEUKEMIA: A RETROSPECTIVE STUDY ON BEHALF OF EUROCORD, SFGM-TC AND CMWP-EBMT

E. Xavier[1,], J. Cornillon[2], A. Ruggeri[1], P. Chevallier[3], J. J Cornelissen[4], N. S. Andersen[5], E. Deconinck[6], N. Maillard[7], D. Blaise[8], S. Nguyen-coq[9], N. Ifrah[10], A. Huynh[11], M. S. Gonzalez Perez[12], J.-Y. Cahn[13], N. Milpied[14], M. Mohty[15], M. Michallet[16], J.-H. Bourhis[17], T. de Revel[18], C. Berthou[19], N. Fegueux[20], D. Bordessoule[21], I. Donnini[22], G. Leone[23], R. Peffault de Latour[24], E. Gluckman[1], N. Kröger[25], J. Schetelig[26], V. Rocha[1]*
[1]Hôpital Saint Louis, EUROCORD, Paris, [2]Hematologie Clinique, Institut de Cancérologie Lucien Neuwirth, Saint Etienne, [3]Hematologie, CHU, Nantes, France, [4]Hematologie, Daniel den Hoed Cancer Centre, Rotterdam, Netherlands, [5]Hematologie, Rigshospitalet, Copenhagen, Denmark, [6]Hematologie, Hôpital Saint Jacques, Besançon, [7]Hematologie, La Miletrie Hospital, Poitiers, [8]Bone Marrow Transplantation and Cellular Therapy, CHU, Marseille, [9]Hematologie, Pitie-Salpetriere Hospital, Paris, [10]Hematologie, CHRU, Angers, [11]Hematologie, Purpan Hospital, Toulouse, France, [12]Hematologie, HCU, Santiago de Compostela, Spain, [13]Hematologie, A. Michallon Hospital, Grenoble, [14]Hematologie, CHU, Bordeaux, [15]Hematologie, Saint-Antoine Hospital, Paris, [16]Hematologie, Edouard Herriot Hospital, Lyon, [17]Hematologie, Institute Gustave Roussy, Villejuif, [18]Hematologie, Percy Hospital, Clamart, [19]Hematologie, CHU Morvan, Brest, [20]Hematologie, CHU Lapeyronie, Monpellier, [21]Hematologie, CHRU, Limoges, France, [22]Hematologie, Ospedale di Careggi, Firenze, [23]Hematologie, Universita Cattolica S. Cuore, Rome, Italy, [24]Bone Marrow Transplantation and Cellular Therapy, Saint-Louis Hospital, Paris, France, [25]Department of Stem Cell Transplantation, University Hospital Hamburg-Eppendorf, Hamburg, [26]Medical Department I, University Hospital Carl Gustav Carus, Dresden, Germany

Introduction: Allogeneic HSCT is the only curative treatment for CLL and is performed in patients (pts) with high-risk features at diagnosis (17p/p53 deletions) or advanced disease. Although only 25-30% of patients have matched siblings, alternative stem cell sources such as umbilical cord blood allow extending HSCT to pts lacking a conventional donor.
Materials (or patients) and Methods: We analyzed 68 pts (49 males; 19 females) who underwent a single (*n*=16) or double (*n*=52) HLA mismatched umbilical cord blood transplantation (UCBT) between 2004 and 2012 in 34 EBMT centers. Median age at UCBT was 57 years (yrs) (27-68). At diagnosis, 56 pts had Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, 8 Prolymphocytic Leukemia and 4 Richter Syndrome. Considering Binet staging, 28% had stage A, 57% B and 15% C; 54% had abnormal (27% del17p/p53, 15% del13q, 5% del11q and 7% others) and 21% normal karyotype (25% data not available). Median time from diagnosis to UCBT was 58 months (3-360). Thirty four pts were in PR at UCBT, 22 in CR and 9 had refractory disease. EBMT-score at transplant was 3-4 in 26% of pts, 5-6 in 71% and 7 in 3%. Sixty-six percent of pts received ≥3 chemotherapy regimens prior UCBT, 34% were refractory to purine analogues and 16% had previous autologous HSCT. The Minnesota RIC regimen (CyFluTBI) was given to 56 pts and ATG, at conditioning, to 15. GVHD prophylaxis was CsA+MMF in 59 pts. Median TNC collected and infused were $3.7x10^7$/Kg (1.8-7.1) and $2.7x10^7$/Kg (1-6.4) for single UCBT and $5x10^7$/Kg (2.6-9.7) and $3.9x10^7$/Kg (0.9-7.8) for double UCBT. Units were HLA matched to the recipient at 5-6 loci in 28% of pts and at ≤4 loci in 72%.
Results: Median follow-up was 30 months (1-98). OS and PFS at 3 yrs were, respectively, 55±7% and 47±7%. The cumulative incidence (CI) of neutrophil and platelet engraftment was 79±5% and 62±6% with a median time for engraftment of 21(5-67) and 43 days (6-189), respectively. CI of aGVHD at 100 days was 43%±6 for grade II-IV and 21±5% for grade III-IV with a median time of onset of 23 days (9-95). Three yrs CI of cGVHD was 33±6% with a median time of onset of 130 days (58-393). CI of relapse and TRM at 3 yrs were, respectively, 13±5% and 40±7%. In univariate analysis use of CyFluTBI regimen was associated with better OS (65% vs 15%, *P*<0.001), PFS (55% vs 15%, *P*<0.001), NE (86% vs 45%, *P*=0.03) and TRM (35% vs 70%, *P*=0.03). Age at UCBT≤57yrs was associated with higher OS (71% vs 41%, *P*=0.02) and lower TRM (26% vs 52%, *P*=0.03). Less than three chemotherapy regimens prior to UCBT was associated with better OS (85% vs 48%, *P*=0.01), PFS (68% vs 38%, *P*=0.04) and lower TRM (15% vs 48%, *P*=0.02). Patients in CR prior UCBT had lower incidence of aGVHD grades II-IV (24% vs 49%, *P*=0.02). No risk factor was associated with cGVHD or relapse. Multivariate analysis was not performed due to low number of patients. Thirty pts died, 25 of transplant related causes (5 infections, 5 PTLD, 3 aGVHD, 2 cardiac and 1CNS toxicity, 1 rejection, and 8 other causes) and 5 of relapse.
Discussion: UCBT is an alternative treatment option for pts with high risk or advanced CLL. Lower relapse incidence suggests that UCBT might be associated with a beneficial GVL effect. The use of CyFluTBI as a conditioning regimen in this setting seems to be associated with good outcomes.
Disclosure of Interest: None Declared.

PH-P088

ALLOGENEIC STEM CELL TRANSPLANTATION FOR PRIMARY MYELOFIBROSIS

A. Zavrelova[1,], L. Miriam[1], J. Radocha[1], P. Belohlavkova[1], P. Zak[1]*
[1]IV. Department of internal medicine, University hospital in Hradec Králové, Hradec Králové, Czech Republic

Introduction: Purpose: To evaluate allogeneic stem cell transplantation results of patients with myelofibrosis at our transplant center.
Materials (or patients) and Methods: Methods: Between years 2002 and 2012 we performed 10 allogeneic stem cell transplantation for myelofibrosis at our center. Median age at transplantation was 57 years (49-62year). Median time from diagnosis to transplantation was 24 months (6-248 months). We used mainly reduced intensity conditioning with targeted busulfan dose (Bu 8mg/kg) except 1 patient (fully ablative regimen). 8 patients had unrelated donor and 2 patients had matched related donor. All patients with unrelated donor were given T cell depletion with thymoglobulin. According to dynamic international prognostic scoring system patients score was low intermideate 1, intermediate 2 and high risk in 2, 4, 2 and 2 patients, respectively. One patient had unfavorable karyotype and other patients had normal karyotype or unclassified changes.
Results: Results: Overall survival in patients was 90% in median observation time of 18 month (1-138 month). Only 1 patient died early after stem cell transplantation from peracute EBV posttransplantation lymfoproliferative disease. 67% experienced aGVHD with quick response to corticosteroid therapy and 67% patients developed chGVHD, which was not debilitating them. We did not register any relapse of disease.
Discussion: Conclusion: Allogeneic stem cell transplantation for myelofibrosis is feasible procedure with extremely good results and very low relapse rate. Due to these results it is possible to do this procedure early in the disease course to decrease transplant related complications and mortality. It is warranted to use T cell depletion with extremely low relapse rate in this kind of patients.
Disclosure of Interest: None Declared.

PH-P089

STEM CELL TRANSPLANTATION FOR CML IN CHILDREN AND ADOLESCENTS FOLLOWING REDUCED INTENSITY CONDITIONING – AN ALTERNATIVE TO LIFE-LONG TKIS?

S. Matthes-Martin[1,2,], P. Sedlacek[3], M. Faraci[4], R. Meisel[5], K. Kalwak[6], J. Stein[7], A. Balduzzi[8]*

[1]St Anna Childrens Hospital, [2]Medical University, Vienna, Austria, [3]Charles University Motol, Prague, Czech Republic, [4]Hospedale Gaslini, Genova, Italy, [5]Childrens University Hospital, Düsseldorf, Germany, [6]Medical University, Wroclaw, Poland, [7]Schneider Childrens Hospital, Petach Tikhva, Israel, [8]Ospedale San Gerardo, Monza, Italy

Introduction: CML is a rare disease in children with an incidence 3-5% of all paediatric leukaemias. Similar to adults, stem cell transplantation is the only curative treatment approach for children with CML. Since the introduction of Tyrosine Kinase Inhibitors (TKI) haematopoietic stem cell transplantation (HSCT) is no longer the first choice of treatment for patients with early phase CML. Imatinib has been approved for use in children and adolescents, and different national CML treatment protocols have emerged.
However life-long medication is necessary in most cases, and about 30% discontinue TKI due to acute side effects, growth impairment or become non-compliant when adolescent. (Millot *et al.* 2011). Progression free survival after 5 years without HSCT is 65% (Champagne *et al.* 2011) For children with CML HSCT still remains an important treatment option.
Materials (or patients) and Methods: The IBFM- protocol (EUDRA_CT 2008-000569-50) for HSCT in paediatric patients with CML consists of a highly immunosuppressive reduced intensity conditioning regimen with the aim to reduce the transplant related mortality and to preserve fertility.The conditioning regimen consists of fludarabin 4 x 40 mg/m^2, thiotepa 2x 5 mg/kg, melphalan 1 x 140 mg/m^2 and thymoglobuline 3 x 2,5 mg/kg. So far 14 patients in CP1 have been included after a median time of TKI treatment of 14 months.
Results: Indications for HSCT were primary insufficient response (*n*=5), secondary response loss (*n*=3), additional rearrangement (*n*=1) and patient choice (*n*=5). Disease status prior to HSCT was bcr/abl< 0.0001 (*n*=2), < 0.001 (*n*=1), < 0.01 (*n*=7) and > 0.1 (*n*=4), one patient had 9% bcr/abl. Donors were in 50% matched siblings and matched unrelated respectively. Grafts were bone marrow in 12 and peripheral blood stem cells in 2 cases. There was no TRM, 2/14 patients experienced a-GvHD > Gr II and 1/14 patients developed extensive c-GvHD, which resolved completely. After a median follow-up of 21 months all 13/14 patients are bcr-abl negative. One patient has given birth to two healthy babies after HSCT.
Discussion: Reduced intensity conditioning for children with CML with a low disease load seems to be feasible and may preserve fertility. If these findings can be confirmed in a larger patient cohort HSCT following a reduced intensity conditioning may constitute an alternative to the life-long continuation with TKI treatment in children and adolescents.
Disclosure of Interest: None Declared.

PH-P090

RUXOLITINIB TREATMENT DECELERATE CYTOKINE PRODUCTION LEADING TO REDUCED T-CELL PROLIFERATION AND KILLING FUNCTION

T. Stübig[1,], H. Alchalby[1], C. Wolschke[1], F. Ayuketang Ayuk[1], M. Heinzelmann[1], I. Triviai[1], A. Badbaran[1], Y. Hildebrandt[1], N. Kröger[1]*

[1]Universitiy Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: In up to 50% of myelofibrosis (MF) cases the JAK2-V617F mutation can be detected, making the JAK protein an interesting target for treatment. Ruxolitinib (Ruxo) a pan-JAK inhibitor was approved for the treatment of MF and showed efficacy in disease treatment, irrespectively of the JAK2V617 mutation status. Beside allogeneic stem cell transplantation (allo SCT) remains the only curative treatment option for MF by using an allo- immune vs MF effect, to improve transplant outcome Ruxolitinib may be used in the context of allo SCT. However the impact of Ruxolitinib on the immune system, especially on T-cells, is poorly understood. Therefor we investigated the effects of Ruxolitinib on T-cells *in vivo* and *in vitro.*
Materials (or patients) and Methods: Healthy donor T-cells were isolated by magnetic cell sorting to pan CD3+, CD4+ and CD8+ fraction. All three different cell subsets were cultured with different dosages of Ruxolitinib (100, 250, 500, 750nM and 1µM) for additional 48h. Thereafter cells were analysed for cell growth, cell death, mRNA expression and killing capacity against myeloid cancer cells.
Additionally, immune profiles of 9 patients were analysed for the changes of the T-cell compartment as well as changes in mRNA expression during the treatment with Ruxolitinib over a period of 3 weeks.
Results: T- cells with Ruxolitinib showed a delay in cell growths compared to control cells, however when analysing the CD4 and CD8 subset, mainly the effector CD8 cells showed a delay after 48h of culturing (2.8 x10^6 cells/ml vs. 1.9 x10^6 cells/ml, *P* =0.03). Surprisingly the rate of AnnexinV positive cells did not differ in both subsets (37.8% vs. 34%). Analysing cell cycle inhibitors we found that CD8 cells express 1.5 times more p21, p27 and p53 when treated with Ruxo compared to their CD4 counter partners, arguing that CD8 cells were more likely to undergo growth arrest.
We next investigated the function of T-cells against myeloid cancer cells. We found co-culturing of Ruxo treated CD3 cells with myeloid target cells resulted in a significant decrease in their killing capacity (50.4% vs 37.2% *P* =0.001). To rule out an effect of Treg suppression of T effector cells we repeated the experiment with purified CD8 cells again Ruxo treated cells showed a significant decrease in their killing capacity (61% vs. 49,1%, *P*= 0.01). To explain the impairment in T-cell function we found that pro-inflammatory cytokines like IL12A and IL23 to be significant reduced (10.4 vs. 7.3 fold expression, *P*< 0.05 and 6.9 vs. 3.7 fold expression, *P*=0.003 respectively).
To confirm the *in vitro* data we analysed nine patients treated with Ruxolitinib. Strikingly to our previous finding we observed a decrease in total CD3 cells after three weeks of treatment (1560/µl vs. 401/µl, *P* =0.01) and CD8 cells (630/µl vs. 203/µl, *P*=0.01). Analysing the mRNA level of cell cycle inhibitors we again found that p53 and p21 were significant up regulated (*P*=0.005 and *P*= 0.03 respectively). Arguing that the growth inhibition is mainly mediated by p53 / cip cascade. Further we could confirm that pro-inflammatory cytokines like IL7 and IL12A were significant down regulated (*P*= 0.03) however IL23 reduction failed a significant level.
Discussion: We conclude that treatment with Ruxolitinib influences T-cell function by inhibition of cytokine expression leading to growth arrest and reduction of T-cell mediated killing.
Disclosure of Interest: None Declared.

PH-P091

EFFICACY OF RESCUE IMMUNOTHERAPY AND SURVIVAL IN PATIENTS WITH HIGH-RISK CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WHO HAD CLINICAL RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

M. Hahn[1,], S. Dietrich[1], I. Herth[1], U. Hegenbart[1], E. Walter[1], M. Rieger[1], P. Stadtherr[1], A. Bondong[1], A. Dick Ho[1], P. Dreger[1]*

[1]Internal Medicine V, University Hospital Heidelberg, Heidelberg, Germany

Introduction: Allogeneic hematopoietic stem cell transplantation is a potentially curative therapy option for patients with high-risk CLL, defined by EBMT criteria (17p-/TP53mut, fludarabine resistant, early relapse, and Richter's syndrome). Structured information about the course and outcome of patients who relapse after HSCT is limited.
Materials (or patients) and Methods: In a single centre retrospective analysis, efficacy of immunotherapeutic rescue strategies and outcome were assessed in those patients who underwent HSCT

for high-risk CLL between June 2005 and April 2013 and had subsequent clinical disease progression or recurrence.
Results: Altogether 77 patients with a median age of 53 (37-68) years were allografted in the 8-year period. Of these, 18 experienced clinical relapse or progression after a median time interval of 10 (3-60) months after HSCT, translating in to 4-year relapse incidence and progression-free survival of 27% and 59%, respectively. The primary rescue strategy was donor lymphocyte infusions (DLI) + rituximab (R), which was administered to 11/18 patients. (DLI could not be given to 7/18 patients because of preceding graft failure (3), early relapse of transformed CLL (3), or donor pregnancy (1)). Of the 11 patients who received DLI, 4 had been given DLI already prior to clinical relapse preemptively upon MRD persistence. The other 7 patients could not be treated pre-emptively because CLL recurrence occurred before withdrawal of systemic immunosuppression. Although all 10 patients who received DLI+R and have informative follow-up showed at least some temporary stabilization, a sustained MRD-negative complete remission (CR) was observed in only two patients (20%). Chronic GVHD subsequent to DLI developed in 3/10 patients (including one of the two long-term responders). As secondary salvage regimen, revlimid + R was given to 5 of the 8 DLI failures, resulting to CLL regression in 2 of them including one durable MRD-negative CR. With a median observation time of 20 (1-50) months, 10/18 patients are alive according to a survival probability of 51% 2 years after relapse.
Discussion: Although individual patients relapsing after HSCT for poor-risk CLL can be put into durable MRD-negative remission with DLI+R, the overall efficacy of this strategy seems to be limited. The addition of revlimid deserves further study, but the exploration of novel agents in this setting is eagerly awaited. With a median survival of 2 years, however, the prognosis of patients relapsing after HSCT does not to be worse than that of patients with high-risk CLL without prior transplant, suggesting that HSCT also in case of subsequent relapse improves the outcome by "resetting the clock" of the disease.
Disclosure of Interest: None Declared.

PH-P092

HIGH EVI1 EXPRESSION PREDICTS SHORT DISEASE-FREE SURVIVAL AFTER HEMATOPOIETIC CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKEMIA

A. Gorbunova[1,], E. Morozova[1], Y. Vlasova[1], M. Barabanshikova[1], I. Barkhatov[1], L. Zubarovskaya[1], N. Mamaev[1], B. Afanasyev[1]*
[1]First Pavlov State Medical University of St. Petersburg, St. Petersburg, Russian Federation

Introduction: High expression of *EVI1* is a negative prognostic indicator of survival in acute myeloid leukemia (AML). More recently, negative effect of *EVI1* overexpression in patients with chronic myeloid leukemia (CML) who received second-generation tyrosine kinase inhibitors (TKIs) after imatinib failure was detected. We report a retrospective study in which the impact of pre-transplant level of *EVI1* on survival after allo-HSCT for CML was evaluated.
Materials (or patients) and Methods: Overall there were 27 patients with CML in chronic phase (*n*=18), accelerated phase (*n*=6) and blast crisis (*n*=3) who received a related or unrelated HSCT after developing resistance to TKI. *EVI1* expression was evaluated on peripheral blood before HSCT by the ratio of *EVI1* transcripts to *ABL* transcripts on a real-time qPCR analysis. Patients were divided into two groups according to their *EVI1* levels, below (*EVI1*-low) or above (*EVI1*-high) the median value.
Results: Before HSCT the median *EVI1* expression value was 0.38 *EVI1*/100*ABL* (range, 0.00-38.42). Although there was a trend for increased *EVI1* expression in advanced phase, blast crisis and second or third chronic phase patients in comparison with those in first chronic phase, this did not reach statistical significance (median, 0.86 [range, 0.00 - 38.42] vs 0.15 [range, 0.03 - 5.20], *P*=0.068). There was no association of *EVI1* expression level with *BCR-ABL* expression level or *BCR-ABL* kinase domain mutations.
After HSCT there was no statistically significant difference between the *EVI1*-high and *EVI1*-low groups in complete molecular response rate, however, patients in *EVI1*-high group had significantly increased incidence of cytogenetic relapse compared with patients in *EVI1*-low group (64% vs. 15%, *P*=0.018).
Gratwohl score, disease stage, age, time from CML diagnosis to HSCT, conditioning regimen, GVHD prophylaxis, donor type and *EVI1* expression level were evaluated as predictors of disease-free survival (DFS) and overall survival (OS). Only high Gratwohl score (*P*=0.029), advanced disease (*P*=0.037) and high *EVI1* level (*P*=0.044) were associated with a shorter DFS in a univariate analysis.
High *EVI1* level and advanced disease stage were confirmed to be independent predictors of DFS by multivariable Cox regression analysis. The hazard ratio (HR) for the *EVI1*-high group as compared with the *EVI1*-low group for DFS was 3.93 (95% CI: 1.17, 13.2) (*P* = 0.026). Gratwohl score was not included in the model because only 4 patients were in the group with low Gratwohl score (1 or 2).
Only advanced disease stage was found to be significant for OS in a univariate analysis, and multivariate analysis for OS was not performed. High *EVI1* expression level had no influence on OS in this patient cohort.
Discussion: High *EVI1* expression is a well-known marker of chemotherapy resistance in AML patients, associated with poor prognosis in about 10% AML cases. Our data suggest that high *EVI1* level in CML patients reflected changes in gene expression pattern during disease progression and affected relapse rate and disease-free survival, but not overall survival and transplant-related mortality.
Disclosure of Interest: None Declared.

PH-P093

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR MYELOFIBROSIS: THE ROLE OF THE SPLEEN

M. Ditschkowski[1], N. Kristin Steckel[1,], A. Emaagacli[2], R. Trenschel[1], T. Gromke[1], D. Wilhelm Beelen[1]*
[1]Clinic for Bone Marrow Transplantation, University Hospital of Essen, Essen, [2]Haematology, Asklepios Klinik Hamburg Altona, Hamburg, Germany

Introduction: Primary and secondary myelofibrosis are chronic myeloproliferative stem cell disorders curable exclusively by allogeneic HSCT. The significance of splenomegaly and the question whether or not to conduct splenectomy (SE) prior to HSCT still is controversely discussed.
Materials (or patients) and Methods: 95 patients (pts) (51 male, 44 female; median age at HSCT 51 years) with primary (*n*=60), post-PV (*n*=15) or post-ET (*n*=20) myelofibrosis underwent allo-HSCT after myeloablative conditioning containing total body irradiation (TBI) (*n*=45), chemotherapy regimen (*n*=28) or reduced intensity conditioning (*n*=22). Donors were HLA-identical (*n*=30) or mismatched (*n*=3) siblings and matched (*n*=44) or mismatched unrelated (*n*=18). Transplants consisted of unmanipulated peripheral blood stem cells (*n*=87), bone marrow (*n*=6) or purified CD34+ cells (*n*=2). GvHD-prophylaxis was performed with CSA + MTX (*n*=51), 31 pts received anti-thymocyte-globulin (ATG) or alemtuzumab (*n*=13).
Results: DIPSS plus scores were allocated to low (*n*=8), int-1 (*n*=19), int-2 (*n*=40) and high (*n*=20) risk groups. 24 pts had splenectomy (SE) prior to SCT, 13 allograft recipients were splenectomised subsequently after SCT (median 40 days post SCT) due to thrombocytopenia. Of the 13 pts with post-transplant SE, 7 were transplanted for primary, 4 for post-ET and 2 for post-PV myelofibrosis. 55 pts (58%) presented with splenomegaly prior to SCT. Median follow-up was 70 months among surviving pts (*n*=47), 1-year TRM was 27% and 5-year (5-y) cumulative relapse incidence was 12%. 5-y overall survival (OS) was calculated 49% and 5-y relapse-free survival (RFS) 50%. Primary graft failure occurred in 4 cases: 2 pts with pre-SCT SE and 2 pts with splenomegaly at SCT. 5-y RFS was significantly superior in not-splenectomised stem cell recipients (56% vs. 40%, *P*=0,042). One year after SCT 83% of the not-splenectomised pts were alive but only 67% of pre-SCT splenectomised and 47% of post-SCT splenectomised pts. However, recipients with post-transplant SE had the highest proportion of DIPSS plus high

risk pts. Post-transplant SE resulted in improved platelet counts in all cases and was not associated with perioperative mortality or morbidity. Cumulative 5-y relapse incidence was significantly (*P*=0,039) lower in pts with splenomegaly at time of SCT (5,4%; 95% CI: 1,8-16,4%) compared to recipients with regular spleen size and splenectomised pts (20%; 95% CI: 11-37%). Distribution of risk groups according to DIPSS plus stratification was comparable in pts with and w/o splenomegaly prior to SCT.
Discussion: Our data point out that splenomegaly at time of SCT may not be considered as a risk factor for increased relapse rates but is on the contrary associated with reduced relapse risk and superior RFS.
Disclosure of Interest: None Declared

PH-P094

TO CONTRAST THE EFFECT OF IMATINIB AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF CHRONIC MYELOID LEUKEMIA

H. Qiu[1], M. Zhou[1], X. Sha[1], G. He[1], Y. Xu[1], J. Cen[1], J. Pan[1], S. Chen[1], A. Sun[1], R. Zhang[1], D. Wu[1,]*
[1]The First Affiliated Hospital of Soochow University, Suzhou, China

Introduction: To compare the curative effect of imatinib and allogeneic hematopoietic stem cell transplantation in the treatment of chronic myeloid leukemia.
Materials (or patients) and Methods: 292 patients of chronic myeloid leukemia received imatinib and 141 patients took allogeneic hematopoietic stem cell transplantation. The clinical data of these patients were retrospectively analyzed so as to compare with the event free survival (EFS) and overall survival (OS) between these two groups with patients in the chronic phase and in advanced phase (including in the accelerate and blast phase).
Results: (1) The EFS, OS, expected 5-year EFS and expected 5-year OS of the group of imatinib(278 patients in the chronic phase) were all higher than the group of allogeneic hematopoietic stem cell transplantation (120 patients in the chronic phase) (88.5% against 70.0%, 93.2% against 80.0%, 84% against 75.0% and 92% against 79.0%). The differences were statistically significant (P values were < 0.05). (2) The EFS of the group of imatinib(14 patients in the accelerate and blast phase) was 42.9%, and the OS was 42.9%. The EFS of the group of allogeneic hematopoietic stem cell transplantation (21 patients in the accelerate and blast phase) was 47.6%, and the OS was 57.1%. To compare the EFS and OS, there were no significant differences between the two groups (P values were >0.05).
Discussion: The EFS and OS of the group of imatinib were significantly higher than that of the group of allogeneic hematopoietic stem cell transplantation for the patients of CML in the chronic phase. Imatinib and allogeneic hematopoietic stem cell transplantation have the similar efficacy for the patients of CML in the accelerate and blast phase.
Disclosure of Interest: None Declared.

PH-P095

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-HSCT) FOR CHRONIC MYELOID LEUKEMIA (CML): THE TUNISIAN EXPERIENCE

L. Torjemane[1,], N. Abdejelil[1], A. Lakhal[1], W. Barhoumi[1], R. Elfatmi[1], S. Ladeb[1], S. Menif[2], T. Ben Othman[1]*
[1]Hematology, Centre National de Greffe de Moelle Osseuse de Tunis, Bab Saadoun, [2]Laboratoire de Biologie Moléculaire, Institut Pasteur de Tunis, Tunis, Tunisia

Introduction: In Tunisia, allo-HSCT for CML has been replaced by Imatinib, as front line therapy, since 2003. So, indication for transplantion was limited for patients with accelerated phase (AP), greater than chronic phase (CP>1), blast crisis (BC) and treatment failure (TF). Here, we compare our results before and during the Imatinib era.
Materials (or patients) and Methods: Between June 1998 and March 2013, 34 patients were transplanted for CML.
Before 2003, 19 patients (group1) with sibling donor and different disease phase (6 CP1, 11AP or CP2, 2BC), received allogeneic bone marrow transplantation. The median age was 24 years (range; 8-34). The median time from diagnosis to transplant was 6 months (range; 2- 19).The EBMT score was mainly low of 0-2 (*n*=15, 78%). The median number of CMN was 2.5x 10^8/kg (range; 1.15- 3.87). Myeloablative conditioning regimens (Bu-Cy, TBI-Cy or Bu-Cy-VP16) was used.
Since 2003, 15 patients (group 2) were transplanted after a first-line therapy by tyrosine kinase inhibitors [Imatinib (*n*=11) and/or 2nd TKI (*n*=4)]. Indications for transplantation were AP ± TF (*n*=7), BC (*n*=7) and CP1. The median time from diagnosis to transplant was 21 months (range; 5- 30). The EBMT score was mainly high of 3-4 (*n*=11, 73%). Only 5 patients achieved major (MMR) or complete molecular response (CMR) with TKIs before transplant. Conditioning regimen are Bu(iv)-Cy and TBI-VP16. Peripheral blood stem cells was the main source of SC (*n*=1) with a median number CD34+ cells of 4x10^6/kg (range; 1.8-7.62). GVHD prophylaxis associated cyclosporine A and short course of methotrexate.
Results: Group1: Mortality was mainly due to transplant related toxicity (*n*=9, TRM=47%) or hematologic relapse (2 patients in BC, 10.5%). Three patients with cytogenetic relapse (30%) received escalating doses of donor lymphocyte infusions (DLI) and could restore durable complete molecular remission (CMR). After a median follow-up of 42 months (range; 1- 178), 8 patients are alive with CMR. The overall survival rate is of 43% at 3 years.
Group2: TRM was significantly lower (*n*=3; 20%). One patient died from relapse with BC (6%). Two patients with molecular relapse (16%) received Imatinib and could restore CMR. After a median follow-up of 38 months (range; 3-87 months), 11 patients (73%) are alive with CMR. Only 1 patient has an extensive chronic GVHD. The overall survival rate is of 74% at 3 years and significantly higher than of the group1 (*P*=.001).
Discussion: Transplantation for CML patients is associated with high mortality. First-line therapy by TKIs, even in the advanced phases and high EBMT score, significantly improved results. Pre-emptive use of TKI after transplantation could restore CMR without risk of cGVHD.
Disclosure of Interest: None Declared.

PH-P096

ALLOGENEIC TRANSPLANTS FOR MYELOFIBROSIS : THE TRANSPLANT SPECIFIC SCORE (TS) PREDICTS OUTCOME, ALSO AFTER STRATIFYING FOR DISEASE SPECIFIC SCORE (DIPSS)

F. Galaverna[1,], A. Raiola[1], F. Gualandi[1], A. Ghiso[1], A. Bacigalupo[1]*
[1]U.O. EMATOLOGIA TRAPIANTO di MIDOLLO, IRCCS Azienda Ospedaliera Universitaria San Martino – IST, Genoa, Italy

Introduction: A recently published disease specific prognostic score (DIPSS) predicts survival of patient with myelofibrosis (MF) (*Blood 2012, 119:2675*). We have previously reported a transplant specific score (TS) based on 3 variables: spleen size, transfusions pre-transplant and donor type (*BMT 2010 Mar;45:458*), which predicts transplant outcome in MF patients.
Aim of the study: The aim of the present study is to assess whether our TS can predict the outcome of patients with MF, after stratification for DIPSS. In other words the question is whether the 2 scores are independent predictors of survival after an allogeneic transplant.
Materials (or patients) and Methods: Patients. We have studied 96 MF patients grafted between 1992 and 2013. The median age was 57 years (23-69); median interval from diagnosis to transplant 866 days (66-3626); median circulating CD34 + cells 100/cmm (0-5280). The conditioning regimen was myeloablative (MA) in 36 patients (37%) and reduced intensity (RIC) in 60 (62%). The donor was an identical sibling (*n*=52), family mismatched member (*n*=18) or an unrelated donor (*n*=26). The DIPSS score was as follows: low (*n*=1), int1 (*n*=19), int2 (*n*=39), high (*n*=36). The Transplant score (TS) was as follows : low (*n*=47), high (*n*=49). Median follow up for all patients is 535 days (8-6630).

Results: Results. Engraftment was achieved in 90 patients (94%); six patients failed to engraft and died with pancytopenia, one despite a second transplant. Acute GvHD II-IV was recorded in 32% of the patients ; moderate/severe chronic GvHD in 19%. The overall actuarial 10 year survival is 33%; the transplant related mortality (TRM) is 22%, the relapse related death is 33%.
In univariate analysis, negative predictors of survival were DIPSS (P<0.0001), TS (P<0.001), spleen size over 22 cm (P=0.004), transfusions >20 (P<0.0001), alternative donor (P=0.04). Age of patient, intensity of the conditioning regimen, interval diagnosis transplant were not predictive.
In multivariate analysis DIPSS (RR 3.0 for high score (P=0.0004) and TS (RR 2.5, for high scores, P=0.005) both maintained their predictive value.
The 10 year actuarial survival for patients with DIPSS low, int1,int2, was 73% for low TS (n=36) and 23% for high TS (n=23) (P=0.0001). For patients with DIPSS high, survival was 22% vs 0% for low TS vs high TS scores (P=0.3).
Discussion: Conclusion. We have shown that transplant score (TS) can improve prediction of survival in MF patients undergoing an allogeneic transplant, in addition to the disease specific scores (DIPSS). This may be relevant for patient councelling and to design clinical trials, aimed to improve the outcome of high TS patients.
Disclosure of Interest: None Declared.

PH-P097
REDUCING SPLEEN SIZE BY JAK INHIBITION PRIOR TO ALLOGENEIC STEM CELL TRANSPLANTATION FOR MYELOFIBROSIS

N. Kröger[1,], H. Alchalby[2], M. Ditschkowski[3], D. Wolf[4], G. Wulf[5], T. Zabelina[1], C. Wolschke[1], T. Stübig[1], F. Ayuk[6] and CMWP*
[1]University Hospital, [2]University Hospital Hamburg, Hamburg, [3]University Hospital, Essen, [4]University Hospital, Bonn, [5]University Hospital, Göttingen, [6]University Hopsital, Hamburg, Germany

Introduction: We investigate early outcome after allogeneic SCT in 22 patients – male (n = 13) and female (n = 9) – with myelofibrosis who received ruxolitinib prior to transplantation in order to reduce spleen size and constitutional symptoms.
Materials (or patients) and Methods: The median age of the patients was 59 years (r: 42 – 74 y) and ruxolitinib was given at doses between 2 x 5 mg (n = 5), 2 x 15 mg (n = 5), and 2 x 20 mg (n = 12) before first (n = 19) or second (n = 3) fludarabine-based reduced intensity conditioning from related (n = 2), and matched (n = 14), or mismatched (n = 6) unrelated donor. Thirteen patients had primary myelofibrosis and 9 post ET/PV myelofibrosis. Before ruxolitinib the patients were classified according to DIPSS as intermediate-1 (n = 3), intermediate-2 (n = 14), or high risk (n = 5). Stem cell source was PBSC (n = 21) or bone marrow (n = 1) with a median CD34+ cell count of 7.1 x 10^6/kg. Before ruxolitinib 21 patients (96%) had constitutional symptoms and all patients had splenomegaly. The median time from start of ruxolitinib to allogeneic SCT was 133 days (r: 27 – 324) and the median treatment duration was 97 days (r: 20 – 316). Most patients (n = 82%) received ruxolitinib until start of conditioning therapy. Four patients (18%) discontinued ruxolitinib between 28 and 167 days before transplantation due to progressive disease or no response (n = 3) or cytopenia (n = 1).
Results: At time of transplantation 86% had improvement of constitutional symptoms and 41% had major response (>50% palpable) of spleen size, 14% had response of spleen size which was less than 50%, and 45% had no response/ lost response or progressive spleen size after ruxolitinib treatment. After discontinuation of ruxolitinib at first day of conditioning regimen no "rebound" phenomenon was seen. One patient transformed to sAML before transplantation despite response of spleen size and constitutional symptoms.
After busulfan (n = 16), treosulfan (n = 3), or melphalan (n = 3) dose reduced conditioning no graft failure was observed and the median time for leukocyte and platelet engraftment was 15 days (r: 10 – 66) and 17 days (r: 8 – 122) respectively. Acute GvHD I-IV was seen in 38% of the patients which was severe (III/IV) in 27%.
During follow-up 4 patients died, 1 patient with sAML at time of transplant due to relapse on day 102 and 3 patients due to therapy-related mortality. One female patient who received a second unrelated HLA-matched transplantation after treosulfan-based regimen died of CMV pneumonitis on day 75. She did not response to ruxolitinib regarding spleen size and constitutional symptoms. A second patient with iron overload and liver fibrosis died of liver toxicity on day 47. This patient initially responded to ruxolitinib but progressed regarding spleen size prior to transplantation. One patient who responded to ruxolitinib regarding constitutional symptoms and spleen size (< 50%) died of GvHD on day 77. The estimated 1-year OS and PFS was 81% (95% CI:72-90%) and 76% (95% CI:67-85%) respectively .
Discussion: Ruxolitinib reduces spleen size and constitutional symptoms in the majority of patients before allogeneic stem cell transplantation. Ruxolitinib did not negatively impact engraftment after transplantation. More patients and a longer follow-up will be presented at the meeting.
Disclosure of Interest: None Declared.

PH-P098
BCR-ABL1 GENE TRANSLOCATION MONITORING IN LONG-TERM SURVIVING CHRONIC MYELOID LEUKEMIA PATIENTS AFTER MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION

A. Ferretti[1], D. Diverio[1], F. Natalino[1], E. Scalzulli[1], A. Di Lascio[1], G. F. Torelli[1], W. Barberi[1], S. Perrone[1], M. Nanni[1], M. Breccia[1], R. Foà[1], A. P. Iori[1,]*
[1]Azienda Policlinico Umberto I, Sapienza University, Rome, Italy

Introduction: Studies on the long-term outcome of patients with chronic myeloid leukemia (CML) who have undergone an allogeneic hematopoietic stem cell transplantation (HSCT) have shown very rare hematological relapses (HR) after 10 years. However, long-term molecular assessment of the disease has never been closely analyzed. In our experience, late HR was observed in 4% of patients at a median of 13 years after HSCT (range 11-15)[1]. We were not able to detect an earlier molecular disease recurrence because no long-term monitoring of the BCR-ABL1 fusion gene was performed. To evaluate whether patients with a long follow-up (more than 10 years) after HSCT, apparently free of leukemia, may have residual leukemic cells we carried out a molecular monitoring.
Materials (or patients) and Methods: Since January 2010 a quantitative real-time polymerase chain reaction (RQ-PCR) analysis was performed, to evaluate the presence of the BCR-ABL1 fusion gene, in all 65 CML patients surviving in continuous complete hematological remission (CCR) at least 10 years after HSCT at our Center. All molecular data are expressed using the International System. Overall, the analysis was performed in 46 patients; 7 refused to participate to the study, 7 were not traceable and 5 were followed at other hospitals.
Results: Of the 46 patients analyzed, at a median follow-up of 220 months from transplant (range133-363), RQ-PCR resulted positive in 9 (19.5%). A patient with a BCR-ABL1 ratio of 62^{IS}, at 23 years after HSCT, presented a previously undetected hematological relapse[1]. A patient with a BCR-ABL1 ratio of 33^{IS} showed a concomitant cytogenetic relapse. The other 7 patients, with a median number of 0.2^{IS} (range 0.006^{IS}-0.7^{IS}), with a normal karyotype and normal blood cell counts, were monitored by RQ-PCR every 3 months: the disease evolved into a HR in 1 patient 5 months later, persisted at the molecular level in 3 and became undetectable in 3.
Discussion: Our study shows that BCR-ABL1$^+$ cells identifiable by RQ-PCR may be detected in CML patients long after a HSCT. However, the prognostic significance of residual BCR-ABL$^+$ cells at low molecular levels and the definition of "low molecular level" after HSCT remains poorly defined; we in fact observed the disappearance of the rearrangement in some patients, as well as the persistence or the evolution into an overt HR in others. The uncertain clinical significance of these molecular findings raises ethical and clinical concerns with regard to disclosing adequate information

to the patients and in terms of therapeutic decisions. Prospective long-term molecular assessments are required to monitor late relapse patterns and to answer the above-mentioned questions.
[1]Iori AP, Breccia M, Girmenia C *et al*. The limit for chronic myeloid leukemia relapse after allogeneic hematopoietic stem cell transplant moves ever forward: when can you safely talk about healing? Leuk Lymphoma. 2013;54:669-70.
Disclosure of Interest: None Declared.

PH-P099

SIGNIFICANCE OF ACHIEVING SECOND CHRONIC PHASE PRIOR TO ALLOGENEIC STEM CELL TRANSPLANT FOLLOWING TYROSINE KINASE INHIBITOR THERAPY: A RETROSPECTIVE STUDY FROM THE CHRONIC MALIGNANCY WORKING PARTY OF THE EUROPEAN GROUP FOR BLOOD AND MARROW TRANSPLANTATION

D. Milojkovic[1,], R. Szydlo[1], J. Hoek[2], D. Beelen[3], R. Hamladji[4], S. Kyrcz-Krzemien[5], A. Bacigalupo[6], D. Niederwieser[7], J. Tischer[8], R. Schwerdtfeger[9], L. Volin[10], G. Gurman[11], H. Greinix[12], Y. Chalandon[13], N. Kroeger[14], E. Olavarria[15]on behalf of CMWP*

[1]Department of Haematology, Imperial College, The Hammersmith Hospital, London, United Kingdom, [2]Department of Med. Statistics & Bioinformatics, Leiden University Medical Centre, Leiden, Netherlands, [3]Department of Bone Marrow Transplantation, University Hospital, Essen, Germany, [4]Service Hématologie Greffe de Moëlle, Centre Pierre et Marie Curie, Alger, Algeria, [5]University Department of Haematology and BMT, Medical University of Silesia, Katowice, Poland, [6]Department of Haematology, Ospedale San Martino, Genova, Italy, [7]Division of Hematology, Oncology and Hemostasiology, University Hospital Leipzig, Leipzig, [8]Med. Klinik III, Klinikum Grosshadern, Munich, [9]KMT Zentrum, Deutsche Klinik für Diagnostik, Wiesbaden, Germany, [10]Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland, [11]Department of Haematology, Ankara University Faculty of Medicine, Ankara, Turkey, [12]Klinik für Innere Medizin I, Medizinische Universität Wien, Vienna, Austria, [13]Hematology Division, Hôpitaux Universitaires de Genève, Geneva, Switzerland, [14]Bone Marrow Transplantation Centre, University Hospital Eppendorf, Hamburg, Germany, [15]Servicio de Hematologia, Hospital de Navarra, Pamplona, Spain

Introduction: In the current management of CML, SCT is reserved for patients who have failed tyrosine kinase inhibitors (TKIs) and advanced phase (AP) disease. Accelerated phase patients are a heterogeneous group in whom SCT is not mandatory as many patients have durable responses to TKI therapy alone. However, since survival clearly correlates with disease phase, TKIs and/or chemotherapy are employed to induce a second chronic phase (CP2) in AP patients in order to maximise the success of allo-SCT. Here we analyse the impact of return to CP on the outcome of CML patients transplanted in the era of TKI therapy.
Materials (or patients) and Methods: We have analyzed 5732 CML patients who underwent allo-SCT reported to the EBMT registry between 2000 and 2011. Of these, 1247 had been treated with a TKI prior to SCT. Imatinib alone had been received by 777, in 418 imatinib had been followed by a second-generation TKI (2G-TKI) and 52 had received a 2G-TKI alone pre SCT. The TKI study population had the following characteristics: male *n*=772 (61.9%), female *n*=475 (38.1%), median age 43.9 years (range 18 -75). At transplant 635 (50.9%) were in first chronic phase (CP1), 314 were > CP1 (25.2%), 168 (13.5%) were in accelerated phase and 130 (10.4%) were in blast crisis. Post TKI, all patients (*n*=1247) received allo-SCT of which 475 patients (38%) were from HLA fully matched siblings.
Results: The best outcome for allo-SCT remains in patients transplanted in CP1, irrespective of TKI therapy, and long-term OS can also be achieved in patients with AP at time of SCT. Despite TKI therapy, a trend towards a difference in 5-yr OS was only documented in patients with CP2 (*P*=0.06, Table). However, in comparing all patients in CP>1 (CP2 and CP3) there was a benefit in 5-year OS for patients who had received TKI pre-SCT (49.5% vs. 41.4%, *P*=0.024).
Discussion: Since the introduction of TKI therapy, there has been little change in the outcome of patients according to disease phase, with a trend towards a difference in improved survival only noted in CP2 patients who have received TKI pre-SCT. Therapy with TKI to CP>1 is associated with a significantly improved outcome post SCT, but disappointingly, advanced phase patients continue to have a poor prognosis.
Disclosure of Interest: None Declared.

PH-P100

RAPID RESOLUTION OF BONE MARROW FIBROSIS ON DAY +100 PREDICTS OUTCOME AFTER ALLOGENEIC SCT IN MYELOFIBROSIS

N. Kröger[1,], H. Alchalby[1], T. Zabelina[1], H. M. Kvasnicka[2], J. Thiele[3], G. Büsche[4], H. Kreipe[4] and CMWP*

[1]University Hospital, Hamburg, [2]University Hospital, Frankfurt, [3]University Hospital, Cologne, [4]Medical School, Hannover, Germany

Introduction: Bone marrow fibrosis is a hallmark of primary or post ET/PV myelofibrosis. Recent studies have shown that allogeneic stem cell transplantation induces rapid bone marrow fibrosis regression suggesting that fibrosis is more a dynamic rather than a static process. So far, no data are reported if fibrosis regression influences outcome after transplantation.
Materials (or patients) and Methods: We studied 94 patients with myelofibrosis who received allogeneic stem cell transplantation at the University Medical Center Hamburg between 2002 and 2010. In 57 patients the complete bone marrow histology prior transplantation and/or on day+30 and +100 after transplantation were available. The median age was 57 years (r: 33–73) and patients were classified as IPSS low-risk (*n* = 1), intermediate-1 (*n* = 5), intermediate-2 (*n* = 18), and high-risk (*n* = 33). Donor source was unrelated (*n* = 46) or related (*n* = 11) and of these 38 were HLA-matched while 19 were HLA-mismatched. 41 had primary and 16 post ET or post PV myelofibrosis. The median number of blasts was 1% (r: 0–17%) and gender was male (*n* = 32) and female (*n* = 25). All patients received busulfan-based dose-reduced conditioning

[PH-P099]

Non-TKI pre SCT		TKI pre SCT		
Phase	5-YR OS (%)	Phase	5-YR OS (%)	p-value
CP1 (n=2,971)	70.9	CP1 (n=635)	70.3	0.67
CP2 (n=566)	42.4	CP2 (n=285)	50.4	0.06
AP (n=522)	49.7	AP (n=168)	53.1	0.56
CP3 (n=64)	32.3	CP3 (n=29)	33	0.11
BP (n=362)	26.5	BP (n=130)	25	0.18
Non-TKI pre SCT		TKI pre SCT		
Phase	5-YR OS (%)	Phase	5-YR OS (%)	
CP1 (n=2,971)	70.9 p< 0.0001	CP1 (n=635)	70.3	p< 0.0001
CP>1 (n=630)	41.4	CP>1 (n=314)	49.5	

regimen. Bone marrow fibrosis was graded according to the European consensus and WHO (MF-0 – 3), respectively, and evaluated by experienced hematopathologists.
Results: Before transplantation 41 patients (72%) had MF grade 3 and 16 (28%) MF grade 2. After engraftment on day+30 (± 10 days) (n = 48), 3 (6%) had complete regression (MF-0) and 7 (15%) near complete regression of bone marrow fibrosis while 17 (35%) had MF-2 and 21 (44%) had MF-3. On day+100 (± 20 days) complete regression (MF-0) was noted in 11 (25%) and near complete regression (MF-1) in 13 (29%) while 12 (27%) and 8 (18%) had still MF-2 or MF-3, respectively. Patients with complete and near complete regression (MF-0 and MF-1) on day +30 had a 5-year estimated overall survival of 100% and those with MF-2 and MF-3 of 75% (P = 0.09). Patients with complete or near complete regression on day+100 had a 5-year estimated survival of 95% in contrast to 60% for those with MF-2 or MF-3 (P = 0.04).
If analyzed by regression by grade at day+100 7 (16%) had a reduction of 3 grades (e.g. MF-3 to MF-0), 12 (27%) of 2 grades, 16 (36%) of 1 grade, and 9 (21%) had no grade reduction at day+100.
Reduction of 2 or 3 grades at day+100 resulted in 95% survival at 5 years while reduction of 1 or no grade had a survival of 70% however this difference did not reach statistical significance (P = 0.1).
There was no difference of fibrosis regression at day+100 between high risk IPSS and low/intermediate risk patients. Furthermore, in those patients with JAK2V617F mutation and complete or near complete regression 42% still had detectable JAK2V617F mutation level in peripheral blood. In contrast, 81% had complete donor cell chimerism if bone marrow fibrosis was MF-0/MF-1 while only 31% had complete chimerism at day+100 if fibrosis was classified as MF-2 or MF-3.
Discussion: This data on fibrosis regression after allogeneic stem cell transplantation suggests that a more rapid regression - independently of IPSS risk score - resulted in a favorable survival and may be used as an early predictive factor for excellent survival.
Disclosure of Interest: None Declared.

Experimental Stem Cell Transplantation and Tumour Stem Cells

PH-P101
HAPLOIDENTIAL STEM CELL TRANSPLANTATION IS AN ATTRACTIVE ALTERNATIVE TREATMENT IN PATIENTS WITH BPDCN

F.-R. Wang[1,], L.-P. Xu[1], D.-H. Liu[1], Y. Wang[1], Y.-Q. Sun[1], X.-J. Huang[1]*
[1]Peking University Institute of Hematology, Beijing, China

Introduction: Blastic plasmacytoid dendritic cell (BPDC) neoplasm is a rare and clinically aggressive tumor with poor prognosis. Though majority of the patients initially respond to combined chemotherapy, relapses with subsequent drug resistance occur in virtually all patients, resulting in a median overall survival of only 1 year. There's no standardized therapeutic approach for BPDCN, but durable responses have only been reported in patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT). Although a HLA-identical sibling donor is preferred, such a donor is unavailable for many patients, especially in China. Haploidentical donor has emerged as a successful alternative source of stem cells. Here we first reported four BPDCN patients allografted as consolidative treatment with an haploidentical donor.
Materials (or patients) and Methods: All four patients were diagnosed according to 2008 WHO classification, without a HLA-matched donor. They all received modified Bu/Cy/ATG as conditioning, including of cytorabine 4g/m^2/day × 2 days (on day -10 and -9); busulfan 3.2 mg/kg/day intravenously × 3 days (on day -8 to -6); cyclophosphamide 1.8 g/m^2/day × 2 days (on day -5 and -4) and semustine 250 mg/m^2 on day -3, along with rabbit antithymocyte globulin (ATG; thymoglobulin; Sanofi), 2.5 mg/kg/d × 4 days (on day -5 to -2 days). The grafts were G-CSF mobilized bone marrow combined with peripheral blood stem cells. All patients received cyclosporine A, mycophenolate mofetil, and short-term methotrexate as prophylaxis for graft-versus-host disease (GVHD).
Results: The median time of neutrophil recovery (>0.5×10^9/L) and platelet recovery (>20×10^9/L) were 12 and 14.5 days. All patients achieved full donor engraftment and attained complete remission at day 30. Acute graft versus host disease (GvHD) was happened in 3 of 4 patients, 2 in grade II and 1 in grade III. Chronic GvHD was observed in 3 of 4 patients, including 2 extensive GvHD. Three of 4 patients had CMV antigenemia at day 27,17 and 28, respectively, but there was no CMV disease diagnosed. No treatment related mortality was happened. All patients were still in ongoing CR with disease-free survivals of 570, 430, 217 and 215 days post transplantation respectively(details in table).
Discussion: The present study was, to the best of our knowledge, the first report to successfully use haploidentical donor in BPDCN patients and modified Bu/Cy/ATG was an effective and safe conditioning. Haploidentical HSCT was an attractive alternative treatment in BPDCN cases and may lead to long-term remission.
Disclosure of Interest: None Declared.

Table. Patients characteristics and treatment outcomes

	Age/ gender	Initial menifestation	Disease status at SCT	Donor (Age/ Relationship)	HLA	Graft CD34+ (×10^6/kg)	Engraftment ANC /BPC (d)	GvHD acute/chronic	Follow-up (Days)/ outcome
Pt1	M/14	Skin, LN, BM (67%)	CR1	42/ mother	3/6	3.3	14/20	No/ extensive	570 /CR
Pt2	F/20	Skin, LN, BM (59%)	CR1	49 /father	3/6	2.5	10/16	over all III (skin, GI tract, liver) /extensie	430/CR,
Pt3	M/38	Skin, LN	CR2	41/ brother	5/6	1.6	11/11	over all II (skin, GI tract)/limited	217/CR
Pt4	F/7	Skin, LN, BM (23%)	CR1	35/ father	3/6	3.3	13/13	over all II (skin, GI tract)/ No	215/CR

PH-P102

IMPROVED RELAPSE-FREE SURVIVAL AFTER T-CELL REPLETE HAPLOIDENTICAL BONE MARROW (BM) AND PERIPHERAL BLOOD STEM CELLS (PBSC) TRANSPLANTATION FOLLOWING MYELOABLATIVE CONDITIONING (MAC) AND POST-TRANSPLANTATION CY (PT-CY) IN HIGH-RISK HEMATOLOGIC MALIGNANCIES

M. D. Simone[1,], A. Severino[1], F. Spirito[1], A. Proia[1], N. Natale[2], A. Shardlow[2], B. Montante[1], M. Garzia[1], A. Locasciulli[2], I. Majolino[1]*
[1]Hematology and Stem Cell Trasplantion, [2]Pediatrics and Pediatric Hematology, AO San Camillo-Forlanini, Rome, Italy

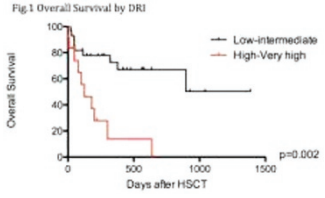
Introduction: The recent alternative approach to haploidentical hematopoietic stem cell transplantation (hHSCT) developed by the Baltimore group, with T-cell replete BM grafts in combination with PT-CY and the results of the Atlanta group (Bashey *et al*, J Clin Oncol 2013) with haplo-mismatched PBSC grafts, have provided an opportunity for nearly all patients to benefit from HSCT. The Genoa group has shown, that a MAC with PT-CY (on day +3 and +5), cyclosporine A (CSA) from day 0 and mycophenolate mofetil (MMF) from day+1, is highly effective on engraftment and remission, with acceptable non relapse mortality (NRM) and GVHD in heavily pretreated hematologic malignancies (HMs).
Materials (or patients) and Methods: We treated 17 high-risk HMs with the same TBF MAC protocol: Thiotepa (10 mg/kg), Busulfan (9,6 mg/kg) and Fludarabine (150 mg/mq) with a GVHD prophylaxis consisting in PT-CY (50mg/kg) iv on day+3 and +4, CSA 2.5 mg/kg/day and MMF 45 mg/kg/day from day+4, in order to respect CY-induced tolerance. In 14 patients stem cells source was unmanipulated haplo-BM cells, while in 3 patients were GCSF-mobilized PBSC. From 2008, 14 adults (M/F=7/7; median age 37.5 years, range 23 to 62) and 3 children (M/F=2/1; median age 7 years, range 4 to 7) with high risk ALL (2 pediatric; 2 adults), AML (1 pediatric; 9 adults), one Richter evolution of CLL, 2 Lymphoma, underwent T-cell replete haplo-BM transplantation. At time of transplant 13 patients were in CR (CR1=9, CR2=2, CR>2=2), 1 in a good PR and 3 had refractory disease. BM grafts contained a median of $6.19x10^8$/kg nucleated BM cells (range 0.7-62.1), $2.44x10^6$/kg CD34+ cells (range 0.55-7.09) and $0,33x10^8$/kg CD3+ cells (range 0.16-0.93). PBSC grafts contained a median of $18x10^8$/kg nucleated cells (range 14.8-20.8), 4.88 x 10^6/kg CD34+ cells (range 0.98-5.71) and $3.39x10^8$/kg CD3+ cells (range 2.37-4.15).
Results: Sustained donor engraftment occurred in 100% of patients for neutrophils, and in 94% for platelets, with full donor T cell and myeloid chimerism by day +30. Only one patient was not evaluable for engraftment. For patients receiving BM grafts, the median time to neutrophil recovery (> $0.5x10^9$/L) was 22 days (range, 13-40) and to platelet recovery (> $20.0x10^9$/L) of 29.5 days (range, 17-49). Patients receiving PBSC had a median time to neutrophil recovery (> 0.5 x 10^9/L) of 17 days (range, 17-19) and to platelet recovery (> 20.0 x 10^9/L) of 30.5 days (range, 28-33). Non-relapse mortality was 17,6%: 2 pt died for sepsis (one before engraftment), 1 for aGVHD. 4 patients had grade I and 6 grade II aGVHD, respectively. Only one patient had a grade III, and another had grade IV fatal GVHD. 4 patients had only limited chronic GVHD. Up to now, only 3 patients have relapsed and of these 2 have died. 12 patients (70.6%) are currently surviving, 11 of them are disease-free with median follow-up time of 199 days (range 40-1021 days) from transplantation.
Discussion: Unmanipulated hHSCT with BM and PBSC with our current protocol is a feasible approach in pediatric and adult patients which promised high engraftment rates, low NRM risks, and low rate of GVHD associated with a durable remission in a high proportion of patients.
Disclosure of Interest: None Declared.

PH-P103

CLOFARABINE AND TREOSULFAN IN CONDITIONING FOR ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANT FROM MATCHED DONORS: A MULTICENTRIC PHASE II STUDY

S. Mastaglio[1,], F. Giglio[1], F. Patriarca[2], S. Deola[3], F. Fagioli[4], B. Forno[1], M. Tassara[1], M. Morelli[1], A. Forcina[1], F. Lorentino[1], A. Assanelli[1], M. Marcatti[1], S. Marktel[1], M. Bernardi[1], C. Corti[1], M. Zecca[5], S. Cortelazzo[3], R. Fanin[2], F. Locatelli[6], F. Ciceri[1], J. Peccatori[1]*
[1]Hematology and Bone Marrow Transplant Unit, San Raffaele Scientific Institute, Milan, [2]Azienda Ospedaliera-Universitaria, Udine, [3]Azienda Sanitaria dell'Alto Adige, Bolzano, [4]Ospedale Infantile Regina Margherita, Torino, [5]Policlinico San Matteo, Pavia, [6]Ospedale Bambin Gesù, Roma, Italy

Introduction: Clofarabine is a new generation deoxyadenosine nucleoside analog with documented both anti-leukemic and immune suppressive properties. Combination of clofarabine and treosulfan has been tested in a multicentric, open-label, non randomized phase II study ("Clotreo", EudraCT 2008-006972-31), aimed at evaluating its tolerability and efficacy in patients with haematological malignancies.
Materials (or patients) and Methods: From November 2009 to November 2013, we enrolled 45 patients (median age 47 years), 37 affected by acute myeloid leukemia, 5 by acute lymphoblastic leukemia and 3 by myelodisplastic syndrome. 28 patients were "low-intermediate", while 17 were "high-very high" risk according to the Disease Risk Index (DRI) (Armand *et al*, Blood 2012). Conditioning regimen was based on: Clofarabine 40 mg/m^2 from day -6 to -2; Treosulfan 14 g/m^2 from day -6 to -4. Allogeneic peripheral blood- or marrow-derived haematopoietic stem cells from a sibling (*n*=23) or a well matched unrelated donor (*n*=21) were infused at day 0. Graft versus Host Disease (GvHD) prophylaxis was performed with Thymoglobuline at the dose of 1.5 or 2.5 mg/kg according to HLA match, Rituximab, Cyclosporine and short course of Methotrexate.
Results: Median follow-up among surviving patients is currently of 217 days (28-1388). Overall the regimen was well tolerated, the most frequent adverse events being body weight gain, skin toxicity and transient renal impairment. 100-day transplant related mortality rate was 20% and did not significantly increase in the long-term follow-up. Engraftment was fast with a median time to neutrophil and platelet recovery of 14 and 15 days respectively (10-27 and 11-156). All evaluable patients reached a full donor chimerism by day 30. The 1 year overall survival (OS) is 50%, with a significant difference when stratifying between patients with "low/intermediate" vs "high/very high" DRI (median OS: 29 vs 5 months respectively, *P*=0.002) as shown in figure 1. 24% of patients experienced acute GvHD, while only 13% developed chronic GvHD. Overall relapse incidence was 44%.
Discussion: Treosulfan and Clorafabine combination is a feasible conditioning and allows a prompt engraftment with rapid achievement of full donor chimerism. Preliminary results show encouraging outcomes especially in patients scored with a "low/intermediate" DRI. This combination is thus worth further clinical investigation in allogeneic HSCT setting focusing on patients with a disease sensitive to Clofarabine anti-tumor activity.
Disclosure of Interest: None Declared.



PH-P104

CORD BLOOD T LYMPHOCYTES EXPANSION FOR THERAPEUTIC USE

C. Pelé[1], V. Mialou[1,], C.-L. Duriez[1], A. Clerc[1], S. Mazet[1], D. Rigal[2], O. Hequet[1]*

[1]Banque de tissus et cellules, [2]Etablissement Français du Sang, LYON, France

Introduction: Allogeneic cord blood (CB) hematopoietic stem cell transplantation (HSCT) is common alternative for HSCT. Donor lymphocytes infusions (DLI) which are a major therapeutic tool to reduce opportunistic infections and increase graft-versus-leukemia (GvL) effect are not available after CB transplant. We present here a means to obtain lymphocytes from CB and use it as therapeutic weapon against malignant disease or viral infection. We develop an expansion protocol, under GMP conditions, from 10% as a fraction of a CB graft likely to expand *Ex vivo* sufficient amounts of T cells for DLI.

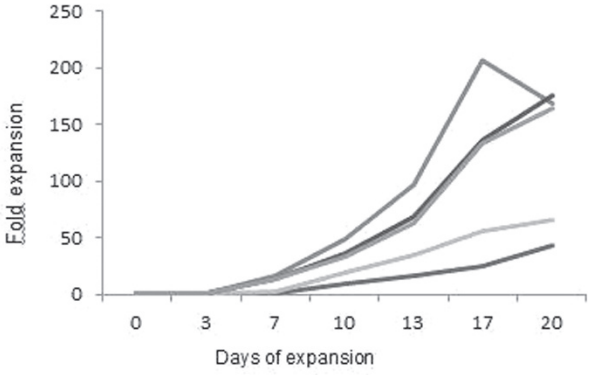
Materials (or patients) and Methods: 10% of the volume from the thawed CB unit was kept for selection and *Ex vivo* expansion T-cells were positively selected by using anti-CD3 mAbs conjugated to magnetic beads and CliniMACS System© (Miltenyi Biotec®) under GMP conditions. After selection CD3+ T-cells were expanded with CD2/CD3/CD28 beads (Miltenyi Biotec®, GMP) and cultured in TexMACS Medium supplemented with IL-2 and IL-7 during 18 to 20 days. Viability, phenotype and function of T cells were assessed by flow cytometry on Becton Dickinson FACS Canto II. After 20 days cells were harvested, counted, washed and beads were removed by automatic cell separation with a kit GMP. The viable cells obtained were numerated in the depleted fraction obtained. Expanded T cells were cryopreserved according to escalating dose scheme at $5x10^5$, $1x10^6$ and $5x10^6$ cells/kg. All steps were performed in closed systems and GMP conditions.

Results: After thawing CB under therapeutic conditions, we selected T-cells with column. Mean purity of the fraction obtained was 98% and median of 10.10^6 CD3+ cells were obtained (range 3-22, *n*=25). We expanded CB-derived T cells to clinically relevant numbers from 5 CB units, with median of 165-fold expansion (range 1-176). Immunological profile of expanded cells showed only CD3+ cells (98%) with 91% of TCRαβ+. No expansion of B cells or NK cells was observed. CD8+ T cells expanded preferentially compared to CD4+ T cells. Expanded T cells were phenotypically and functionally immature with higher percentage of CD45RA+ but exhibited activated phenotype with increased expression of CD25 and CD69. Amount of T-cell obtained after expansion was sufficient for cryopreservation according to conventional dose escalating lymphocytes protocol in all cases. Thawing of expanded cells showed rates of viable CD3+ cells compatible with a therapeutic use.

Discussion: These data suggest that obtaining T-lymphocytes clinically relevant doses for therapeutic use from a small sample of thawed CB is feasible respecting the GMP conditions in all *Ex vivo* steps. T-lymphocytes expansion is obtained with a combination of IL-2, IL-7 and magnetic beads coupled to CD2/CD3/CD28 mAbs that significantly enhance proliferation and activation of thawed CB T cells. The function of thawed expanded T cells must be assessed and compared to the function of thawed T cells obtained from peripheral blood by cytapheresis and used in routine for DLI. These results will show whether thawed expanded cells can be used as a therapeutic tool after HSCT (prevention/treatment of leukemia relapse, viral priming to treat viral infection) or whether they need to received additional *Ex vivo* treatments.

Disclosure of Interest: None Declared.

Fig 1: The kinetics of the cord blood T-cell expansions is shown. Each line represents a single cord blood unit



PH-P105

MINOR-HISTOCOMPATIBILITY-ANTIGEN UTY AS TARGET FOR GRAFT-VERSUS-LEUKEMIA AND GRAFT-VERSUS-HAEMATOPOIESIS IN THE CANINE-MODEL

D. Bund[1,2,], F. Goekmen[1,2], J. Zorn[3], R. Buhmann[1,2], H.-J. Kolb[1,2], H. Schmetzer[1]*

[1]Department of Hematopoetic Cell Transplantation, MEDIII, University of Munich-Grosshadern, Munich, [2]Helmholtz Center Munich, German Research Center for Environmental Health, Haematopoietic Stem Cell Transplantation, [3]Helmholtz Center Munich, German Research Center for Environmental Health, Research Unit Comparative Medicine, Munich, Germany

Introduction: In haploidentical-SCT (stem-cell-transplantation) male-patients with female-donors have better prognosis compared to female-to-male-combinations due to Y-encoded minor-histocompatibility-antigens recognized by female-allo-immune effector-lymphocytes in the context of a graft-versus-leukemia-(GvL)-effect. We provide data in a dog-model that the minor-histocompatibility-antigen UTY might be a promising target to further improve GvL-immune-reactions after allogeneic-SCT.

Materials (or patients) and Methods: Canine (c) purebred-beagle-dogs' PB and BM were studied. T2-cells (HLA-A2+, TAP-deficient) were used. These human-(h)-UTY-sequence-derived HLA-A2-binding-peptides were investigated: W248 (WMHHNMDLV), T368 (TLAARIKFL), K1234 (KLFEMIKYC). *In vitro*: Autologous-cDCs were generated with best of three DC-methods (*Calcium-Ionophore*, *Picibanil*, *Cytokines*). Generation cUTY-specific-CTLs: CD3+T-cells were co-cultured with autologous-mature cDCs+hUTY-peptides (weekly restimulation for 21 days; +hIL-2, +hIL-7). Cytotoxicity and antigen-specificity were determined by [^{51}Cr]-release- and cIFN-g-ELISPOT-assays. Cells were quantified day 0 and of harvest using anti-cmAbs/hmAbs (FACS), UTY-mRNA-expression via RT-PCR-analysis. *In vivo*: A female-dog was immunized with PBMCs from a DLA-identical-male-dog (day 0 and 14). PB-derived T-cells were harvested 35 days post 2nd-injection followed by analysing UTY-specific-reactivity.

Results: Female cUTY-specific-CTLs were stimulated *in vitro* using autologous-DCs loaded with three HLA-A2-restricted UTY-derived-peptides (≤2.9-fold-expansion) and specific T-cell-responses were determined in 3/6 female-dogs. CTLs specifically recognized/lysed autologous-female peptide-loaded-DCs (900 spots/100,000 T-cells (median)/≤47.9%), but not naive autologous-female-DCs and -monocytes (p≤0.026). They mainly recognized BM and to a lower extent DCs, monocytes, PBMCs and B-cells from DLA-identical-male-littermates and peptide-loaded T2-cells in an MHC-I-restricted manner (up to p≤ 0.046). UTY-mRNA was only expressed in male-cells. A UTY-/male-specific-reactivity was also obtained *in vivo* after stimulation of a female-dog with DLA-identical-male-PBMCs.

Discussion: We demonstrated natural UTY-processing/presentation in dogs. Female-dog-CTLs were specifically stimulated by HLA-A2-restricted-UTY-peptides, thereby enabling recognition of DLA-identical-male-cells, mainly BM-cells. These observations suggest UTY as a promising candidate-antigen to improve GvL-reactions in the course of immunotherapy. Next-generation-sequencing and specialised-bioinformatics-algorithms are now focus for human-individualised-leukemia-treatment (T-cell-receptor-

Profiling, detection/selection of T-cell-receptor-clones or DC-based-immunotherapies).
Disclosure of Interest: None Declared.

PH-P106

IMMUNMODULATION OF BLASTS IN AML-PATIENTS (PTS) WITH CLINICALLY APPROVED RESPONSE MODIFIERS TO IMPROVE ANTILEUKEMIC T-CELL REACTIVITY: AN *EX VIVO* SIMULATION OF THE CLINICAL SITUATION

H. Schmetzer[1,], Z. Stankova[1], D. Deen[1], A. Hirn[1], Y. Vokac[1], T. Kroell[1], D. Bund[1], R. Buhmann[1], A. Hausmann[1], C. Schmid[2], J. Tischer[3]*
[1]Department Of Hematopoetic Transplantations, Med Dept 3, University Hospital Munich, Munich, [2]Department Of Hematopoetic Transplantations, Municipal Hospital Augsburg, Augsburg, [3]Department Of Hematopoetic Transplantations, Municipal Hospital Augsburg, Munich, Germany

Introduction: Problem: Allogenic SCT/DLI are promising T-cell based therapies to cure AML-pts. Antileukemic T-cell-reactivity has to be improved/re-established in pts *in vivo*. *Ex vivo* leukemia-derived DC (DC_{leu}) are the most effective antileukemic T-cell-stimulators.
Materials (or patients) and Methods: Aim and Methods: We generated DC_{leu} *ex vivo* from AML blasts from heparinised whole blood ('**WB-DC**', to simulate the *in vivo* situation) from 65 AML-pts in active stages of the disease using standard methods ('Picibanil', 'MCM-Mimic', 'Ca-ionophore', 'IFNa') or 11 minimalized cocktails ('**WB-minicock-DC**', combinations of 1-3 selected cytokines, antibiotics, bacterial lysates, or other clinically approved response-modifiers) and to correlate proportions of DC- or T cellsubsets and cytokine profiles with results with their *ex vivo* stimulatory capacity for antileukemic T-cells and the pts' response to immunotherapy (SCT/DLI).
Results: 1. **Generation of DC:** we could identify 4 of 11 **minicocks**, that allowed the generation of DC/DC_{leu} from blast-containing WB-samples with at least one of the methods. Some of the cocktails induced *ex vivo* blast-proliferation in individual pts. Proportions of DC-subtypes (e.g DC/DC_{leu}/mature DC) were comparable to proportions generated with standard DC methods. 2. **Antileukemic functionality:** In 21 cases T-cells stimulated with 1 to 3 '**WB-minicock-DC**' resulted in 56% cases with blastlysis; in 6 pts 2-3 cocktails could be studied in parallel and in at least one of the cocktails a blastlysis could be achieved. Blast lysis (vs non-lysis) correlated with higher proportions of DC-subtypes: (DC,DC_{leu} blastconversion to DC_{leu}), higher proportions of T-cell-subtypes (viable, CD8 Tcells), higher concentrations of IL-12 and IFNg but lower concentrations of IL-6 and IL-8 3. **Clinical correlation:** AML-pts successfully responding to immunotherapy (SCT or DLI therapy) presented with higher proportions of DC, DC_{leu} and $CCR7^+$ mature DC compared to pts without successful immunotherapy.
Discussion: DC/DC_{leu} can be generated regularly from MNC or WB and with at least 1 to 4 of 11 minicocks containing combinations of 1-3 selected, clinically approved responsemodifiers. T-cells stimulated with 'WB-minicock-DC' achieved antileukemic function, although not with every cocktail. A patient-individual testing of the best cocktail as well as the achieved antileukemic (*ex vivo*) function can contribute to define cocktails of response modifiers to be applicated to AML pts to achieve or sustain remission.
Disclosure of Interest: None Declared.

PH-P107

NEONATAL BONE MARROW TRANSPLANTATION STRATEGY FOR THE CURE OF HURLER DISEASE SKELETAL PHENOTYPE

A. Pievani[1,2,], I. Azario[1,2], B. Rambaldi[2], S. Cersosimo[3], V. Scagliotti[1,2], A. Biondi[4], S. Tomatsu[5], M. Riminucci[3], M. Serafini[1,2]*
[1]Dulbecco Telethon Institute at Centro Ricerca Tettamanti, [2]Centro Ricerca Tettamanti, Department of Paediatrics, University of Milano-Bicocca, Monza, [3]Department of Molecular Medicine, Sapienza University, Roma, [4]Department of Paediatrics, University of Milano-Bicocca, San Gerardo Hospital, Monza, Italy, [5]Department of Biomedical Research, Alfred I. duPont Institute Hospital for Children, Wilmington, DE, United States

Introduction: Hurler syndrome (MPS-IH) is a rare autosomal recessive lysosomal storage disease caused by mutations in the IDUA gene, resulting in the deficiency of alpha-L-iduronidase (IDUA) enzyme activity with a consequent intracellular accumulation of glycosaminoglycans (GAGs). Among a broad spectrum of clinical manifestations, MPS-IH is characterized by a range of skeletal abnormalities known as dysostosis multiplex. The introduction of hematopoietic stem cell transplantation (HSCT) has significantly improved the life-span of Hurler patients. However, the musculoskeletal manifestations are only partially responsive to HSCT. Since MPS-IH is a progressive storage disorder that affects the skeleton during childhood, it may be necessary to undertake transplantation in MPS-IH infants (identified by prenatal or neonatal diagnosis). Therefore, in the present study, we have firstly analyzed the skeletal phenotype of MPS-I mice and then investigated whether HSCT in newborn mice prevents the subsequent accumulation of storage material in the lysosomes and the derived bone damage.
Materials (or patients) and Methods: 1- to 2-day-old mutant mice were conditioned using a single administration of 20 mg/kg busulfan and then injected via the superficial temporal vein with 2×10^6 bone marrow cells from wild type donors. Bones of transplanted mice were examined radiographically and microscopically at 37 weeks of age. Age-matched WT and untreated mutant mice were used as controls.
Results: Untreated MPS-I mice at 37 weeks of age develop a flattened facial profile and thickening of the digits and palmar regions. Radiographs reveal thickness of the zygomatic arches and long bones. Femurs from MPS-I mice were wider at 1.36 ± 0.10-fold normal ($n = 8$; $P < 0.01$) and more sclerotic compared to WT siblings. Similar features were observed for the tibia (1.23 ± 0.10-fold normal; $n = 8$; $P < 0.01$), humerus (1.46 ± 0.08-fold normal; $n = 8$; $P < 0.01$) and radius/ulna (1.19 ± 0.05-fold normal; $n = 8$; $P < 0.01$). Furthermore, pathological evaluation of cross sections demonstrated that femurs of MPS-I mice have abnormal osteocytes and a thick cortex. Transplantation of normal murine bone marrow cells into preconditioned MPS-I neonates led to a high engraftment level at 37 weeks after HSCT (peripheral blood, 58.9 ± 40.3%; $n = 14$) and a long-term multilineage donor hematopoiesis recovery. Similar engraftment levels were obtained in transplanted WT neonates (62.1 ± 33.7% $n = 8$; $P > 0.05$), as expected. Neonatal HSCT allowed restoration of IDUA activity in peripheral organs. Indeed, IDUA activity levels detected in heart, lungs, spleen, kidneys and liver were 18%, 22%, 70%, 35% and 57%, respectively, of those detected in unaffected animals. Radiographic analysis has shown that the width abnormality of femurs was significantly normalized (1.14 ± 0.08; $n = 6$). This was confirmed also for the tibia (1.05 ± 0.13-fold normal; $n = 6$), humerus (1.08 ± 0.10-fold normal; $n = 6$) and radius/ulna (0.99 ± 0.08-fold normal; $n = 6$). Preliminary histological and microCT analyses of long bones seem to confirm the correction of the bone phenotype in transplanted MPS-I mice.
Discussion: We conclude that neonatal HSCT would be an attractive therapeutic option to prevent severe bone dysplasia in MPS-IH.
Disclosure of Interest: None Declared.

PH-P108

EXPRESSION OF SURFACE-ASSOCIATED METALLO-PROTEINASE 82 KDA-PROMMP-9 VARIANT ON LEUKEMIC BLAST CELLS FROM PATIENTS WITH AML, ALL, AND CLL

T. guenther[1], W. sutanto[1], J. schmohl[2], F. schuster[3], D. kraemer[4], K. neuhaus[4], C.-H. koehne[4], A. hausmann[5], T. pitsch[6], H. salih[2], C. ries[6], H. Schmetzer[1,]*

[1]Department Of Hematopoetic Transplantations, Med Department 3, University Hospital Munich, Munich, [2]Department For Hematology, University Hospital Tuebingen, Tuebingen, [3]Department For Pediatric Oncology, University Hospital Of Duesseldorf, Duesseldorf, [4]Department For Hematology, Municipal Hospital Oldenburg, Oldenburg, [5]Municipal Hospital Munich-schwabing, Munich, [6]Department For Cardiovascular Prevention, University Of Munich, Munich, Germany

Introduction: Background: Cellular trafficking (e.g. invasion through matrix barriers and penetrations of blood vessel walls) in healthy organisms is highly regulated and restricted. Matrix metalloproteinases (MMPs) mediate the proteolytic breakdown of these barriers. MMP-9 degrades collagen type IV and denatured collagens, and processes TGFß, IL-8 and IL-1ß into their active forms. A surface associated 82-kDa variant of MMP-9 was found to be expressed in leukemic cells (Ries *et al*, Biochem J, 2007). The role of MMP-9 variant expression on leukemic cells is still unclear.
Materials (or patients) and Methods: Aim and Methods: We generated a monoclonal antibody that specifically recognizes the aberrant MMP-9 form. By use of this antibody we analysed the expression of MMP-9 variant on blasts from patients (pts) with AML (*n*=22), ALL (*n*=20) and CLL (*n*=21) compared to healthy controls by FACS analyses and correlated our findings with prognostically relevant subgroups.
Results: 1. Results in AML: we could identify a variable coexpression of MMP-9 on blasts in different FABtypes, however a significantly higher expression on pooled 'favorable' (30% coexpression) compared to unfavourable FABsubtypes (20%, $P<0.05$) and favourable (40%) vs unfavourable NCCN risk group (8%, n<0.005). Expression was also higher in pts studied at first diagnosis (19%) vs pts at relapse/persisting disease (10%, $P<0.05$), in pts <60 years (25%) vs pts>60 years (16%, *P*=ns) and in responders to initiated induction therapy (21%) vs nonresponders (8%, *P*=ns). A cut-off value of coexpression (11%) could be evaluated that allowed a separation of pts in those with a more favourable (>11%)/unfavorable prognosis (<11%, NCCN).
2. Results in ALL: we could identify a variable coexpression of MMP-9 on blasts in different EGILsubtypes, however a lower expression on pooled 'favorable' (8% coexpression) compared to unfavourable GMALLsubtypes (24%, *P*=ns) risk types. Significantly lower coexpression was found on blasts from female (13%) vs male pts (28%, $P<0.05$) and on pts with primary (9%) vs secondary ALL (28%, $P<0.05$). Expression was also lower in pts studied at first diagnosis (21%) vs pts at relapse/persisting disease (42%, *P*=ns). A cut-off value of coexpression (9%) could be evaluated that allowed a separation of pts in those with a more favourable (<9%)/unfavorable prognosis (>9%, GMALL).
3. Results in CLL: we could identify a low coexpression of MMP9 on CLL-blasts (10-12%) independent of different subtypes and risktypes (data not shown). A predictive cut-off value could not be evaluated.
Discussion: These findings demonstrate opposing roles of MMP-9 variant in AML and ALL cells. It can be speculated, that in AML MMP-9 variant-mediated processing of regulatory factors on the surface of blast cells contributes to a favourable outcome, whereas in ALL degradation of matrix barriers by MMP-9 variant may increase the invasive capabilities of ALL blast cells and thus explain a rather poor prognosis. Our data suggest usefulness of MMP-9 variant as a prognostic marker in certain AML and ALL subgroups.
Disclosure of Interest: None Declared.

PH-P109

MULTI-GENOTYPING OF MINOR HISTOCOMPATIBILITY ANTIGENS (MHAGS) IN ALLOGENEIC STEM CELL TRANSPLANTATION AND THEIR ROLE IN DETERMINING GRAFT VERSUS HOST DISEASE (GVHD) AND GRAFT VERSUS LEUKEMIA (GVL) EFFECT

F. Cattina[1,], C. Skert[1], S. Bernardi[1], M. Malagola[1], E. Ottaviani[2], M. T. Bochicchio[2], I. Iacobucci[2], S. Soverini[2], F. Bonifazi[2], G. Bandini[2], M. Arpinati[2], M. R. Motta[2], E. Toffoletti[3], D. Damiani[3], A. Santoro[4], F. Fabbiano[4], D. Pastore[5], G. Specchia[5], G. Console[6], M. Bruno[6], G. Carella[7], A. Malagoli[7], V. Guadagnuolo[2], C. De Benedittis[2], C. Bergonzi[1], C. Fili[1], A. Turra[1], R. Ribolla[1], V. Cancelli[1], E. Alghisi[1], S. Perucca[1], A. Di Palma[1], G. Martinelli[2], D. Russo[1]*

[1]Chair of Hematology, Bone Marrow Transplant Unit, University of Brescia, AO Spedali Civili Brescia, Brescia, [2]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, [3]Division of Hematology and BMT, Udine, [4]U.O. di Ematologia e UTMO Ospedali Riuniti Villa Sofia-Cervello, Palermo, [5]Hematology, A.O.U. Policlinico di Bari, Bari, [6]Azienda Ospedaliera Bianchi Melacrino Morelli, Reggio Calabria, [7]Chair of Rheumatology and Clinical Immunology, University of Brescia, Brescia, Italy

Introduction: The outcome of allogeneic stem cell transplantation (Allo-SCT) is closely related to graft versus host disease (GvHD) and graft versus leukemia (GvL) effects which, in part, are mediated by minor Histocompatibility Antigens (mHAgs). Twenty-six mHAgs have been identified and reported to be differently and variably correlated with GVHD or GVL. These antigens are: UGT2B17 (correlated with GvHD), ACC-1, ACC-2, ACC-6, C19orf48, HB-1, LB-ADIR-1, LB-LY75-1K, LB-MR1-1R, LB-MTHFD1-1Q, LB-PTK2B-1T, LRH1 (correlated with GvL), HA-1, HA-2, HA-8, CD31 (correlated with both GvHD and GvL) and ACC-4, ACC-5, CTL7A7, DPH1, DRN7, HA-3, HEATR-1, P2RX7, LB-ECGF-1H UTA2-1 (with not well determined clinical significance). Nowadays a simultaneous method to genotype a so large panel of mHAgs has never been employed.
The aim of this work has been to develop a feasible method to genotype all the 26 mHAgs described so far and to test them for their correlation with GVHD and GVL in a group of donor/recipient pairs submitted to allo-SCT.
Materials (or patients) and Methods: For the multi-genotype of 23 mHAgs we designed 3 multiplex and samples analyzed by mass-spectrometry through Maldi-Tof IPlex Gold technology. This assay is relatively fast and it requires a small amount of DNA. For the other three mHAgs we performed other three assays: two based on capillary sequencing of PCR products (for LB-MR1-1R and LRH1) and the last one based on PCR alone (for UGT2B17). By these methods, we tested the 26 mHAgs in 70 donor/recipient pairs at least 6/6 matched at 4-digit high resolution typing, that underwent allo-SCT (sibling or MUD) because of Philadelphia positive CML (*n*=46) or ALL (*n*=24).
Results: Maldi-Tof IPlex Gold technology proved a high degree of efficiency. Out of a total of 3220 SNPs an evaluable genotype was obtained in 3176 (98.6%). Also the other assays were efficient (417/420, 99.3%). As expected, sibling pairs showed most identity of MUD pairs. Notably, donor/recipient mismatch on ACC-5, UGT2B17, DPH1 and LRH1 can drive a pathogenetic mechanism that leads to GvHD increase ($P<0.05$). Next we identified that LB-ADIR1 can improve RFS ($P<0.05$) as GvL effect. This is potentially important (*P*=ns, for the low patients number) especially for ALL-Ph+ patients because this mismatch can enhance GvL in a subgroup that is otherwise less or un-responsible to allo-immunotherapy.
Discussion: Our data generated by a multi-genotype technique confirm the role of mHAgs in addressing graft reactions; in some cases only GvL without GvHD. This suggest that a study of mHAgs (particularly ACC-5, UGT2B17, DPH1, LRH1 and LB-ADIR1) could be performed at the donor recruitment time in order to better and prospectively investigate the role of the known and new mHAgs involved in GvHD and GvL effects. Work supported by Lions Club "Bassa Bresciana" and BCC di Pompiano e Franciacorta Founds.
Disclosure of Interest: None Declared.

PH-P110

FEASIBILITY OF ALPHA BETA T- CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATIONS FROM MATCHED RELATED AND UNRELATED DONOR GRAFTS AND ENGRAFTMENT IN PATIENTS WITH POOR RISK LEUKEMIA

L. Van Der Wagen[1,2,], L. te Boome[1], K. Westinga[3], I. Slaper-Cortenbach[3], J. Kuball[1]*
[1]Hematology, [2]Immunology, [3]Department of Clinical Pharmacy/Cell Therapy Facility, UMCU, Utrecht, Netherlands

Introduction: Life-threatening GVHD is still a major complication of allogeneic haematopoietic stem cell transplantation (allo-SCT). αβT-cells are considered to be main contributors in GVHD. Depletion of αβT- cells have so far been reported in haploidentical stem cell transplantations. First results have been promising with good engraftment and low incidence of GVHD. In this study we aimed to assess the feasibility of αβT-cell depletion in the context of MUD and MRD. Main purpose is to develop transplantation protocols which result in a more profound expansion of both NK and γδ-T cells. These cellular subsets, which are at the border of the innate and adoptive immune system, are considered to contribute to the graft-versus-leukemia (GvL) effect as well as to control viral reactivations after allo-SCT. In this way, we hope to decrease GvHD whilst preserving GvL effects in allo-SCT in patients with poor risk leukemia.
Materials (or patients) and Methods: Initial proof runs for the generation of αβT-cell and CD19 depleted grafts have been performed in 4 healthy donors. In the first 5 patients (cohort I) grafts for transplantation have been depleted with GMP-grade anti-αβTCR and anti-CD19 antibodies. The subsequent grafts for patients in cohort II (*n*=4) and III (*n*=3) have been selectively depleted with GMP-grade anti-αβTCRs antibodies only. Three conditioning regimens have been investigated (I): fludarabine 120 mg/m^2 + cyclophosfamide 4800 mg/m^2, (II): fludarabine 120 mg/m^2 + busilvex AUC=90 and (III): ATG (Genzyme®) 4 mg/m^2 + fludarabine 120 mg/m^2 + busilvex AUC=90 followed by abT-cell depleted grafts from matched related or unrelated donors. No additional immune suppression was given after allo-SCT.
Results: Products for 12 patients have been successfully processed and used for αβT-cell depleted allo-SCT between 2011 and 2013. A ~4 log depletion of αβT-cells has been observed in the product with a recovery of ~75% of CD34+ cells. In cohort I, primary engraftment (chimerism > 95%) was 40%. Engrafted patients showed a rapid reconstitution of γδT-cells and αβT-cells with a broad αβT-cell repertoire as determined by spectratyping. Due to the rapid reconstitution of αβT-cells in engrafted patients, CD19 depletion was omitted in further cohorts. To further improve engraftment, the condition regimen of the next cohort was dose-intensified (cohort II). 75% of cohort II showed a swift engraftment. Again a dominance of γδT-cells was observed which associated with a rapidly reconstituting αβT-cell repertoire. In order to further increase engraftment cohort III was additionally treated with an early application of ATG (day -10/-9) and no graft failure has been observed so far. In the CD19-depleted group, two EBV reactivations occurred. No other viral re-activations have been observed.
Discussion: αβT-cell depletion is feasible in the context of MRD and MUD. An intensified conditioning with additional host T-cell depletion seems to be beneficial for a profound engraftment. abT-cell depletion associates with a swift and dominant reconstitution of γδT-cells as well as a rapidly restoring αβT-cell repertoire that displays a broad reactivity. Cohort III is currently expanded to confirm whether this regimen results in solid engraftment. Subsequently we aim to use αβT-cell depleted allo-SCT as a platform for post-transplant immune interventions.
Disclosure of Interest: None Declared.

PH-P111

EXTRACELLULAR HMGB1 PROMOTES THE MIGRATION OF CORD BLOOD CD34+ CELLS VIA SDF-1/CXCR4 AXIS

X. Zhu[1,], L. Yang[1], Z. Sun[1], X. Wang[1], X. Liu[1]*
[1]Hematology, Anhui Provincial Hospital, Hefei, China

Introduction: This study was aimed to investigate the effect of HMGB1 (high mobility group protein) and/or CXCL12(stromal cell derived factor) on the migration of cord blood CD34 + cells, and to explore whether HMGB1 promotes cord blood CD34+ cells migration via CXCL12/CXCR4 axis.
Materials (or patients) and Methods: Cord blood mononuclear cells were isolated by Ficoll-Paque density centrifugation. CD34+ cells were collected by a positive immunoselection procedure(CD34 MicroBeads) according to the manufacturer's instructions. The purity of the isolated CD34+ cells was detected by flow cytometry. In vitro chemotaxis assays were performed using Transwell cell chambers to detect cells migration. 1×10^5/well cord blood CD34+ cells were added into the upper chambers. Different concentrations of HMGB1 and/or CXCL12 (0,10,25, 50,100,200 ng/ml) were used respectively to detect the optimal concentrations of HMGB1 and/or CXCL12 for inducing migration of cord blood CD34+ cells. Freshly isolated cord blood CD34+ cells express CXCR4(CXCL12 receptor), and HMGB1 receptors TLR2,TLR4 and RAGE. To explore which receptors were required for the synergy of HGMB1 and CXCL12 on cells migration, we used anti-CXCL12, anti-CXCR4, anti-RAGE, anti-TLR2 and anti-TLR4 antibodies to detect the effect of HGMB1 alone or with CXCL12 on cord blood CD34+ cells migration.
Results: The purity of CD34+ cells isolated from cord blood mononuclear cells by magnetic cell sorting was 97.4%. 25 ng/ml CXCL12 did not induce migration of cord blood CD34+ cells, whereas optimal migration was observed at 100 ng/ml. HMGB1 alone did not induce migration up to 50 ng/ml. We determined, by dose finding experiments, that the the best synergistic concentrations for cells migration are 100 ng/ml HMGB1 combined with 50 ng/ml CXCL12. The blocking experiments showed that both the anti-CXCL12 (4μg/ml) and anti-CXCR4 (5μg/ml) antibodies could block cell migration induced by HMGB1 alone or combined with CXCL12. But cord blood CD34+ cells in the presence of anti-RAGE,anti-TLR2 and anti-TLR4 antibodies, did not modify the response to CXCL12 in the presence of HMGB1.
Discussion: Both HMGB1 and CXCL12 can induce cord blood CD34+ cells migration. HMGB1 enhances CXCL12-induced migration exclusively via CXCR4 and in a RAGE- and TLR-independent manner. The exact mechanism needs to be further explored.
Disclosure of Interest: None Declared.

PH-P112

EXPANDED CORD BLOOD T-CELLS USED AS DONOR LYMPHOCYTE INFUSIONS AFTER UMBILICAL CORD BLOOD TRANSPLANTATION

S. Berglund[1,2,], J. Gertow[1], M. Uhlin[1], J. Mattsson[1,2]*
[1]Therapeutic Immunology, Karolinska Institute, [2]Centre for Allogeneic Stem cell Transplantation, Karolinska University Hospital, Stockholm, Sweden

Introduction: Umbilical cord blood transplantation (UCBT) is increasingly used and gives comparable results to transplantation with other stem cell sources. Donor lymphocyte infusion (DLI) is an effective treatment option for relapsed malignancies after hematopoietic stem cell transplantation with other stem cell sources, but is not available to UCBT recipients. *In vitro* cultured cord blood DLI present an attractive putative option for UCBT recipients.
Materials (or patients) and Methods: In this study, we explored the use of *in vitro* cultured T-cells from the cord blood graft as an alternative to conventional DLI for UCBT recipients. T-cells from 5% of a cord blood unit used for UCBT were cultured for 7-11 days with either 600 IU IL-2/ml or 100 IU IL-2/ml and 20 ng IL-7/ml. The cultured cord blood DLI (CB DLI) was given to four patients as treatment for mixed chimerism with threatening rejection (*n* = 2),

minimal residual disease (MRD, $n = 1$), and for a patient with graft failure with severe infection. The patients had received a UCBT due to AML, ALL or PNH/MDS. The patient receiving treatment for MRD was given T-cells cultured with IL-2 and IL-7, while the remaining three were given cells cultured with IL-2. To our knowledge, *in vitro* cultured and activated UCB T-cells produced in this manner have not been used for clinical DLI treatment previously. In this study, we address the safety and feasibility of this treatment and study the outcome in the first four patients to receive the cultured CB DLI.
Results: No adverse reactions were seen at transfusion. Graft-versus-host disease (GVHD) after CB DLI could not be excluded in one patient (Patient 1), while the remaining three did not show any signs of GVHD. In the patient treated with CB DLI due to MRD (Patient 4), the malignant cell clone was undetectable for several months after infusion (see figure). In one patient with mixed chimerism, Patient 3, the percentage of recipient cells decreased in temporal association with DLI treatment (see figure). No obvious effect of the CB DLI treatment was detected in the remaining two patients.
Discussion: In conclusion, we saw no ascertainable adverse effects of treatment with CB DLI. However, we could see beneficial effects that may be directly associated to the treatment. CB DLI produced *in vitro* in this manner appear to be a safe and feasible alternative to DLI in UCBT recipients.
Disclosure of Interest: None Declared.

PH-P113
NK CELL (FROM PRIMARY NK CELL CULTURES) MEDIATED KILLING OF MULTIPLE MYELOMA CELLS *IN VITRO*
T. Martin, Christian Binder[1], Y. Hildebrandt[2], C. Giffey[2], K. Jungk[2], T. Heinrich Eiermann[1], H. Sierich[1], N. Kröger[2,]*
[1]HLA Laboratory, [2]Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: Multiple myeloma (MM) is a plasma cell malignancy and natural killer (NK) cells have demonstrated anti-MM activity *in vitro*. Anti-myeloma effect of NK cell against MM cells may be due to the interaction between killer cell immunoglobulin-like receptors (KIR) against different human leukocyte antigens (HLA), like HLA-Cw, on the target cells. The most important inhibiting KIRs KIR2DL2 and KIR2DL3 binding to HLA-Cw group C1 and KIR2DL1 binding to C2, control the NK cell mediated killing of target cells, if their corresponding C1 or C2 is absent or present on these cells. Therefore selecting donor NK cell infusions according KIR expression may be an effective cell-mediated immunotherapy for patients with MM.
Materials (or patients) and Methods: We isolated NK cells from buffy coats of respective five healthy blood donors by NK cell specific columns. In the next step we isolated KIR2DL1 respectively KIR2DL3 bearing (specific) NK cells by fluorescence activated cell sorting (FACS). These primary NK cells were cultivated in cell cultures and were used as models for human NK cells *in vitro*, in weekly experiments. To cultivate the primary NK cell lines, we tested different IL-2 containing interleukin cocktails. Our purpose was to establish a cell culture system for primary NK cell lines. As target cells we used the MM cell lines MOLP-8 (C1/C2), KMS-12-BM (C1/C1) and RPMI-8226 (C1/C2). To demonstrate the effectiveness NK cell lines against these target cells, we made chromium release assays in three independent experiments. For control experiments, we down regulated the expression of both KIR with the help of specific siRNA.
Results: To cultivate the primary NK cell lines *in vitro*, we established a medium, containing IL-2, IL-15 and IL-21 and were able to cultivate primary NK cell lines up to 4 weeks. KIR expression mediated cytotoxicity of our NK cell lines against MM cells we could demonstrate with chromium release assays. The transient KIR expression silencing was effective for four up to five days.
Discussion: Allogeneic NK cells with the potential to mediate anti-leukemic or anti-myeloma effects can be delivered in the context of an HSCT or adoptively transferred following *ex vivo* stimulation. In addition to the expansion and adoptive transfer of unmanipulated NK cells, NK cells can also be engineered to express certain receptors (like KIR2DL2/KIR2DL3 or KIR2DL1 and others) to increase their ability to recognize and eliminate MM cells. Primary NK cell lines may offer a more effective therapy for multiple myeloma in future.
Disclosure of Interest: None Declared.

Hematopoietic Stem Cells

PH-P114
WHY ARE UNRELATED HAEMATOPOIETIC STEM CELLS COLLECTIONS POSTPONED OR CANCELLED?
A. Isabel Spínola[1,], F. Bordalo[1], S. Lopes[1], F. Amado[1], S. Ferreira[1], S. Roncon[1]*
[1]Therapy Cellular Service, Instituto Português Oncologia do Porto Francisco Gentil, EPE, Porto, Portugal

Introduction: The search for an unrelated haematopoietic stem cells (HSC) donor, many times from international registries, is a juggling act between the Donor Center (DC), the Collection Center (CC) and the Transplant Center (TC). The DC, together with the CC and the TC spend time, energy and sometimes sacrifice to plan the best strategy for a successful and on-time HSC transplant. Beyond economic costs, the frustration that comes along with a cancelled or a postponed allogeneic transplant is quite wide.
Objective: To analyse the main causes to postpone and/or cancel HSC transplantation.
Materials (or patients) and Methods: Our data was obtained retrospectively from all cancelled or postponed requests that our CC received between January 2007 and October 2013 for unrelated peripheral blood HSC and bone marrow collections, from the national registry (CEDACE). A descriptive analysis was performed using Microsoft Excel 2007.
Results: In this period, we received 323 requests (for 284 patients); 14% were postponed and 40% of them were after cancelled; 11% were cancelled without previous reschedule.
The cancelled requests were concerning to 51 patients (13 females, 37 males, 1 unknown), with a median age of 41 (range 1-75) years old, with 5 patients under 18. They presented the following diagnoses: acute leukemia 51%; Hodgkin and non-Hodgkin lymphoma 26%; others 29%. The median time between request and cancellation was 31 (5-177) days.
The motive analysis showed they were patient-related in 51% of cases, donor-related in 29% and TC-related in 18%. In the first group, the main reasons were: worsening of the clinical status not directly related with the hematological malignancy in 14 patients, relapse or disease progression in 6 and death in 4; 2 patients refused to proceed with the transplant.
Donor-related reasons were mainly: 7 refused to collect HSC without any further explanations, 5 were excluded because of acute or previous illness, 1 had a positive infectious disease marker, 1 was pregnant and 1 migrated to an African country.
The major cause for cancelation by the TC was having found a more compatible HLA donor.
Discussion: The postponed or cancelled HSC requests are mainly due to patient's clinical condition. When the reasons are patient or TC-related, it would be grateful for the Collection Center to have more feedback in order to maintain our donor motivated for a subsequent donation.
Disclosure of Interest: None Declared.

PH-P115

NEGATIVE SELECTION BY APOPTOSIS ENRICHES PROGENITORS IN NAÏVE AND EXPANDED HUMAN UMBILICAL CORD BLOOD GRAFTS

I. Yaniv[1,2,], K. Mizrahi[3], J. Stein[1,2], N. Askenasy[3]*

[1]BMT Unit, Pediatric Hematology Oncology, Schneider Children's Medical Center of Israel, Petah-Tikva, [2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, [3]Frankel Laboratory, Center for Stem Cell Research, Petah-Tikva, Israel

Introduction: Hematopoietic progenitors express high levels of TNF family receptors after transplantation, however are inherently insensitive to apoptotic signaling. The TNF superfamily receptors are largely responsible for transduction of trophic signals, which improve the efficiency of engraftment and enhance reconstitution.

Materials (or patients) and Methods: In this study we assessed the use of Fas and TNF receptor signaling in preparation of umbilical cord blood (UCB) grafts for transplantation (*n*=38).

Results: $CD34^+$ progenitors and T cells display outstanding survival, whereas ~30% and >50% B lymphocytes and myeloid cells undergo spontaneous apoptosis within 24 and 48 hours respectively. Although the impact of exposure to toxic doses of FasL and TNF-a was undetectable in measurements of apoptosis, removal of dead cells after 2 days of incubation with the ligands revealed a 2-fold increase in frequency of $CD34^+$ progenitors and colony forming cells (CFU). The sensitivity of progenitors to apoptosis was also unaffected by Fas cross-linking following TNF-induced upregulation of the receptor, and CFU frequency was equally increased. Pretransplant exposure of UCB cells to either one of the ligands resulted in increased myeloid progeny in NOD.SCID xenochimeras. Most significant enrichment in $CD34^+$ progenitors and corresponding increase in CFU frequency were observed when FasL was applied during the final week of *ex vivo* expansion under the influence of nicotinamide, without impairing SCID reconstituting cell activity.

Discussion: These data emphasize differential sensitivities of UCB progenitors and lineage-positive cells to apoptotic signaling mediated by the Fas and TNF receptors, which might be useful in improving the efficiency of *ex vivo* expansion and UCB cell engraftment.

Disclosure of Interest: None Declared.

PH-P116

TRYPAN BLUE ACCURATELY DEFINES THE VIABILITY OF CFU-GM CELLS IN CORD BLOOD UNITS

S. Ulrika Frändberg[1,2,], I. Ögärd[2], E. Anghem[2], L. Palmqvist[2]*

[1]The National Swedish Cord Blood Bank, Clinical Immunology and Transfusion Medicine Sahlgrenska University Hospital Göteborg, [2]Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine University of Gothenburg, Göteborg, Sweden

Introduction: The Colony forming unit assay (CFU) is a progenitor cell cultivation assay used to approximate stem cell content and potency in donated cord blood units (CBU), we investigated the accuracy of the routinely performed viability measurement by Trypan blue staining prior to cultivation.

Materials (or patients) and Methods: CBU were processed to Cord blood buffy coat (CBB) using the Sepax instrument (Biosafe) and 0. 5 ml of CBB was sampled and frozen in a separate vial in liquid nitrogen. The material was briefly thawed and viability was measured by adding 0.1% Trypan Blue (T8154, Sigma), 100-200 cells were manually counted by microscopy and the fraction of stained cells was determined. For each CBB two different cultivation protocols were performed in duplicate; one with 50.000 mononuclear cells (cNoT) and the other with 50.000 *viable* mononuclear cells as adjusted for the fraction of viable cells measured by Trypan Blue (cWiT) per ml of medium (Methocult GF H84434, Stem Cell Technologies) 37 CBU were investigated and calculations performed using SPSS (IBM) version 21.

Results: After 14 days in an incubator the growing Granulocyte-Monocyte colonies (CFU-GM) in both protocols were counted. Since only viable CFU-GM cells can form colonies, the number of growing colonies in the cNoT protocol was expected to be lower compared to the cWiT protocol relative the fraction of viable cells in the corresponding sample given that the viability measurement by Trypan Blue was accurate. We therefore theoretically calculated the number of *expected* colonies (cCalc) in the cNoT protocol as given by; cCalc CFU-GM colonies = (fraction of viable cells by Trypan Blue * cWiT CFU-GM colonies) The cCalc results were correlated to the number of actually growing colonies in the cNoT protocol. The Pearson correlation coefficient between the cCalc colonies and the cNoT colonies was 0.93 (P<0.0001).

Discussion: A highly accurate viability measurement is necessary to obtain correct CFU-GM assay results. In this work we show that the fast, cheap and simple Trypan Blue viability stain is an accurate method to determine the viability of CFU-GM cells in CBU prior to release for transplantation and thus eliminating the need to implement more expensive and time-consuming methodology such as flow cytometry.

Disclosure of Interest: None Declared.

PH-P117

VALIDATION OF THE REVISED DISEASE RISK INDEX FOR PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) AFTER PARTIAL T-CELL DEPLETION

Y. Beauverd[1,], E. Roosnek[1], Y. Tirefort[1], M. Ansari[1], C. Dantin[1], O. Tsopra[1], S. Masouridi-Levrat[1], Y. Chalandon[1]*

[1]Blood and Marrow Transplant Program, Geneva University Hospital, Geneva, Switzerland

Introduction: Disease and status at transplant time is a major determinant for HSCT outcomes. A revised disease risk index (revDRI) was recently proposed (Armand *et al*, Abstract 548, ASH 2013). The revDRI contains 4 risk groups (low, int, high, very high) and the 3 first risk groups are subdivided in 2, yielding a 7-risk score (table 1), which is prognostic for OS. We investigated if the revDRI is also valid in our center, where 63% of patients are transplanted with T-cell depleted grafts (TDEP).

Materials (or patients) and Methods: We analyzed 416 patients (59% male, median age of 46.5 (2-70)) transplanted between Jan 1998 and Oct 2012 for the different hematological malignancies shown in Table 1 . PBSC (87%), BM (11%) or cord blood (2%) grafts were from identical siblings (49%), MUD (34%), MMUD (12%) or alternative donors (5%). 65% of patients received a myeloablative - and 35% a reduced intensity conditioning. T cell depletion (TDEP) was performed for 63% of patients with *in vitro* CAMPATH. GVHD prophylaxis consisted mostly of calcineurin inhibitor ± MTX, or MMF.

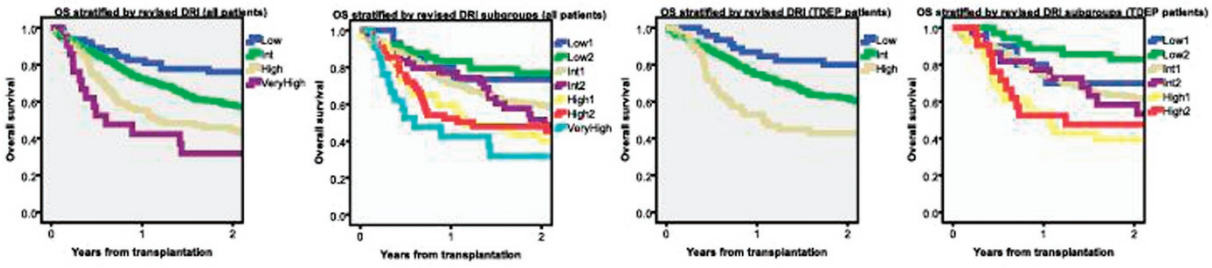
Results: 2-year OS for all patients and TDEP was 57±5% and 62±6% respectively. 2-year OS for revDRI and subgroups, for all patients and TDEP patients, are described in the table 1. Impact of revDRI was confirmed in multivariate analysis (HR for int, high and very high was 2.2 (1.3-3.8), 3.3 (1.9-6.0) and 4.7 (2.3-9.5), P<0.01). SC source, age, donor type and TDEP also impacted OS in multivariate analysis. TDEP had a impact with less acute GvHD grade 2-4 incidence (21 vs 43%, P<0.01) and with a trend to less severe chronic GvHD (11% vs 17%, P=0.22).

Discussion: Our study confirms the impact of the revDRI in term of OS in a cohort of patient mainly transplanted with TDEP graft. There is even a slight positive impact of TDEP on OS (low and int revDRI) and aGVHD. The revDRI may help better to define the patients who may benefit from TDEP.

Disclosure of Interest: None Declared.

[PH-P117]

revDRI subgroup	All pts. (%)	TDEP pts. (%)	All pts. 2yOS	TDEP pts. 2yOS	revDRI	All pts. (%)	TDEP pts. (%)	All pts. 2yOS	TDEP pts. 2yOS
Low-1: Hodgkin Lymphoma, Indolent B-NHL, MCL or CLL, any CR	3.6	3.8	73% (50-96)	70% (55-85)	Low	15.4	17.6	76% (65-87)	80% (68-92)
Low-2: Indolent B-NHL or CLL, PR; AML Fav cyto,any CR; CML, Chronic Phase	11.8	13.8	77% (65-89)	83% (70-96)					
Int-1: T-NHL, any CR; ALL, 1st CR; AML Int cyto, any CR; Myeloproliferative neoplasms, Any Stage; Low-risk MDS, Any cyto, Early Stage; Multiple myeloma, CR/VGPR/PR; Aggressive B-NHL, any CR; Hodgkin lymphoma or MCL, PR	46.2	52.9	59% (52-67)	62% (54-70)	Int	56	61.3	58% (51-65)	62% (54-69)
Int-2: Aggressive B-NHL or T-NHL, PR; Low-risk MDS Int cyto, Advanced Stage or High-risk MDS Int cyto, Early Stage; CML, Advanced Phase; Indolent B-NHL or CLL, Advanced Stage; Aggressive NHL, PR	9.9	8.4	49% (32-71)	53% (32-75)					
High-1: High-risk MDS Int cyto, Advanced Stage; AML Fav cyto, Advanced Stage; Burkitt lymphoma, CR; AML Adv cyto, CR; ALL, 2nd CR	9.9	10.7	40% (26-56)	39% (21-58)	High	23.3	30.6	45% (34-55)	41% (27-55)
High -2: High-risk MDS Adv cyto, Any Stage or Low-risk MDS Adv cyto, Advanced Stage; Hodgkin Lymphoma, MCL or T-cell NHL, Advanced Stage; ALL, ≥3rd CR; AML Int cyto, Advanced Stage; Multiple myeloma, Advanced Stage	13.5	8	45% (31-60)	42% (21-64)					
Very High : CML, Blast Phase; ALL, Advanced Stage; Aggressive NHL, Advanced Stage; AML Adv cyto, Advanced Stage; Burkitt lymphoma, PR or Advanced Stage	5.3	2.3	27% (7-46)	NA	Very High	5.3	2.3	27% (7-46)	NA



PH-P118

EXTRACORPOREAL PHOTOPHERESIS FOR PAEDIATRIC GRAFT VS HOST DISEASE FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PRIMARY IMMUNODEFICIENCY-A SINGLE CENTRE EXPERIENCE.

J. Guest[1,], H. Robson[1], M. Slatter[1,2], A. Gennery[1,2]*
[1]Great North Children's Hospital, [2]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) is curative for many primary immunodeficiencies (PID). Whilst cure and survival reach 90% in some cohorts, acute graft versus host disease (aGvHD) confers no additional advantage to the patient in the way of anti-tumour effect, and remains a significant cause of morbidity and mortality. We report on a series of 8 patients treated at our centre since December 2012, for predominantly acute or persistent acute steroid-resistant or -dependent aGvHD.

Materials (or patients) and Methods: As all patients were <30 kg, the CellexÔ was primed with packed red blood cells and normal saline for each procedure, with ACDA citrate dextrose as anticoagulant. Extracorporeal photopheresis (ECP) was commenced after failure of conventional treatment. Immunosuppression was weaned as clinically indicated.

Results: Patient characteristics and responses are detailed (Table). All tolerated the procedure with no severe adverse events. 4/8 had a complete response (CR) with symptom resolution and reduction in immunosuppression. 1 (4) had a partial response (PR)

Patient D	Weight kg	Conditioning	HLA match	GvHD	Rx	ECP cycle	Outcome
1 NEMO	16.4	C1H/T/F	8/10 A,Cmm URD PBSC	Skin IV	MP 2/kg tacrolimus MMF IFx C1H ATG	15	CR Prednisolone 5/0 MMF od tacrolimus
2 artemis SCID	6.8	C1H/T/F	10/10 URD UCB	Skin III	MP 2/kg tacrolimus MMF IFx	16	No response Re-transplanted
3 CID PTLD	8.5	T/F	10/10 URD UCB	Skin III	MP 2/kg tacrolimus MMF IFx	5.5	CR Prednisolone 5/0 MMF bd
4 T-B- SCID Adenovirus	12	T/F	9/10 Amm URD UCB	Skin II (gut)	Pred 2/kg tacrolimus MMF bd IFx	12	PR Prednisolone 7.5 MMF tacrolimus IFx 4/52
5 CD40L crypto	19	C1H/T/F	9/10 DQmm BMT URD	Liver IV	MP 2/kg tacrolimus Sirolimus MMF IFx ATG	16	CR SBR 280->70 Sirolimus Pred 5/0 IFx 4/52
6 CGD adenovirus	29.5	T/F	Sib 12/12	Gut IV	MP 2/kg CSA MMF IFx 1/52 ATG C1H	12	PR MP 0.5/kg CSA IFx 2/52
7 CID PTLD	18.4	C1H/T/F	9/10 URD BM	Skin III	MP 2/kg tacrolimus MMF IFx C1H	6.5	CR MP 0.1/kg IFx **
8 RAG Omenn	8.2	T/F	Sib 12/12	lung	Pred 3/kg MMF IFx ATG Sirolimus Imatinib	6	PR Prednisolone 1/kg MMF IFx Sirolimus Imatinib

SCID, severe combined immunodeficiency; Crypto, cryptosporidium; PTLD, post transplant lymphoproliferative disease; C1H/T/F, Campath, Treosulfan, Fludarabine; URD, unrelated donor; PBSC, peripheral blood stem cells; UCB, umbilical cord blood; Rx, treatment; MP, methylprednisolone; MMF, mycophenolate mofetil; IFx, infliximab; ATG, anti-thymocyte globulin; SBR, serum bilirubin; CSA, ciclosporin.

**Died of PTLD, GvHD controlled.

with symptom improvement and immunosuppression reduction, but ECP was temporarily discontinued because of unrelated surgical complications. The clinical response of patient 6 improved despite concomitant severe adenovirus enteritis, with histological improvement. Best responses were seen in patients with skin GvHD, but response in other organ disease was also seen.

Discussion: ECP is effective treatment for small children undergoing HSCT for PID who develop aGvHD, well tolerated with a blood-prime, with resolution of GvHD possible. Earlier intervention after failure of steroid treatment may improve results further.

Disclosure of Interest: None Declared.

PH-P119

A MEAN TO OPTIMIZE TRANSPLANT REGIMEN FOR NEUROBLASTOMA: THE ROLE OF PRE-TRANSPLANT MIBG SCINTIGRAPHY IN TARGETED ADMINISTRATION OF 131I-MIBG ACCOMPANIED BY AUTOLOGOUS STEM CELL TRANSPLANTATION

A. A. Hamidieh[1,], D. Beiki[2], P. Paragomi[1], B. Fallahi[2], M. Behfar[1], A. Fard-Esfahani[2], A. S. Hosseini[1], A. Shamshiri[1], M. Eftekhari[2], A. Ghavamzadeh[1]*

[1]Hematology, Oncology and Stem Cell Transplantation Research Center, [2]Research Center for Nuclear Medicine, Tehran University of Medical Sciences, Tehran, Iran, Republic Of Islamic

Introduction: MIBG has emerged as an effective component in transplant regimen of neuroblastoma. Along its therapeutic role, ^{131}I-MIBG scintigraphy is an imaging modality mostly applied at disease presentation and in primary assessments of tumor. However, none of previous studies has evaluated the role of pre-transplant MIBG scintigraphy in decision making for treatment of neuroblastoma. In this study, we selected therapeutic approach based on pre-transplant ^{131}I-MIBG scintigraphy.

Materials (or patients) and Methods: The current study targeted high risk neuroblastoma from April 2008 to November 2013. Thirty days before launching our therapeutic modality, MIBG scintigraphy was performed. According to MIBG scan results, MIBG avid patients were consecutively enrolled in the study. Myeloablative chemotherapy comprised of etoposide (1200 mg/m^2), carboplatin (1500 mg/m^2) and melphalan (210 mg/m^2) two weeks following administration of therapeutic MIBG (12 mCi/kg). In addition all patients received 13-cis retinoic acid after autologous stem cell transplantation (ASCT).
Results: Thirteen enrolled patients had demonstrated various responses to previous treatments including 10 patients with very good partial response (VGPR), and 3 patients with partial response (PR). Mean age at diagnosis was 42.5 months (range, 17-65) and Mean age at transplantation was 60.2±21.3 (range, 34-92) months in patients. The median time to neutrophil engraftment after ASCT was 10 days (range, 9-13 days) and median time to platelet engraftment was 13 days (range, 10-20 days). None of the cases failed to engraft after ASCT. In studied patients, 3-y-OS was 66% ± 21% while 3-y-EFS was 53% ± 20%.
Discussion: These findings may underline the efficiency of pre-ASCT MIBG scintigraphy in high-risk neuroblastoma patients. Patients with MIBG avid lesions at pre-ASCT stage may benefit from exploitation of therapeutic MIBG combined with high dose chemotherapy. Furthermore, therapeutic MIBG may not be a necessary component of pre-ASCT regimen in MIBG non-avid patients.
Disclosure of Interest: None Declared.

PH-P120
IMAGING FEATURES OF POSTERIOR REVERSIBLE ENCEPHALOPATHY SYNDROME (PRES) AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS: A PROSPECTIVE STUDY.

A. A. Hamidieh[1,], A. R. Radmard[2], S. Kooraki[2], M. Behfar[1], A. P. Hashemi Taheri[2], A. Ghavamzadeh[1]*
[1]Hematology, Oncology and Stem Cell Transplantation Research Center, [2]Department of Radiology, Tehran University of Medical Sciences, Tehran, Iran, Republic Of Islamic

Introduction: Posterior reversible encephalopathy syndrome (PRES) is a well-known complication of hematopoietic stem cell transplantation (HSCT). The impact of PRES after HSCT in pediatrics is not fully recognized. This study investigates the radiologic spectrum of PRES and its clinical associations in post-HSCT pediatric patients.
Materials (or patients) and Methods: Seventy-four children (45 Males, 29 females) with mean age of 84 (4-180) months underwent HSCT between March and November 2013 in our center. The underlying disease was inherited abnormalities of RBC in 30, leukemia in 18, bone marrow failure syndromes in 15, primary immunodeficiency syndromes in 8 and inborn errors of metabolism in 3. The most common pre-HSCT conditioning regimen included Busulfan and Cyclophosphamide and none of the patients received TBI. Cyclosporine was used in all patients for GVHD prophylaxis. Patients have been closely followed up with the mean follow-up time of 4 months. Brain CT-SCAN and subsequent MRI were performed in all children with neurologic symptoms. Follow-up MRI was performed two months later in patients with the diagnosis of PRES.
Results: Of 74 patients, currently 62 patients (83.7%) are alive. 12(16.22%) post-HSCT children (6 males, 6 females) with mean age of 111(36-180) months developed neurologic symptoms with the most common being seizure (12) and headache(8). Brain imaging showed PRES in these patients; of whom 6 had Major Thalasemia, 4 Fanconi Anemia, one AML and one Diamond-Blackfan Anemia. Hemorrhagic foci were seen in MRI of two patients with PRES. Follow-up MRI of 4 patients showed resolution in three, while persistent findings in one. Short-term Mortality rate was significantly higher among subjects with PRES (6 patients, 50%, *p* value: 0.003).
Discussion: PRES is a serious neurologic complication following HSCT in children and it is associated with increased mortality rate. Patients with certain underlying diseases and transplant conditions are more likely to develop PRES.
Disclosure of Interest: None Declared.

PH-P121
RESULTS OF HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF PEDIATRIC BRAIN TUMORS

A. Gevorgian[1,], E. Morozova[1], I. Kazantsev[1], T. Iukhta[2], S. Safonova[2], Y. Punanov[2], O. Goloschapov[1], M. Estrina[1], E. Babenko[1], S. Shiriaev[1], L. Zubarovskaya[1], O. Zheludkova[3], B. Afanasyev[1]*
[1]Raisa Gorbacheva Memorial Institute for Children Oncology, Hematology and Transplantation, [2]Federal State Institution N.N. Petrov Research Institute of Oncology, Saint-Petersburg, [3]Federal Research Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation

Introduction: Central nervous system (CNS) tumors are the second most common pediatric malignancies with an about 30% 5-year overall survival rate in high-risk group. The aim of this study was to assess the effectiveness of single or tandem high-dose chemotherapy (HDCT) with autologous hematopoietic stem-cell transplantation (auto-HSCT) in this patient group.
Materials (or patients) and Methods: From October 2006 to December 2013, 37 pediatric patients with high-risk or relapsed medulloblastoma (*N*=22), supratentorial PNET (*N*=5), germinoma (*N*=4), pineoblastoma (*N*=2) and atypical teratoid rhabdoid tumor (*N*=3), choriocarcinoma (*N*=1) received HDCT with auto-HSCT after induction chemotherapy, radiotherapy and surgical treatment. At the moment of HDCT 15 patients were in complete remission (CR), 16 patients were in partial remission (PR) and 6 patients had stable disease (SD). Patients with germinoma received single auto-HSCT, patients with medulloblastoma, pineoblastoma, ATRT or supratentorial PNET received single or tandem auto-HSCT depending on age. The conditioning regimen for single auto-HDCT consisted of cisplatin, etoposide, and ifosfamide, or carboplatin, etoposide and thiotepa +/– intraventricular etoposide, or thiotepa and temozolomide. In tandem HDCT, the first conditioning regimen was carboplatin and etoposide with intraventricular/intrathecal metotrexat, the second was thiotepa and cyclophosphamide with intraventricular/intrathecal metotrexat. Bone marrow (*N*=21), peripheral blood stem cells (*N*=11) or both (*N*=5) were used for stem cell sources. The mean transplanted CD34+ cell dose was 5.5 x 10^6/kg (range, 1.0–11.3 x 10^6/kg).
Results: The median follow-up is 20 months (range, 1–95). The median time to engraftment was 15 (range 12–30) after auto-HSCT. A half (*N*=3) of the patients with SD at the moment of auto-HSCT died of disease progression. Thirteen of 31 patients with CR or PR relapsed 1-23 months after HDCT, the other 18 patients are currently in CR. The following therapy toxicity was observed: liver toxicity grade 3-4 (*N*=16), skin toxicity grade 3-4 (*N*=9), severe mucositis grade 3-4 (*N*=22), nausea/vomiting grade 3-4 (*N*=11), infectious complications grade 3-4 (*N*=23). Four patients died of toxicity. 8-years Overall survival (OS) in all groups was 52% and 8-years disease free survival (DFS) was 49%. DFS was significantly better among high-risk patients in 1st CR compared to patients in 2nd or following CR: 65% and 36%, accordingly (*P*=0.02). Patients in CR or PR at the moment of HDCT had better DFS rate than patients in SD: 61%, 53% and 25% (*P*=0.00), respectively.
Discussion: HDCT with auto-HSCT in pediatric patients with high-risk CNS tumors may be a feasible option for patients in CR or PR after induction chemotherapy. It is ineffective as a salvage therapy in refractory patients.
Disclosure of Interest: None Declared.

PH-P122

PRIOR MD-ARAC CHEMOTHERAPY AFFECTING PERIPHERAL BLOOD HEMATOPOIETIC STEM CELL MOBILIZATION IN AML

R. Feng[1,], M. Xie[1], X. Wei[1], H. Jing[1], M. Dai[1], Y. Zhang[1], Q. Wei[1], X. Li[1], Y. Wei[1], F. Huang[1]*

[1]Hematology, Nanfang Hospital, Southern Medical University, Guangzhou, China

Introduction: Prior chemotherapy had been reported to affect the efficiency of peripheral blood stem cell (PBSC) mobilization. As one of the major chemotherapeutic drugs for AML, the impact of cytarabine on mobilization remains unknown. Herein, we retrospectively analyzed AML patients performed PBSCs apheresis to explore the relation between prior medium-dose cytarabine (MD- AraC) chemotherapy and mobilization.

Materials (or patients) and Methods: We retrospectively analyzed 90 patients with *de novo* AML, underwent mobilization in the haematology department of Nanfang hospital from August 1999 and November 2012. Median age at mobilization was 38 years (range, 12-60 years). Before mobilization, all patients had received various chemotherapeutic regimens, including induction and consolidation or intensive chemotherapies. According to the course of MD-AraC chemotherapy, patients were divided into group I, II III. With a complete response, patients received the same mobilization regimen, EA (cytarabine 1.0 g in IV, every 12 hours × 3 ~5 days; etoposide 0.1~0.2 g in IV, every 12 hours × 3~5 days) chemotherapy combined granulocyte-colony stimulating factor(G-CSF) 5~10 μg/kg/d. G-CSF starting after completion of chemotherapy 4 to 5 days when WBC down to the bottom until the end of the collection. Apheresis was scheduled to start when the WBC count recovered ≥4.0 × 10^9/L or the CD34 cells ≥ 0.01% WBC of PB. After 24~48 h following high-dose conditioning regimens (mostly busulfan/cyclophosphamide), grafts were infused. The efficacy of PBSC mobilization, hematopoietic reconstitution, survival rates were assessed for each AraC group.

Results: The median doses of CD34 cell in those three AraC groups were 4.7×10^6/Kg, 2.8×10^6/Kg, 2.2×10^6/Kg, respectively (P=0.006). In addition, patients collected ≥2.0×10^6/kg numbers of CD34 cells in groups I need the lowest leukapheresis, total blood volume processed, G-CSF total dose and days (P<0.05). A significantly greater proportion of good mobilization (≥2.0×10^6 CD34 cells/kg with at most 3 leukapheresis procedures) in the group I (39/46, 84.8%) compared with the group II (13/22, 59.1%) and group III (10/19, 52.6%) (χ^2=8.918, P=0.012). The sex, age, cytogenetic risk, the pior chemotherapy courses, the prior courses of the various chemotherapeutic agent drugs except MD-AraC did not correlate with mobilization response. Multivariate analysis revealed the course of prior MD-AraC chemotherapy was an independent predictive factor for HSC mobilization[OR 0.627, 95% CI 0.421-0.935, P=0.022]. However, the MD-AraC chemotherapy has no effect on hematopoietic reconstitution and survival in AML patients treated with auto-HSCT.

Discussion: Exposuring to the bone marrow toxic drugs is the major factor negatively effected mobilization, there mitoxantrone, fludarabine, lenalidomide, platinum, alkylating agent, carmustine, nucleoside analogue, melphalan were reported. As in previous studies, prior exposure to MD-AraC also was an independent negative predictor for mobilization and without survival benefit. The conventional chemotherapy not accurately presented a significant influence in the mobilization in AML. In preparation for Auto-HSCT, MD-AraC must be taken into account.

Disclosure of Interest: None Declared.

PH-P123

EXPLORING THE HETEROGENEITY OF THE HEMATOPOIETIC STEM AND PROGENITOR CELL POOL:

S. Ulrika Frändberg[1,2,], C. Boreström[1], S. Li[1], L. Palmqvist[1]*

[1]Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine Sahlgrenska Academy, [2]Stem cell laboratory, Clinical Immunology and Transfusion Medicine Sahlgrenska University Hospital Göteborg, Göteborg, Sweden

Introduction: Choosing the perfect cord blood unit (CBU) for transplantation is based on tissue typing and the stem cell potential of the unit. CBUs are routinely assessed at collection with regards to total nucleated cells, CD34+cell count and progenitor cell cultivation protocols such as the time-consuming Colony-Forming-Unit assay. Efforts are however continuously made towards finding better ways of defining true stemness of CBUs.

Side population phenotype (SP) and activity of the Aldehyde Dehydrogenase enzyme (ALDH) are functional markers of stemness that can be assayed using flow cytometry.

Here, we have developed a protocol for the simultaneous determination of CD34+, SP and ALDH+ populations in relation to immature leucocytes, i.e. the weakly CD45+ population of cells with low side scatter properties (CD45dimSSClow), inspired by the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines for determination of CD34+ cell count.

Materials (or patients) and Methods: 30 randomly selected CBUs were investigated in this study. 200 μl cord blood buffy coat was sampled from each CBU. After lysis of erythrocytes, 1.5×10^6 viable cells were stained with Hoechst 33342 (Life Technologies, Carlsbad CA, USA) for 90 minutes at 37°C. After a few minutes on ice, cells were stained with ALDH reagent according to manufacturers´ instructions for 30 minutes at 37°C (Stem Cell

[PH-P122]

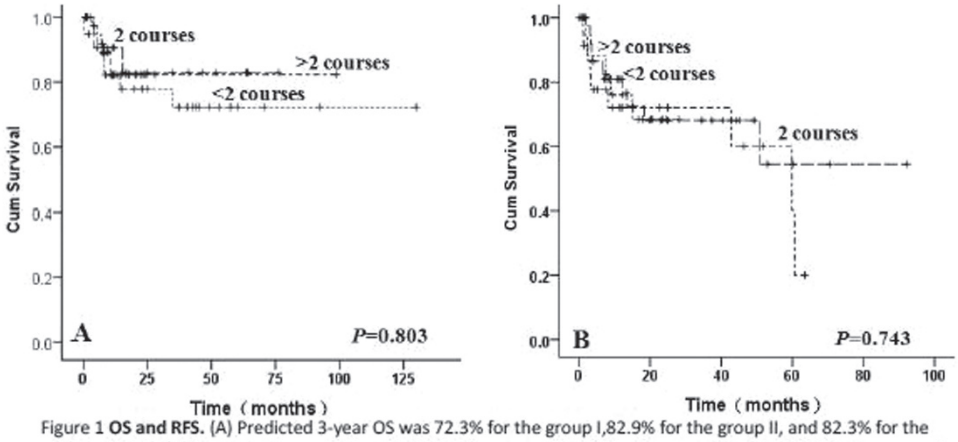


Figure 1 **OS and RFS.** (A) Predicted 3-year OS was 72.3% for the group I,82.9% for the group II, and 82.3% for the group III(P = 0.803). (B) Predicted 3-year RFS as 60.1% for the group I,72.1% for the group II, and 64.8% for the group III (P = 0.743).

Technologies, Vancouver, Canada). Subsequently, the cells were put on ice and stained with the viability stain 7-AAD and antibodies directed against the CD45 and CD34 (BD, Franklin Lakes NY, USA) antigens for 30 minutes. Samples were analyzed on a FACSAriall (BD, Franklin Lakes NY, USA) equipped with a 375nm near UV-laser. All calculations performed using IBM SPSS Statistics version 21.
Results: There was no overlap between the SP and ALDH+ populations in any of the investigated units. The majority of SP cells were CD34 negative, whereas the ALDH+ population was dominated by CD34+ events. The mean size of the CD45dimSSClow population was approximately 10% of the total number of 7-AAD negative i.e. viable events. The CD34+ and ALDH+ populations amounted to a few percent of the CD45dimSSClow population and were approximately of the same size. The SP population was significantly smaller
Discussion: Taken together, we show that simultaneous staining for CD45, CD34, SP and ALDH+ cells is feasible using small amounts of cord blood buffy coat in a time frame possible to implement in routine laboratory work. In our study, the sizes of the ALDH+ and CD34+ populations in relation to the number of CD45dimSSClow events were approximately the same whereas the SP was smaller. There were also differences in immunophenotype between the SP and ALDH+ populations inferring that they represent cells with diverse biology. Since the stem and progenitor pool in each CBU is small the implementation of the CD45dimSSClow gating strategy enhances the relative size of the populations and therefore possibly the sensitivity of the measurement. In conclusion this study illustrates the heterogeneity of the stem and progenitor pool in CB both in quantity i.e. population size and quality i.e. immunophenotype and functional properties.
Disclosure of Interest: None Declared.

PH-P124
THE EFFECT OF MOBILISATION REGIMEN AND DAY OF COLLECTION ON REGULATORY T CELL LEVELS IN HAEMATOPOIETIC PROGENITOR CELL APHERESIS PRODUCTS

J. Catherine Wells[1,], J. Vincent Johnston[1], S. ann-Marie Ingleton[1], B. Jayanti Patel[1], M. Ann Smith[1]*
[1]Stem Cell Transplant Lab, The Royal Marsden Hospital, Sutton, United Kingdom

Introduction: Regulatory T cells (Tregs) may play an important role following haematopoietic progenitor cell (HPC) transplantation. In allogeneic recipients, higher Treg numbers have been shown to protect against graft-versus-host-disease (GVHD) but in both allogeneic and autologous recipients can also suppress immune responses to tumour antigens leading to higher rates of relapse. Previous studies have shown that Treg levels in the graft and also during immune reconstitution may affect transplant outcome.
Materials (or patients) and Methods: This study considered $CD3^+$ $CD4^+CD125^{high}CD127^{low}$ $FoxP3^+$ Treg levels in autologous HPC apheresis harvests (*n*=109) following one of three mobilisation regimens: G-CSF(Lenograstim), G-CSF and cyclophosphamide and G-CSF and Plerixafor (*n*=11, *n*=82 and *n*=16 respectively). Additionally Treg levels were compared in 75 allogeneic harvests from sibling (*n*=22) and unrelated (*n*=43) donors and 10 non-mobilised donor lymphocyte donations.
Tregs were measured using multi-colour flow cytometry and expressed as Tregsx10^6/ml, Tregs as a ratio of CD34 or CD3 positive cells and Tregs as a percentage of CD4 cells.
Treg levels on two consecutive harvest days were also compared.
Results: No significant differences were observed in absolute Treg numbers/ml or ratio quantifications in autologous harvests mobilised by either G-CSF or G-CSF plus cyclophosphamide. Harvests mobilised by G-CSF/Plerixafor showed significantly higher levels of Tregs/ml ($0.42x10^6$/ml +/– 0.09) than those mobilised by G-CSF alone ($0.18x10^6$/ml +/– 0.05) (*P*=0.034). Also a significant increase in Tregs was observed when comparing the Treg:CD34 ratio following G-CSF/Plerixafor compared with G-CSF/cyclophosphamide regimens (0.66 +/– 0.15 and 0.28 +/– 0.04 respectively) (*P*=0.026). The levels of Tregs as a percentage of $CD4^+$ cells did not differ significantly with any mobilisation protocol. Day of harvest did not affect Treg levels collected regardless of mobilisation regimen.
In allogeneic harvests, no significant difference was noted between Tregs/ml in the non-mobilised and mobilised harvests (*P*=0.21). However, expressing the data as either Treg:CD3 ratios or Tregs as a percentage of CD4 cells showed lower levels in products from G-CSF mobilised donors. These results which verge on significance (*P*=0.057 and *P*=0.058 respectively) may be due to proportionately higher levels of CD3 and CD4 cells following G-CSF.
Discussion: Overall these data indicate that neither the G-CSF or cyclophosphamide components of autologous mobilisation regimens elevate Treg levels. However, the higher absolute levels noted with G-CSF/Plerixafor may be associated with the higher white cell counts observed with this regimen. As the majority of patients in this cohort (93/109), received G-CSF or G-CSF/cyclophosphamide, it is reassuring to surmise that such products may not be associated with tumour suppression. It may however be worthy of note that Plerixafor mobilisation induces higher Treg levels/ml and as a ratio of the CD34 cells collected in the autologous setting.
G-CSF used in allogeneic donors does not appear to affect absolute Treg levels which may be of clinical relevance to tumour tolerance and/or GVHD.
Disclosure of Interest: None Declared.

PH-P125
ADOPTIVE THERAPY WITH DONOR LYMPHOCYTE INFUSION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION – EXPERIENCE OF THE POLISH PEDIATRIC GROUP FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION (PPGHSCT)

J. Gozdzik[1,2,], K. Rewucka-siewiera[3], A. Krasowska-Kwiecien[1,2], A. Pieczonka[4], R. Debski[5], A. Zaucha-Prazmo[6], K. Drabko[6], J. Kowalczyk[6], M. Wysocki[5], E. Gorczynska[3], K. Kalwak[3], A. Chybicka[3], J. Wachowiak[4] and PPGHSCT*
[1]Dept. Clinical Immunology, Chair of Clinical Immunology and Transplantation Polish-American Institute of Pediatrics, Jagiellonian University Medical College, [2]Department of Transplantation, University Childrens Hospital, Krakow, [3]Chair and Clinic of Bone Marrow Transplantation, Pediatrics Oncology and Hematology, Medical University, Wrocław, [4]Chair and Clinic of Pediatrics Oncology, Hematology and Transplantation, Medical University, Poznan, [5]Chair of Pediatrics, Hematology and Oncology, Nicolaus Copernicus University, Medical College, Bydgoszcz, [6]Department of Pediatric Hematology, Oncology and Transplantology Medical University, Lublin, Poland

Introduction: Almost 30 years have passed since the first use of donor lymphocytes infusion (DLI) after allogeneic HSCT. Nevertheless, the therapy still remains poorly recognized. Mainly due to numerous indications and small number and huge diversity of patients (pts.) treated with DLI. The efficacy and safety of DLI in pediatric pts. is particularly poorly studied. The aim of our study was to describe experience of PPGHSCT in respect of DLI.
Materials (or patients) and Methods: The study included 51 pts., who underwent DLI in the years 1993-2011. The study group comprised 23 girls and 28 boys, aged median 9 (1-22) years, grafted because of oncohematological (45) and nonproliferative disease (6). The indications for DLI were as follows: 1) increasing recipient chimerism after nonablative HSCT (*n*=18), 2) modulation of reduced intensity conditioning regimen (*n*=2), 3) persistence or progression of minimal residual disease (MRD; *n*=3), and 4) relapse after HSCT (*n*=28).
Results: DLI was carried out in median 6.0 (0.5–79.0) months after HSCT. The time of initiation of DLI was different depending on the indications type (*P*=0.04). Median time from completion of immunosuppressive therapy to DLI was 49 (-28–1758) days. No differences were demonstrated in respect of interval between the completion of immunosuppressive treatment and start of DLI depending on the indications type (*P*>0.05). The source of DLI was peripheral blood cells collected by apheresis: nonstimulated and unselected (*n*=22), or G-CSF mobilized (*n*=29). The DLI was

administered as a single dose (*n*=19), or in fractions (*n*=32). In case of fractionated DLI, it was administrated an average of 2 (1–11), on median each 5 (2-36) weeks. The dosage of DLI was determined based on the count of CD3(+) cells. The total dose of CD3(+) cells was a median 28.0 (0.1-730.0) x10^6/kg recipient's body weight (k.b.w.). The number of administrated CD3(+) cells per a dose was a median 6.0 (0.1–240.0) x10^6/k.b.w. The total dose of CD3(+) cells and the number of doses were not different between the indications (*P*>0.05). Thirty six pts. received only DLI and 15 pts. DLI with cytoreductive (*n*=12) or specific (*n*=3) therapy. The efficacy of DLI was assessed based on clinical parameters and imaging studies, as well as by testing a donor chimerism by PCR and/or monitoring of MRD. The time for assessment of DLI efficacy ranged from 0 to 70 (median 3) months. During the evaluation, 18 (35%) pts. experienced complete remission, in 3 (6%) pts. progression of the disease slowed down, 19 (37%) pts. showed relapse and progression of disease and 11 (22%) pts. rejected the graft. DLI was found as effective in 41% of cases. The efficacy of DLI was affected only by the total dose of CD3 cells (*P*=0.02). Other factors like: time from HSCT to DLI, method of application (one dose vs. fractionated), source of cells, occurrence of GvHD after HSCT or type of the indication were not important to the outcome of DLI (*P*>0.05). In 18 (35%) pts. after DLI, complications of different intensity were found (including sever bone marrow aplasia – 5 pts. and GvHD – 13 pts.), due to which 2 (4%) patients died.

Discussion: DLI shows its efficacy in a significant percentage of children undergoing HSCT. It has low mortality rate due to complications. However, this method requires standardization.

Disclosure of Interest: None Declared.

PH-P126

CHANGES IN THE GUT MICROBIOME IN SEVERE COMBINED IMMUNODEFICIENCY THROUGH HAEMATOPOIETIC STEM CELL TRANSPLANTATION

J. Lane[1,2,], C. Stewart[3], S. Cummings[3], A. Gennery[1,2]*

[1]Institute of Cellular Medicine, Newcastle University, [2]Great North Childrens Hospital, [3]Northumbria University, Newcastle upon Tyne, United Kingdom

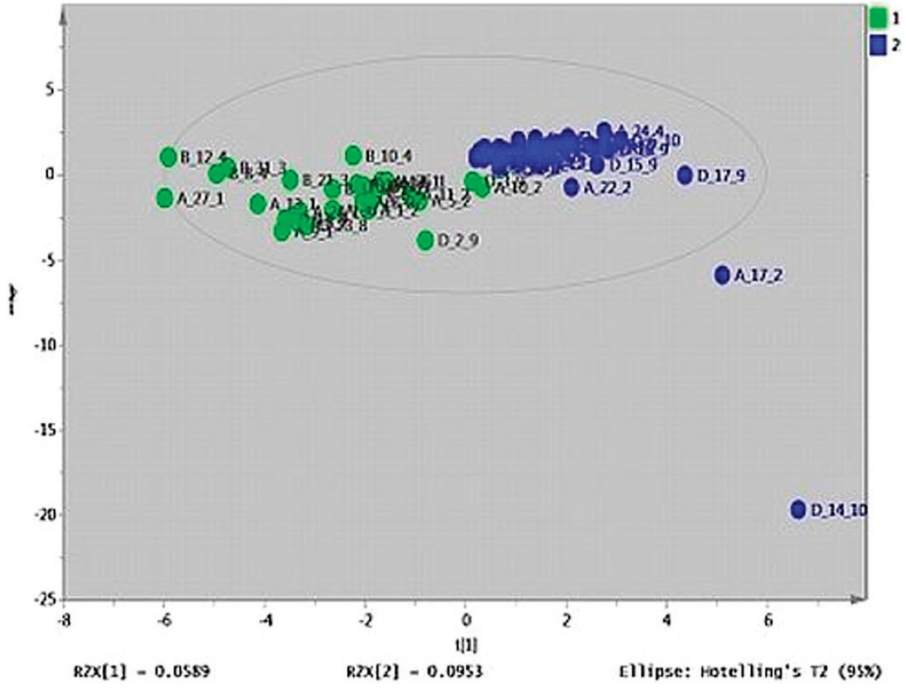
Introduction: With sequencing technology improvements, the gut microbiome has been a topic of study in many diseases with an immunological component. In adult haematopoietic stem cell transplant (HSCT) patients, a change in the gut microbiome through HSCT has been associated with GvHD-related intestinal inflammation and bacteraemia. We performed a longitudinal prospective pilot study to explore changes in gut microbiome in severe combined immunodeficiency (SCID) patients through HSCT.

Materials (or patients) and Methods: Stool samples were analysed longitudinally from 3 SCID patients (Babies A, B and D) based on occurrence of clinical events (conditioning, HSCT, neutrophil engraftment, pyrexia, antibiotic therapy, GvHD). Bacterial profiles were generated based on the 16S ribosomal DNA gene using both denaturing gradient gel electrophoresis (DGGE) and Illumina MiSeq next generation sequencing.

Results: Combining all 3 patients in a partial least squares discriminant analysis (PLS-DA) showed that pre- and post-HSCT samples grouped separately (Figure). In all 3 patients a low Shannon diversity index was observed which fluctuated throughout HSCT. Baby A was 8 months old on admission and 9 months at HSCT. Pre-HSCT, the bacterial profile was a mix of *Staphylococcus* and *Clostridium* XIVa. Post-HSCT the bacterial profile was dominated by *Staphylococcus*. Pyrexia on day -3 occurred in conjunction with the appearance of *Escherichia* in stool. Baby B was 8 days old on admission and 6 weeks old at HSCT. Prior to conditioning samples were dominated by *Bifidobacterim* and *Streptococcus*. Post-HSCT, *Enterococcus* and *Staphylococcus* dominated. The change between pre- and post-HSCT coincided with pyrexia, central line infection and antibiotic use. Growth of *Enterococcus* post-HSCT coincided with disseminated adenovirus infection. Baby D was 6 days old on admission and 5 weeks old at time of BMT infusion with no conditioning. At 3 weeks of age there was a dominance of *Enterococcus* in stool, which gradually declined over time post-HSCT. Pyrexia, GvHD and disseminated CMV developed from day 33 post-HSCT, when *Enterococcus*, *Klebsiella* and *Eschericha* were dominant in stool samples.

Discussion: Despite the individual nature of the gut microbiome in each patient we observe a clear difference in the bacterial community following the HSCT process. The diversity was shown to be unstable over the duration of the study. This small study represents the first such study to explore the gut microbiome in HSCT in SCID but effects of the microbiome on complications post HSCT in SCID and other primary immunodeficiencies warrants further work.

Disclosure of Interest: None Declared.



PH-P127

IMPLEMENTATION OF NONCRYOPRESERVED GRAFTS IN AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH MULTIPLE MYELOMA; SINGLE CENTER EXPERIENCE

B. Georgievski[1], S. Genadieva Stavric[1], Z. Stojanoski[1], A. Pivkova Veljanovska[1], L. Cadievski[1,], L. Cevreska[1]*
[1]University Clinic for Hematology, Skopje, Macedonia, The Former Republic Of Yugoslav

Introduction: Autologous hematopoietic stem cell transplantation is a well established and almost inevitable step in the treatment of patients with multiple myeloma, proven by the improvement of survival rates presented in a sea of data from an enormous number of trials. Cryopreservation of harvested peripheral blood stem cells is an everyday routine in almost every transplant center. But is it possible to avoid additional costs, and DMSO intoxication of the patients without influencing the effectiveness of the procedure. We are caring to present our modest results of using noncryopreserved hematopoietic stem cells for transplantation of patients with multiple myeloma. Our motive was influenced by the fact that the viability of stem cells is not significantly changed if stored at 4°C for at least 5 days.
Materials (or patients) and Methods: Knowing the fact that the duration of the conditioning regimen for transplantation of patients with multiple myeloma (melphalan 200 mg/m^2) is short, we anticipated that the use of noncryopreserved hematopoietic stem cells in this setting would be more than appropriate and a good opportunity for simplifying the transplantation procedure without influencing it's effectiveness. We have evaluated 14 patients diagnosed with multiple myeloma, 9 males (64%) and 5 females (36%), with an average age of 51.9 years (range 39-65).
Results: The majority of patients (64.3%) were treated according to the CTD (Cyclophosphamide, Thalidomide, Prednisone) regimen, and the reevaluation of the disease status before stem cell transplantation (SCT) showed that 9 patients (64.3%) were in very good partial response (VGPR) and 4 patients (28.5%) with complete response (CR). The median time from diagnosis to SCT was 7.8 months (range 4-18). The average number of apheresis procedures was 1.7 (range 1-3), and the average number of collected cells was 3.1x10^8/kg TT mononuclear cells (range 4.0-2.0). G-CSF mobilizing regimen was used in most of the patients. The number of days to confirmed engraftment in our group of patients was 11.5 (range 9-15). The amount of blood transfusions was on average 0.7 (range 0-4), and transfusion of thrombocytes (mainly pooled thrombocytes) 24.7 units (range 0-82). When we compared this results with the control group of patients, searched retrospectively, where cryopreserved cells were used, we couldn`t confirm any significant difference in the duration of aplasia (median time to engraftment 11.6 days (range 8-20)), nor any significant difference in transplant related mortality or post transplant complications. There was a difference in the amount of transfused blood products but that was related to complications not related to the transplant procedure.
Discussion: We must conclude that the use of noncryopreserved hematopoietic stem cells in transplantation of patients with multiple myeloma represents a safe, equally effective and relatively simple procedure, that has, among other positive values, cost-effective advantages and eliminated DMSO intoxication of the patient. However, for performing an optimal SCT in this manner, a good coordination and timing between teams for stem cell collection, administration of high-dose chemotherapy conditioning regimen, storage and application of noncryopreserved stem cells, and good supportive treatment of the patients in the transplant unit is essential.
Disclosure of Interest: None Declared.

PH-P128

FEAM COMPARED WITH BEAM AS CONDITIONING REGIMEN IN AUTOLOGOUS TRANSPLANT:SINGLE CENTRE EXPERIENCE

P. Vincenzo[1,], M. Anna[1], D. F. Rosa[1], F. Mariapaola[1], G. Giuseppina[1], S. Silvia[1], C. Daniela[1], R. Bernardo[1], D. R. Clara[1], M. Mariarosaria[1], C. Stefania[1], R. Giulia[1], O. Angelo[1]*
[1]Haematology, Az Osp Card Panico, Tricase (LE), Tricase, Italy

Introduction: BEAM is the standard conditioning regimen for lymphoma patients undergoing autotransplant. Fotomustine could be used as substitute of BCNU in this regimen
Materials (or patients) and Methods: Here we present the results in 34 consecutive patients treated with FEAM compared with 54 treated with BEAM. Patients were similar in terms of diagnosis, median age, number of previous therapies, disease status at transplant, number of CD34+cells infused (3.6x10^6/kg).
Results: Hematopoietic engraftment and treatment related toxicity are shown in table1. Thirty-four (77%) BEAM patients required more than 20 days to recovery platelets vs 11(46%) FEAM patients, (*P*=0,01). Mucositis (98% vs 82%, *P*=0.019) and diarrhea (98% vs 85%, *P*=0.044) seem occurred more frequently in the BEAM group. Almost all patients in the two groups had fever during neutropenia. The median days of hospitalization were 22 in FEAM and 25 in BEAM group:54% of patients treated with BEAM and 35% with FEAM needed more than 24 days of hospitalization, *P*=0.06. The incidence of TRM was 9% in BEAM and 12% in FEAM cohort, *P*=0.9. At univariate analysis, older age was only factor influenced negatively TRM. In patients <65years, TRM was 7% in BEAM and 4% in FEAM, *P*=0.05. The 90-day overall response rate (ORR) was 86% (71% CR) and 85% (65% CR) in patients treated with FEAMand BEAM, respectively. No difference was observed regarding 90 day ORR between the two groups.After 31 months of median follow-up (1-98), the estimated 2y EFS was 57%, without difference among the two groups.
Discussion: In our experience FEAM ensured a reduction of mucositis, diarrhea and a more rapid platelets engraftment with a reduced hospitalization. We observed a higher incidence of TRM in both groups, being 32% (FEAM) and26% (BEAM) of our patients older 65years. In younger patients the TRM did not differ from that reported in the literature and in patients treated with FEAM the TRM occurred less frequently.In terms of efficacy, ORR and EFS of FEAM was comparable to BEAM, however longer follow-up is needed to evaluate fully its efficacy and long term safety.
Disclosure of Interest: None Declared.

PH-P129

LONG-TERM FOLLOW UP OF BONE DENSITY AND REPRODUCTIVE HEALTH IN FEMALE SURVIVORS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

S. Naessén[1,] on behalf of Yes, I. Bergström[2], K. Leblanc[3], P. Ljungman[4], B.-M. Landgren[5]*
[1]Women and Child Health, Division of Obstetrics and Gynecology, [2]Clinical Science, Intervention and Technology, [3]Hematology, Karolinska University Hospital and Division of Hematology, Department of Medicine, Huddinge, Karolinska Institute, [4]Hematology, Karolinska University Hospital and Division of Hematology, Department of Medicine, Huddinge, Karolinska Insitute, [5]Clinical Science, Intervention and Technology, Karolinska Institute, Stockholm, Sweden

Introduction: Hematological malignancies are a heterogeneous group with variable incidence and prognosis. The overall survival and cure rates of patients with HMs have improved dramatically for ex through stem cell transplantation either autologous or allogeneic. To achieve these results there is a need for prompt intensive treatment, but it affects fertility and bone density. The risk of developing premature ovarian failure is a major concern in long-term survivors and there is therefore a great need for information on fertility issues.
This descriptive study, conducted in the Stockholm region, assessed bone mineral density and reproductive health in

[PH-P128]

Table 1: Hemopoietic engraftment and toxicity

	FEAM 34	BEAM 54	P
Hematopoietic engraftment			
Neutrophils (> 500 x 10e 9/L)	10 (8-22)	10 (7-21)	0,9
>12 days	2 (6%)	3 (6%)	
Neutrophils (> 1000 x 10e9/L)	10 (8-25)	11 (8-26)	0,59
>15 days	3 (50%)	3 (50%)	
Plts (> 20000 x 10e9/L)	13 (9-32)	13 (8-60)	0,44
>15 days	7 (27%)	15 (32%)	
Plts (> 50000 x 10e9/L)	18 (14-40)	17 (11-180)	0,01
>20 days	11 (46%)	34 (77%)	
Febrile neutropenia	29 (85%)	42 (84%)	
Days of onset, median (range)	4 (-1; 7)	5 (1; 22)	0,87
Median duration days, range	3 (1-10)	3 (2-23)	
FUO	18 (62%)	19 (45%)	
Mucositis	27 (82%)	42 (98%)	0.019
Diarrhea	29 (85%)	42 (98%)	0.04
Renal Toxicity	1 (3%)	4 (9%)	0.3
Hepatic Toxicity	1 (3%)	2 (5%)	0.7
Neurological Toxicity	2 (6%)	3 (7%)	0.43
Hospitalisation median day, range	22 (6-59)	22 (20-61)	
> 24 days	12 (35%)	28 (54%)	0.06

hematological cancer survivors after haematopoietic stem cell transplantation (HSCT).

Materials (or patients) and Methods: Thirty-seven premenopausal women having undergone autologous or allogeneic HSCT were consecutively included in the study between1998 and 2010; they were followed annually until 2011. The diagnoses were acute lymphoblastic leukaemia (*n*=6), acute myeloid leukaemia (*n*=9), chronic lymphocytic leukaemia (*n*=1), chronic myeloid leukaemia (*n*=12), Hodgkin lymphoma (*n*=4), non- Hodgkin lymphoma (*n*=5). Fertility preservation options were offered before cancer treatment to most women. The mean age at diagnoses was 27 and at the final evaluation 39 years. Twenty-nine women (78.4 %) with a mean age of 25 (21-43) years had never been pregnant. All women were in a menopausal state (POF) due to given therapy. Hormone substitution was given and bone mineral density was measured repeatedly.

Results: Twenty-six patients received allogeneic HSCT and eleven autologous HSCT. Before allogeneic HSCT, nineteen patients received myeloablative conditioning; seven had reduced-intensity conditioning. Eleven patients got total body irradiation. Eight patients were transplanted with grafts from an HLA-identical sibling donor while 18 had unrelated donors. Three patients developed venous thrombosis after the HSCT; two malignancies occurred after allogeneic HSCT, one malignant brain tumor, one cancer in situ cervices. During follow-up, bone mineral measurements showed a slight increase in the spine while no decrease was seen in femoral neck and total hip values over time. Resumption of menstruations occurred in three women allowing spontaneous pregnancies, two babies were born and one pregnancy was terminated. Additionally, three pregnancies were achieved with oocyte donation, surrogacy, and adoption respectively.

Discussion: The risk of infertility has not been extensively studied after HSCT. The type and intensity of cytotoxic agents and the cumulative doses are, besides age, the most important factors determining the likelihood of gonad failure. A resumption of menstrual cycles does not guarantee normal fertility. However, the oestrogen substitution is crucial in women with POF. This study highlights the effects on bone density and fertility after HSCT for hematological malignancies. Since the number of patients who regained their menstruation and/or became mothers is very small, it is difficult to draw conclusions regarding important background factors. Early preservation of fertility is important issue, as the management of general health in these groups. A multidisciplinary collaboration between hematologists and Assisted Reproductive Techniques teams is needed.

Disclosure of Interest: None Declared.

PH-P130

SINGLE CENTRE EXPERIENCE OF SECOND ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR HAEMATOLOGICAL DISORDERS

F. Seymour[1,], A. Gudger[1], H. Hussein[1], P. Dyer[1], J. Patel[1], J. Khwaja[1], K. Holder[1], L. Baker[1], L. Bratby[1], R. Lovell[1], B. Kishore[1], D. Milligan[1], E. Nikolousis[1], S. Paneesha[1]*

[1]Haematology, Heart of England NHS Trust, Birmingham, United Kingdom

Introduction: Second allogeneic stem cell transplants are uncommon but can deliver a potent graft versus malignancy effect and re-establish normal haematopoesis in patients who are fit enough to undergo the procedure.

Materials (or patients) and Methods: Our retrospective study included 27 patients (M: 16, F: 11) with a median age at second transplant of 43 (range: 19-66). The most common indication for transplantation was acute leukaemia in 13 patients (52%). Second transplantation was performed for relapsed disease in 11 patients (40%) and graft failure in 14 patients(52%) whereas a secondary haematological malignancy was the indication for transplantation in two patients (8%). Matched sibling donors were used for 16 patients (59%) of second transplants while the remaining 11 (41%) were fully matched unrelated donor transplants. 12 transplants were T depleted with either alemtuzumab *in vivo* (8/12) or Alemtuzumab *ex vivo* (4/12). 7 transplants (26%) were carried out using myeloablative conditioning whereas reduced intensity conditioning was used in 20 patients(74%) . The same donor was used for both transplants in 7 (26%) cases. 9 patients received a T deplete protocol for their first transplant and subsequently underwent T replete second HSCT.

Results: The median time for neutrophil engraftment was 14.5 days (range 2-52 days). Severe grade 3 or 4 acute graft versus host disease occurred in 15% patients whereas chronic graft versus host disease was observed in 26%. Median overall survival was 12 months (Range: 0 to 101) with 55% of patients surviving more than 12 months after HSCT. 63% of deaths were related to non relapse mortality (NRM) whereas 32% were due to progressive disease.

Discussion: Our single centre retrospective analysis suggests second allogeneic transplantation can achieve reasonable overall survival although as expected non relapse mortality is significant. In view of the smaller number of patients in our single centre study this needs to evaluated from the registry data to identify which group of patients derive significant benefit from second HSCT. This information will enable clinicians with informed decision making for patients undergoing second allogeneic HSCT.

Disclosure of Interest: None Declared.

PH-P131

DISEASE RISK INDEX (DRI) AND HEMATOPOIETIC CELL TRANSPLANTATION COMORBIDITY INDEX (HCT-CI) PREDICT SURIVIVAL AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION: A COMPARATIVE STUDY WITH EBMT RISK SCORE IN 183 CONSECUTIVE PATIENTS

M. T. Lupo-Stanghellini[1,], E. Sala[1], M. Morelli[1], M. Marcatti[1], A. Assanelli[1], M. Carrabba[1], S. Marktel[1], F. Lunghi[1], E. Guggiari[1], F. Giglio[1], C. Corti[1], M. Bernardi[1], J. Peccatori[1], F. Ciceri[1]*

[1]Bone Marrow Transplantation Unit, Ospedale San Raffalele, Milan, Italy

Introduction: Optimization of pre-transplant risk assessment is a crucial issue to improve the allo-HSCT decision making process. To date 2 major algorithms are in use in clinical practice: the EBMT risk score and the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) score. Recently a disease risk index (DRI) - Armand *et al*, Blood 2012;120:905 - was defined to calibrate HSCT outcome across studies and centers. Here we are presenting the results of a retrospective study designed to evaluate the strength of the 3 aforementioned score in stratification of transplant outcome after an haploidentical HSCT (haplo-HSCT).

Materials (or patients) and Methods: We included 183 adult patients (pts) who underwent an haplo-HSCT for hematologic malignancies, between 2004 and 2013 and were reported to the local database. Risk assessment score and outcome analysis included all consecutive pts receiving an haplo-HSCT as 1st allogeneic transplantation. Pts receiving haplo-HSCT as 2nd or 3rd HSCT were excluded from the present analysis. Their median age was 48 (range, 15-77) years. The cohort included a broad representation of diseases (116/183 acute leukemia, 22 Hodgkin lymphoma) and disease status. Donor source was mobilized peripheral blood stem cell in all the cases. Only 5 pts were conditioned with a non-myeloablative regimen. The median follow-up for survivors was 25 months.

Results: The overall survival (OS) at 2-y was 30% and the transplant related mortality at 100-days 23%. The 2y OS according to EBMT / HCT-CI / DRI risk score are reported in table.

The evaluation of the HCT-CI impact after DRI stratification was still able to show a significant difference in outcome showing better survival for pts with low DRI score and low HCT-CI score as expected.

Discussion: DRI score and HCT-CI score predict survival after haplo-HSCT. The integrated application of DRI and HCT-CI may improve the definition of transplant eligibility for pts candidate to allogeneic HSCT form alternative donors including family haploidentical source.

Disclosure of Interest: None Declared.

PH-P132

CD 34+ CELL NUMBER AND CELL VIABILITY IMPACT ON ENGRAFTMENT

S. Jurenec[1,], M. Lukić[2], V. Zatezalo[3], D. Radić-Krišto[3], B. Jakšić[3]*

[1]Transfusion Medicine, Clinical Hospital Merkur, [2]Clinical transfusion, Croatian Institute for transfusion medicine, [3]Department of hematology, Clinical Hospital Merkur, Zagreb, Croatia

Introduction: Cell viability determination is an important quality indicator of peripheal blood stem cell (PBSC) product. Cryopreservation of PBSC with dimethylsulfoxid (DMSO) is associated

[PH-P131]

	0-2	%pts	3-4	%pts	>/=5	%pts	p
EBMT score	47%	16	29%	51	25%	33	0.07
HCT-CI score	41%	58	29%	31	0%	11	0.001
DRI score	Low-Intermediate		High		Very-High		
	49%	30	24%	60	13%	10	0.0009
DRI score	HCT-CI 0-4		HCT-CI >/=5				
Low-Int	55%		0%				0.0001
High-Very High	25%		0%				

with decreased viability of cells. Trypan blue assay show the percentage of all survived cells without possibility of determining the number of survived CD34+ cells. Furthermore, it is assumed that the stem cells are more resistant to cryopreservation and it is assumed that not survived cells belong to granulocytes. The aim of this study was to determine influence of reinfused CD34+ cell number and cell viability performed with trypan blue assay, on the speed of hematopoietic recovery.
Materials (or patients) and Methods: We analyzed 114 PBSC products with leukapheresis performed on cell separator in the period from January 2011 until November 2013. Absolute numbers of CD34+ cells were enumerated on flow cytometer. PBSC products were cryopreserved with 10 % DMSO. Before freezing, cell viability was determined by trypan blue assay. Products were gradually frozen with programmed average speed of 2.3°C/min to a temperature of -196°C and stored in liquid nitrogen. PBSC autologous transplantation was performed in 114 patients with median age of 56 years (19-72) and diagnosed multiple myeloma (*n*= 51), non-Hodgkin's lymphoma (*n*= 37), Hodgkin's disease (*n*= 22) and acute myeloid leukemia (*n*= 4). On the first posttransplantation day each patient received pegylated filgrastim 6mg sc. Time elapsed from transplantation to hematopoietic recovery (leukocytes >1x10^9/L, neutrophils >0.5x10^9/L and platelet count >20x10^9/L at least two days after platelet transfusion) as well as number of transfused blood cell products was measured.
Results: The median number of CD34+ cells infused was 5.6x 10^6/kg of body weight (range 1.8- 24.1) with cell viability in the PBSC product before freezing 83.65% (range 22.3-100). Average time to leukocyte and neutrophil engraftment was 10.4 days with range 8-27 and 8-26, respectively and to platelet engraftment 11.1 days (range 8-22). Number of transfused red blood cells (RBC) units was 1.4 (range 0-10) and for platelets 12.7 (range 0-72). Statistical correlation was confirmed between the number of CD34+ cells infused and the time of hematopoietic recovery of leukocytes (P <0.0001), neutrophils (P <0.0001) and platelets (P < 0.02), but with no significant correlation between cell viability in the PBSC product and leukocyte (P= 0.25), neutrophil (P = 0.49) and platelet engraftment (P = 0.54). No difference was found in time to hematopietic recovery among patients with different diagnoses, age, gender, number of RBC and platelet transfusions.
Discussion: Our data indicate that cell viability determined by trypan blue assay before freezing PBSC product, in spite of detecting variability in total cell population cannot be an indicator of the number of survived CD34+ cells. The best indicator of the hematopoietic speed recovery after transplantation is the number of infused CD34+ cells.
Disclosure of Interest: None Declared.

PH-P133
PHARMACOKINETICS OF TREOSULFAN AND ITS MONOEPOXIDE IN CHILDREN UNDERGOING HSCT

F. Główka[1,], T. Wróbel[1], A. Kasprzyk[1], M. Romański[1], J. Wachowiak[2], D. Szpecht[2], K. Kałwak[3], A. Wiela-Hojeńska[4]*
[1]Department of Physical Pharmacy and Pharmacokinetics, [2]Department of Oncology, Hematology and Pediatric Transplantation, Poznan University of Medical Sciences, Poznan, [3]Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation, [4]Department of Clinical Pharmacology, Wroclaw Medical University, Wroclaw, Poland

Introduction: Treosulfan (TREO) is an alkylating agent which has been applied in preparative regimens before HSCT since 2000. In the clinical trials it has demonstrated a strong myeloablative action and more favorable toxicity profile in comparison to busulfan. TREO is a pro-drug of which biologically active epoxy-derivatives are formed under physiological conditions of pH and temperature without enzymes contribution. Up till now data on levels of TREO epoxides in patients are very scarce. This study describes pharmacokinetics of both TREO and its active monoepoxide (S,S-EBDM) in children undergoing HSCT.
Materials (or patients) and Methods: Fifteen children aged between 0.4 and 18 years with hematologic malignancies and non-malignant diseases who received 26 courses of TREO were enrolled in the pharmacokinetic study. TREO was administered as an 1 h or 2 h intravenous infusion at doses of 12 g/m^2 b.s. (18 courses in 8 patients) and 14 g/m^2 b.s. (8 courses in 7 patients). Blood samples were drawn before the infusion and at 0.5 - 8.0 h following a start of the drug administration. After collection the blood samples were immediately adjusted by 1 M citric acid to pH below 5 to avoid artificial *ex vivo* activation of TREO. Plasma concentrations of TREO and S,S-EBDM were determined by an HPLC method with tandem mass spectrometry detection. Pharmacokinetic parameters were calculated in WinNonlin 6.2.
Results: Changes in plasma concentrations of TREO were best described by a two-compartment model, whereas levels of S,S-EBDM were best fitted by a one-compartment model. Values of C_{max} and area under the curve (AUC) of TREO as well as S,S-EBDM increased with the dose of TREO. Following 2 h infusion of TREO at dose of 12 g/m^2 (n = 8) and 14 g/m^2 (n = 8) the mean C_{max} values of the parent drug were 1926 ± 906 and 3070 ± 2129 μM, whereas the mean C_{max} values of S,S-EBDM amounted to 14 ± 11 and 18 ± 11 μM, respectively. In turn the mean AUC of TREO after the dose of 12 g/m^2 and 14 g/m^2 was 5664 ± 2458 and 8644 ± 4849 μM × h, whereas the values obtained for S,S-EBDM equaled to 43 ± 26 and 59 ± 34 μM × h, respectively. The mean biological half-lives of TREO and S,S-EBDM were 1.77 ± 0.51 h and 1.83 ± 1.15 h (n = 26), respectively.
Discussion: The changes in S,S-EBDM concentrations in the pediatric patients' plasma followed TREO levels, though concentrations of the epoxide were two-order lower in comparison to the parent drug. This might be explained by the fact that formation of S,S-EBDM from TREO *in vivo* proceeds simultaneously with its elimination. Importantly, biological half-lives of the parent drug and its epoxy-transformer were comparable, therefore S,S-EBDM is supposed to be completely eliminated from the patients' blood within relatively short time, similar to TREO.
Disclosure of Interest: None Declared.

PH-P134
VALIDATION OF THE SPECTRA OPTIA® SEPARATOR FOR HEMATOPOIETIC STEM CELL COLLECTION: IMPACT OF PLERIXAFOR

A.-M. Eller Thomsen[1], B. Kuno Moller[1], E. Kay Segel[2], B. Sorensen[1,]*
[1]Department of Clinical Immunology, Aarhus University Hospital, Aarhus N, [2]Department of Hematology, Aarhus University Hospital, Aarhus C, Denmark

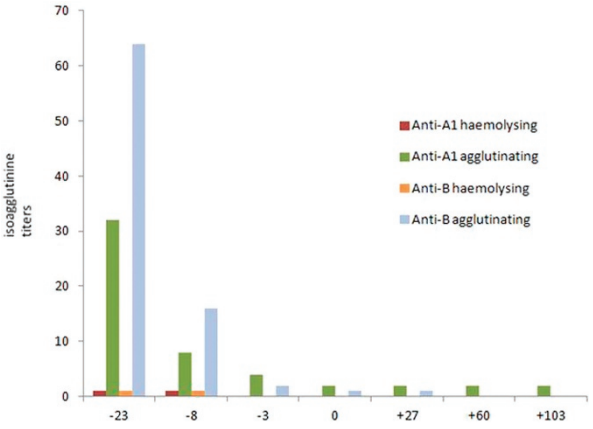
Introduction: Peripheral blood stem cells are the major source of stem cells for autologous and allogeneic hematopoietic stem cell (HSC) transplantation. In June 2012, we implemented the cell separator Spectra Optia for stem cell harvest, and since May 2013 we have used it exclusively for apheresis procedures at our center. As a standard, donors are mobilized with G-CSF, and stem cell mobilization in patients is performed with G-CSF after chemotherapy. However, in some patients, particularly after heavy chemotherapeutic pretreatment, this approach fails to yield the target number of HSC. In such cases, plerixafor is added to the G-CSF regimen. We evaluated the performance of the Spectra Optia device in the allogeneic and autologous settings and in plerixafor mobilized patients.
Materials (or patients) and Methods: In this retrospective study, 46 autologous mobilized patients underwent 124 leukapheresis procedures with Spectra Optia (Terumo BCT). Seven patients, who poorly mobilized or failed at G-CSF induced peripheral blood stem cell collection, plerixafor was used as mobilization agent (in 18 apheresis procedures). We also performed 10 stem cell collections in 8 allogeneic donors.
Procedure performance was evaluated on CD34+ cells collection efficiency (CD34 CE2) (calculated as 100 x (CD34+ cells/kg collected x body weight)/ (CD34+/μL x blood volume processed))). Data are presented as median (min-max).
Results: In the standard mobilization regimen cohort, median CD34+ cell precount was 36/μL (5-545/μL) with 9x10^9/L leukocytes (0.4-75.4x10^9/L). CD34+ cell yield was 2.8x10^6/kg (0.3-18.3x10^6/kg)

corresponding to a CD34 CE2 of 62% (21-139%). Sixty-eight procedures (64%) yielded a CD34+ cell dose above $2x10^6$/kg in a single apheresis. A median of 9944 mL (3109-16833 mL) whole blood was processed (2.3 times total blood volume (TBV) (0.5-3)) in a median of 201 min (107-277 min).
Despite a comparatively lower CD34+ cell count in the plerixafor cohort (10/µL (2-22/µL)) before the procedure, a median yield of $0.8x10^6$/kg CD34+ cells was collected in a single apheresis with Spectra Optia. We reached a similar CD34 CE2 (64% (18-144%)) as for standard mobilization. A median of 11902 mL (3550-17545 mL) of whole blood was processed (2.4 TBV; 1-2.9) in 220 min (91-252 min).
The median run time was slightly longer (261 min (148-278 min)) in the donor cohort. A median of 13211 mL (5743-18903 mL) of blood was processed (2.8 TBV; 1.5-3), and the CD34 CE2 (54%; 18-84%) was not statistically different to the one observed in autologous HSCT patients.
Discussion: We observed good clinical performance of the Spectra Optia device in harvesting allogeneic and autologous stem cells. Our experience showed that Spectra Optia delivered median CD34+ cell collection efficiencies above 50%, even in poor mobilized patients additionally treated with plerixafor.
Disclosure of Interest: None Declared.

PH-P135
SUCCESSFUL ANTI-A/B IMMUNOADSORPTION IN A PATIENT WITH MAJOR ABO-INCOMPATIBLE ALLOGENEIC BONE MARROW TRANSPLANTATION

C. Schindera[1,], J. Halter[2], T. Kühne[1], N. von der Weid[1], A. Buser[2], J. Passweg[2]*
[1]Division of Oncology/Haematology, University Children's Hospital UKBB Basel, Switzerland, [2]Department of Haematology, Basel University Hospital, Basel, Switzerland

Introduction: Major ABO-incompatible allogeneic stem cell transplantation may lead to acute and delayed haemolytic reactions, delayed red blood cell (RBC) engraftment or pure red cell aplasia. Preventive measures to overcome these risks are graft manipulation by *in vitro* RBC depletion or *in vivo* absorption of recipient's anti-A/B antibodies by slow infusion of donor-type RBCs pre transplantation. Furthermore, the use of an anti-A/B immunoadsorption column allows specific depletion of anti-A/B-titers before transplantation.
Materials (or patients) and Methods: We report on an 18 year old male patient with Fanconi anemia who underwent allogeneic HSCT with an unrelated matched (10/10) bone marrow transplant after reduced intensity conditioning with fludarabine, cyclophosphamide, ATG, and methylprednisolone. Immunosuppressive protocol consisted of cyclosporine A (started on day -7) and MMF (started on day -7). Because of major ABO incompatibility (donor AB positive, recipient 0 positive) and impossibility of red cell depletion in the stem cell product, immunoabsorption with Glycosorb®-ABO column processing twice the plasma volume with COBE Optia was performed on days -5 to -1 after administration of rituximab once on day -21.
Results: Neutrophil engraftment was on day +28. Bone marrow on day +30 showed complete remission and full donor chimerism. Bone marrow on day +100 showed complete remission, but mixed donor chimerism (85% donor type). Anti-A and anti-B isoagglutinine titers pre- and posttransplantation were as shown below. There was normal reticulocyte engraftment without signs of posttransplant pure red cell aplasia or haemolysis.
Discussion: Antigen-specific immunoadsorption with Glycosorb®-ABO column allowed an effective reduction of antibody titers in a patient with major ABO-incompatible allogeneic HSCT for Fanconi anemia. Further evaluation is needed to define the most useful approach to apply this technique in major AB0-incompatible allogeneic HSCT.
Disclosure of Interest: None Declared.



PH-P136
OUTCOME AND PROGNOSTIC INDICATORS OF ADULT PATIENTS WITHEMATOPOIETIC STEM CELL TRANSPLANTS (HSCT) ADMITTED TO THE INTENSIVE CARE UNIT (ICU)H

H. Abujazar[1,], O. Al-Rawi [1], R. Najar[2], F. Hawari [3], A. Addasi [3], F. Abdel–Rahman[1]*
[1]Bone Marrow Transplantation, [2]Pharmacy, [3]Intensive Care Unit, King Hussein Cancer Center, Amman, Jordan

Introduction: Intensive care unit (ICU) admission for bone marrow transplant patients immediately following transplantation is an ominous event. The procedure sometimes yields improved long-term survival, yet it can entail significant morbidity during the initial recovery. We tried to assess the outcome of adult hematopoietic stem cell transplantation (HSCT) patients who were admitted to ICU.
Materials (or patients) and Methods: We retrospectively reviewed the files of all adult patients underwent HSCT between January 2007 and December 2012, at a bone marrow transplantation (BMT) unit in a tertiary care medical center with a comprehensive cancer program. The primary endpoint was mortality at 6 months.
Results: A total of 313 patients received bone marrow transplantation; 42 (13.4%) of which received ICU care during BMT hospitalization. The most common cause of ICU admission is sepsis related issues. 19 (45.2%) of patients who required ICU died within 6 months. 10 of dead patients (52.6%) underwent allogeneic HSCT. Worse ICU prognosis was evident with patients who required mechanical ventilation and high APACHI II score.
Discussion: The prognosis of hematopoietic stem cell transplant admitted to the intensive care unit BMT hospitalizations is poor but should not be considered futile. The results from our study support the concept that ICU care should not be systematically withheld from bone marrow transplant recipients.
Disclosure of Interest: None Declared.

PH-P137

PHASE 2 TRIAL OF HIGH-DOSE GEMCITABINE, BUSULFAN AND MELPHALAN WITH AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) FOR PATIENTS WITH REFRACTORY HODGKIN'S LYMPHOMA (HL)

Y. Nieto[1,], U. Popat[1], P. Anderlini[1], C. Hosing[1], B. Andersson[1], B. Valdez[1], E. J Shpall[1], S. Ahmed[1], M. Qazilbash[1], P. Kebriaei[1], A. Alousi[1], R. Bassett[2], Y. Oki[3], M. Fanale[3], F. Hagemeister[3], B. Dabaja[4], V. Reed[4], C. Pinnix[4], P. Tewari[5], L. Worth[5], R. Champlin[1], R. B Jones[1]*

[1]Stem Cell Transplantation and Cellular Therapy, [2]Biostatistics, [3]Lymphoma and Myeloma, [4]Radiation Oncology, [5]Pediatrics, The University of Texas MD Anderson Cancer Center, Houston, United States

Introduction: More active HDC is needed for ASCT for refractory or poor-risk relapsed HL, where BEAM consistently offers poor results. We previously developed a regimen of infusional gemcitabine with busulfan and melphalan (GemBuMel), exploiting their synergy based on DNA damage repair inhibition (Nieto, BBMT 2012). The encouraging results seen in refractory HL patients led us to conduct a phase 2 trial of GemBuMel in this population.

Materials (or patients) and Methods: Eligibility: HL pts ages 12-65 and ≥1 of the following: 1) Primary refractory tumors (PD during or within 3 mo of 1st line chemo), 2) CR1 <12 mo, 3) relapse within a prior irradiation field, 4) extranodal tumor at relapse/PD, 5) B symptoms at relapse/PD, 6) bulky (>5 cm) relapse/PD, 7) persistent PET+ lesions after 2nd-line chemo, 8) 2nd relapse or beyond. Gem infused on d-8 & -3 with a loading dose of 75 mg/m^2 followed by a fixed dose rate of 10 mg/m^2/min over 4.5 hrs (2,775 mg/m^2/d). Each Gem dose immediately followed by Bu or Mel. Bu infused IV from d-8 to -5 (target AUC=4,000/d). Mel infused at 60 mg/m^2/d on d-3 & -2. ASCT on day 0. Response assessment of PET+ lesions was done at 1 mo. Involved field radiotherapy (IFRT) was considered for lesions >5 cm at HDC or persistently PET+ at 1 mo.

Results: Results: 65 pts, median age 31 (13-65) were enrolled between 6/11 and 10/13. 26 pts had primary refractory tumors and 39 poor-risk rel, with prior CR1 <6 mo (*N*=13), 6-12 (*N*=13) or >12 mo (*N*=13). Median # prior chemo lines: 2 (2-6). # prior rel/PD: 1: 46 pts, >1: 19 pts.

Prior xRT / Relapse within prior xRT field	16 / 8
Extranodal relapse/PD	27
B symptoms at relapse/PD	4
Bulky relapse	27
PET+ at HDC	22
Status at HDC: CR/ PR / SD / PD	43 / 15 / 3 / 4

There were no treatment-related deaths. Toxicities were manageable and reversible: mucositis (48% G2, 39% G3), skin (22% G2, 7% G3), self-limited transaminitis (34% G2, 11% G3) and hyperbilirubinemia (27% G2, 18% G3) (no cases of VOD). No cardiac,lung, renal or CNS toxicities. Neutrophils and platelets engrafted at median d+10 (8-12) and d+12 (9-21), respectively. Response and CR rates were 82% and 68%, respectively. Post-HDC IFRT to mediast+supraclav (*N*=3) and mediast+sternum (*N*=1) at 30-40 Gy started on median d+42 (41-53), with good tolerance. At median f/u of 16 mo (2-30), 22 pts have relapsed at median 5 mo (only 1 after 12 mo). 1-yr and 2-yr. EFS rates were 62% and 60%, respectively. 1-yr and 2-yr OS rates were 98%.

EFS univariate analyses: PET+ at HDC (PET 41% vs. PET- 79%, *P*=0.006), primary refractoriness (50% vs. 77%, *P*=0.01) and B symptoms at relapse/PD (25% vs. 69%, *P*=0.04) correlated with worse EFS, whereas bulky relapse, extranodal relapse or # relapses were not significant. Multivariate analyses: PET+ [HR 2.6 (95% CI, 1.1-6.1) *P*=0.03] and primary refractoriness [HR 2.3 (1-5.6), *P*=0.05] were independent adverse EFS predictors.

Discussion: This prospective phase 2 trial shows a 60% 2-yr EFS in pts with refractory/poor-risk relapsed HL. A randomized phase 3 trial comparing GemBuMel to BEAM in this population is planned.

Disclosure of Interest: Y. Nieto Conflict with: Graint-in-aid from Otsuka Pharmaceuticals, U. Popat: None Declared, P. Anderlini: None Declared, C. Hosing: None Declared, B. Andersson: None Declared, B. Valdez: None Declared, E. Shpall: None Declared, S. Ahmed: None Declared, M. Qazilbash: None Declared, P. Kebriaei: None Declared, A. Alousi: None Declared, R. Bassett: None Declared, Y. Oki: None Declared, M. Fanale: None Declared, F. Hagemeister: None Declared, B. Dabaja: None Declared, V. Reed: None Declared, C. Pinnix: None Declared, P. Tewari: None Declared, L. Worth: None Declared, R. Champlin: None Declared, R. Jones: None Declared.

PH-P138

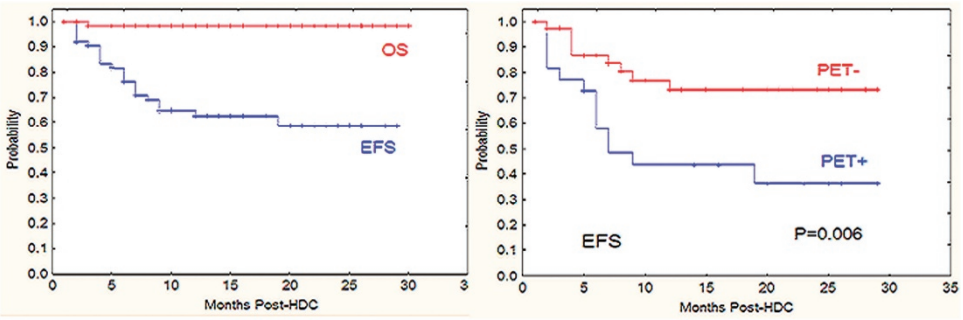
HAPLOIDENTICAL STEM CELL TRANSPLANTATION (HAPLO-SCT) WITH REDUCED INTENSITY CONDITIONING (RIC) REGIMENS AND HIGH DOSE CYLOPHOSPHAMIDE POST-TRANSPLANT (PT-CY) AS GVHD PROPHYLAXIS IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN´S DISEASE

J. Gayoso[1,], P. Balsalobre[1], C. Castilla[2], M. Jesús Pascual[3], M. Kwon[1], D. Serrano[1], L. López-Corral[4], J. Antonio Pérez-Simón[5], A. Bermúdez[6], C. Martínez[7], C. Pascual[1], I. Heras[2], C. Solano[8], A. Figuera[9], M. Antonia Sampol[10], C. Ferrá[11], P. Herrera[12], I. Buño[1], J. Luis Díez-Martín[1] and GETH (Grupo Español de Trasplante Hematopoyético)*

[1]Hematology, HGU Gregorio Marañón, Madrid, [2]Hematology, HU Morales Messeguer, Murcia, [3]Hematology, HU Carlos Haya, Málaga, [4]Hematology, HU Clínico, Salamanca, [5]Hematology, HU Virgen del Rocío, Sevilla, [6]Hematology, HU Marqués de Valdecilla, Santander, [7]Hematology, HU Clínic, Bacelona, [8]Hematology, HU Clínico, Valencia, [9]Hematology, HU La Princesa, Madrid, [10]Hematology, HU Son Espasses, Palma de Mallorca, [11]Hematology, HU Can Ruti, Badalona, [12]Hematology, HU Ramón y Cajal, Madrid, Spain

Introduction: Allogeneic transplantation is the only curative option for patients with high risk hematologic malignancies. HAPLO-HSCT offers a therapeutic option to most of these patients with the advantages of quick availability, easy programation

[PH-P137]



and logistics, and a committed donor. This procedure has shown promissing results in patients diagnosed with relapsed or refractory Hodgkin´s lymphoma (Burroughs LM *et al*. Biol Blood Marrow Transplant 2008; 14:1279-1287).
Materials (or patients) and Methods: We retrospectively evaluate the results of HAPLO-HSCT with RIC regimens (Fludarabine 30 mg/m^2 x 5 days (-6 to -2), Cyclophosphamide14,5 mg/kg x2 days (-6 to -5), Busulfan IV 3,2 mg/kg x 1-2 days (BUX, days -3 to -2) or 200 cGy TBI on day -1) and GVHD prophylaxis based on HD-CY (50 mg/kg on days +3 and +4) and a calcineurin inhibitor plus mycophenolate from day +5 performed in GETH centers to patients diagnosed with relapsed or refractory Hodgkin´s lymphoma.
Results: From March-2009, 29 HAPLO-HSCT have been performed in patients diagnosed with relapsed or refractory Hodgkin´s disease in 11 GETH centers. Median age was 31 years (18-53), 19 were males and all were in advanced phases of their disease. Autologous HSCT was previously employed in 90% of them, and allogeneic HSCT in 10%. Disease status at HAPLO-HSCT evaluated by PET was complete remission in 8 (28%) and persistent disease in 21 (72%). Bone marrow was the stem cell source in 15 (52%) and peripheral blood in 14 (48%), without T-cell depletion in all cases. The haploidentical donor was the patient´s mother (13), father (2), brother (8), sister (5) or daughter (1). The RIC regimens employed included 1 dose BUX (11), 2 doses BUX (14) or 200cGy TBI (4). Median neutrophils engraftment was day +17 (11-44) and platelets >20K was day +26 (11-150). Main toxic complications were grade II-III muchositis in 50%, febrile neutropenia in 75% and CMV reactivations in 58% with a transplant related mortality rate of 7% (2/29) at day +100 and 17% (5/29) at 6 months post-transplant. Acute GVHD grade II-IV affected to 7/28 patients at risk (25%), with grade III-IV in 3/28 (11%). Chronic GVHD was present in 3/19 (16%), being extensive in 1/19 (5%). After a median follow-up of 9 months (0.3-49), 13/22 (59%) remain alive and in complete remission. Relapse or progression occurred in 6/28 (21%). Immune reconstitution was fast and complete in those evaluated.
Discussion: Haplo-HSCT with PT-Cy is a useful tool in the treatment of patients with relapsed or refractory Hodgkin´s lymphoma, rendering long-lasting remissions with limited toxicity, low GVHD incidence and early immune reconstitution.
Disclosure of Interest: None Declared.

PH-P139

SECOND ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN PATIENTS WITH RELAPSED LYMPHOMA AFTER FIRST ALLO-SCT. A RETROSPECTIVE STUDY OF THE EBMT LYMPHOMA WORKING PARTY.

K. Horstmann[1,], A. Boumendil[2], H. Finel[3], J. F. Apperley[4], F. Ciceri[5], J. Finke[6], A. Van Hoof[7], N. Ifrah[8], S. Kyrcz-Krzemien[9], G. Milone[10], G. J Mufti[11], R. Or[12], N. H Russell[13], P. Dreger[1]on behalf of lymphoma and Lymphoma Working Group*

[1]University Hospital of Heidelberg, Heidelberg, Germany, [2]European Society for Blood and Marrow Transplantation, [3]EBMT Lymphoma Working Party, Paris, France, [4]Hammersmith Hospital, London, United Kingdom, [5]San Raffaele Scientific Institute, Milan, Italy, [6]University Medical Center Freiburg, Freiburg, Germany, [7]University Hospital St-Jan, Brugge, Belgium, [8]CHU d'Angers, Angers, France, [9]Medical University of Silesia, Katowice, Poland, [10]Grupo Argentino de Tratamiento de Leucemia Aguda, Buenos Aires, Argentina, [11]King's College London, London, United Kingdom, [12]Hadassah Hebrew University Medical Center, Jerusalem, Israel, [13]Nottingham City Hospital, Nottingham, United Kingdom

Introduction: *The aim* of this registry-based retrospective study was to analyze the outcome of second alloSCT (alloSCT2) performed in patients with malignant lymphoma who had relapsed after a first alloSCT (alloSCT1). Another purpose was to identify prognostic factors which might influence outcome after alloSCT2.
Materials (or patients) and Methods: *Primary endpoint* was disease-free survival (DFS) measured from alloSCT2; secondary endpoints were overall survival (OS), non-relapse mortality (NRM), and incidence of relapse (REL).
Eligible were patients >=18 years who were registered within the EBMT and had received an alloSCT2 for relapse of lymphoma between 2000-2011 with a minimum interval of 3 months between alloSCT1 and alloSCT2. Centers with eligible patients were contacted to provide additional treatment and follow-up information.
Statistical analysis was descriptive and employed log rank comparisons for univariate assessment of the impact of baseline characteristics on survival endpoints.
Results: 229 patients were identified who fulfilled the inclusion criteria. Additional information upon center request was provided for 84 them, resulting in exclusion of 19 patients who had been re-grafted for reasons other than relapse. The final study sample of 65 patients had a median age of 39 (18-67) years, a median interval from diagnosis to alloSCT1 of 21 (4-107) months, and a median interval from alloSCT1 to alloSCT2 of 18 (3-169) months. Diagnosis was Hodgkin's lymphoma (HL) in 29%, diffuse large B cell lymphoma (DLCL) in 14%, T cell lymphoma (TCL) in 12%, lymphoblastic lymphoma in 12%, follicular lymphoma (FL) in 11%, mantle cell lymphoma (MCL) in 11%, and other lymphoma in 11% of the patients. Remission status at alloSCT2 was CR/PR in 28% and more advanced in 72%. The same donor was used in 55% of the second allotransplants, whereas an alternative donor was used in 45%. Conditioning was myeloablative in 32% and less intensive in 68% of the alloSCT2 procedures. With a median observation time after alloSCT2 of 73 (13-73) months, DFS, OS, REL, and NRM were 26%, 37%, 55%, and 19% at 2 years; and 19%, 27%, 61%, and 19% at 5 years after alloSCT2. 45 patients died (26 from relapse and 19 from other reasons, mainly multi organ failure due to GVHD, infectious complications or toxicity). 15% of the patients suffered from grade 3/4 acute GVHD. The cumulative incidence of chronic GVHD was 34% at 2 years. Whilst DFS and OS were adversely affected by non-remission at alloSCT2 (P=0.017 and P=0.004) and interval between alloSCT1 and alloSCT2 <12 months (P=0.011 and P=0.001), underlying diagnosis (HL vs. DLCL/FL/MCL vs. TCL) and donor (same vs. alternative donor) had no significant impact.
Discussion: Although disease recurrence remains a problem, second allogeneic transplantation is a reasonable option in patients with lymphoma relapse after a first allogeneic transplantation, resulting in long-term remission in a substantial proportion of patients – in particular if the interval between alloSCT1 and alloSCT2 is 12 months or longer.
Disclosure of Interest: None Declared.

PH-P140

AUTOLOGOUS STEM CELL TRANSPLANTATION WITH BEEAM (BENDAMUSTINE, ETOPOSIDE, CYTARABINE, MELPHALAN) IN AGGRESSIVE NHL AND HODGKIN´S LYMPHOMA

T. Noesslinger[1,], M. Moestl[1], E. Koller[1], C. Tinchon[2], A. Mulabecirovic[2], W. Linkesch[3], E. Sallaberger[1], A. Hundsamer[1], F. Keil[1]*

[1]Department of Hematology and Oncology, Hanusch Krankenhaus, Vienna, [2]Department of Hematology, LKH Leoben, Leoben, [3]Department of Hematology, University of Graz, Graz, Austria

Introduction: In relapsed DLBCL and other aggressive Lymphoma Autologous Stem Cell Transplantation (ASCT) is standard of care (PARMA and Coral trial). It results in PFS in 50% of patients who proceed with ASCT (only 50% who relapse are eligible for ASCT). **B**CNU, **E**toposide, **A**ra-c, **M**elphalan is a standard conditioning regimen but BCNU is associated with interstitial pneumonia (range 2 to 20%) and a RR of death 2.27 in BCNU containing regimens compared with Busulfan or TBI based regimens. A less toxic regimen might improve the results in relapsed Lymphome patients. Bendamustine showed promising results in indolent Lymphoma and CLL. Here we report promising results with bendamustine replacing BCNU in the BEAM regimen described as BendaEAM -recently published in a phase two dose finding study (Visani, Blood 2011).
Materials (or patients) and Methods: Thirty-one patients with Hodgkin´s (HL) (n=7) or Non-Hodgkin (n=24) lymphoma were consecutively treated with Benda EAM on two consecutive days at a dose of 200 mg/m^2 per day. Eight patients were diagnosed with diffuse large B-cell lymphoma (DLBCL), seven patients with

mantle cell lymphoma, four patients with an anaplastic T-cell lymphoma, four patients with follicular lymphoma and one patient with an undefined NHL. There were twenty male and eleven female patients with a median age of 52 years (range 22-70), with 20% above the age of sixty. The median lines of previous therapies were 2 (range: 1-4). 29 patients were treated with BeEAM and 2 patients with mantle cell lymphoma received additionally Zevalin.
Results: All patients had chemosensitive disease and before transplantation twenty –six patients (84%) were in complete (CR) and 5 (16%) in partial remission. A median number of $4,12*10^6$ CD34+ cells/kg (range: 2,20-9,96) was infused.
All patients showed engraftment with a median time to achieve an absolute neutrophil count > $1*10^9$/L of 10 days (range 7-13) and to platelets >$20*10^9$/L of 11 days (range 5-26). The median time of fever was 6 days (range: 0 -22). The most common grade 3 and 4 toxicity during the whole treatment period were diarrhoea (*n*=10), mucositis (*n*=7) and febrile neutropenia (*n*=6), followedby nausea (*n*=4) and cardiologic toxicities (*n*=3). There were no pulmonary toxicities observed and no transplant related mortality occurred. The median time of follow-up is 15 months. After at least day +100 20 patients (65%) were still in CR, while 11 patients (35%) showed progression after a median time of 5 months after transplantation (range 2-14), Until today five patients (16%) died (4 DLBCL, 1 HL), all due to lymphoma progression. The 1-year PFS is 68% and the 1-year OVS 87%.
Discussion: Thus Benda EAM seems to be feasible with a promising response rate und a randomized trial comparing BendaEAM with BEAM is warranted.
Disclosure of Interest: None Declared.

PH-P141
PROGNOSTIC SIGNIFICANCE OF EBMT SCORE AND SERUM SOLUBLE IL-2R LEVEL ON OUTCOMES AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR ADULT T-CELL LEUKEMIA/LYMPHOMA

M. Tokunaga[1,], N. Nakano[1], A. Kubota[1], M. Tokunaga[1], T. Itoyama[1], T. Makino[1], S. Takeuchi[1], Y. Takatsuka[1], A. Utsunomiya[1]*
[1]Department of Hematology, Imamura Bun-in Hospital, Kagoshima, Japan

Introduction: Adult T cell leukemia/lymphoma (ATL) is a poor prognosis T cell malignancy. Recent studies show that allogeneic hematopoietic stem cell transplantation (allo-HSCT) has improved overall survival of ATL, but the prognostic factors of transplantation are less well understood. To confirm pre-transplant prognostic factors, we analyzed retrospectively 70 allo-HSCT for acute and lymphoma types of ATL patients in our institute.
Materials (or patients) and Methods: Seventy ATL patients (40 male, 30 female) consecutively received initial allo-BMT and PBSCT expect CBT between June, 1998 and May, 2013 at Imamura Bunin Hospital. This time we studied about backgrounds (age, gender, subtypes of ATL and performance status (PS) at HSCT) of the patients, details of HSCT (disease status at HSCT, stem cell sources, donor type, HLA compatibility, conditioning regimens, duration from initial therapy to HSCT, Anti-HTLV-I antibody of donor, HCT-CI and EBMT score), laboratory findings (lymphocyte count, hemoglobin level, platelet count, serum sIL2-R, BUN and Ca level) and survival duration retrospectively. The overall survival after HSCT (OS) was analyzed with Kaplan-Meir method and Cox regression analysis about OS was performed. Statistical significance defined $P<0.05$. Statistical analysis ware performed with STATA Version 12 software (Stata Corp).
Results: Median age was 52 years (32-65). Sixty-three patients were diagnosed as acute type ATL, seven as lymphoma type. Fifty-eight patients were categorized as 0-1 of PS and 12 as ≥2. Disease status at transplantation resulted in CR in 29 patients, PR in 10, SD in 5 and PD in 26 respectively. Fifty patients ware received an allo-BMT and 20 PBSCT. Thirty-six patients of 70 received stem cell from unrelated donors. Fifty patients used myeloablative conditioning (MAC) regimens. It is 5 patients that anti-HTLV-I antibody of donor was positive. The median study observation period was 300 days (10-5047). OS at 1 year and 3 years were 49% and 34.7% respectively. Courses of death were 17 disease progression, 12 infection, 8 GvHD, 5 TMA and 3 others. Adverse prognostic factors for OS by the univariate analysis were PS ≥2, HCT-CI ≥3, EBMT score ≥5, HLA mismatch, sIL2-R ≥10000 U/ml, and Ca ≥10 g/dl. EBMT score and sIL-2R were significant adverse prognostic factors by multivariate analysis (Hazard ratio: 2.5 and 4.0 respectively). Kaplan Meier survival analysis indicates OS curves are significantly divided between three groups of ATL patients undergone HSCT, according to EBMT score and sIL-2R (1. sIL2-R<10000 and EBMT score <5, 2. sIL2-R≥10000 or EBMT score ≥5 and 3. sIL2-R≥10000 and EBMT score ≥5) ($P<0.000$).
Discussion: This retrospective analysis estimates that EBMT score and serum sIL-2R level are promising prognostic factors of pre-transplant ATL patients.
Disclosure of Interest: None Declared.

PH-P142
TANDEM TRANSPLANT AUTO/AUTO OR AUTO/ALLO IN REFRACTORY AND VERY UNFAVORABLE RELAPSE OF HODGKIN LYMPHOMA (HL) PATIENTS: ANALYSIS OF EARLY DEATHS IN A PROSPECTIVE MULTICENTRIC NON INTERVENTIONAL STUDY OF THE LYSA SFGM-TC

P. Brice[1,], B. Deau[2], S. Bologna[3], A. Delmer[4], S. Lissandre[5], P. Quittet[6], D. Chaoui[7], L. Oberic[8], J. Cornillon[9], A. Clavert[10], S. Clementine[11], M. Robin[12] and LYSA and SFGM-TC*
[1]oncohematology, Hopital Saint Louis, [2]Hematology, Hopital Cochin, Paris, [3]Hematology, CHU BRABOIS, Nancy, [4]Hematology, CHU, Reims, [5]Hematology, CHU, Tours, [6]Hematology, CHU ST ELOI, MONTPELLIER, [7]Hematology, CH, Argenteuil, [8]Hematology, CHU, TOULOUSE, [9]Hematology, CH, Saint etienne, [10]Hematology, CHU, angers, [11]Hematology, CHU, PIERRE BENITE, [12]Hematology, Hopital Saint Louis, Paris, France

Introduction: Patients with refractory HL or early and disseminated relapse have a very poor outcome with a 5-years EFS of 46% with tandem autotransplantat.[1] Reduce intensity conditioning (RIC) followed with allogeneic transplantation in this setting was associated with a 4-year PFS at 24%[2] mainly because lack of stable remission at the time of allotransplant.
Fort these reasons we decided to propose to this high-risk group tandem transplantation with a second allotransplant if an HLA matched donor was available. A donor research was initiated at registration including HLA-matched sibling donor and HLA-matched unrelated donor (10/10).
Materials (or patients) and Methods: Patients received second-line chemotherapy to obtain response (assessed by PET scans) then a BEAM regimen followed 2 or 3 months later by RIC transplantation (fludarabine and busulfan regimen) or a second autotransplant (BAM regimen).
From November 2010 to October 2013, 94 patients (from 25 French centers) have been included and we will focus on the 75 first patients with available data.
Patients characteristics are: median age at 28 yrs [18-52], sex ratio at 1, 48 % of patients had primary refractory disease (biopsy proven in 50%) and 52% relapse with a median time to relapse at 6 months. First line chemotherapy was ABVD in 56 cases (intensified with BEACOPP/VABEM in 12 cases), BEACOPP in 19, eighteen patients received radiotherapy. Second line was mostly MINE, DHAP or ICE and 42% of patients received one second line chemotherapy before the BEAM regimen, the remaining received 2 to 4 lines (with Brentuximab vedotine in 9 patients) and before BEAM.
Results: 41% of patients had and HLA donor (familial in 39%), six patients did not receive any transplant for refractory disease and 16 patients progressed early after BEAM and received chemotherapy (with brentuximab vedotin in 8 cases). 70% of patients received the second transplant(allogeneic in 62%) (in tandem in 80% of cases or after CT for early relapse). 22 patients did not receive the second transplant (toxicities $n = 5$, early progression $n = 4$ insufficient stem-cell collection $n= 5$ or physician/patient decision $n = 9$). On intent to treat analysis 30% of patients had at

least one progression after inclusion. Twelve patients died in the study, 3 deaths from sepsis in patients in CR after the protocol (2 in tandem auto/allo). 12 patients had AGVH grade 4 in 2 cases in patients progressive before the transplant). One further patient progressed after autotransplant, received further CT and secondary allo and died from sepsis.
Eight patients died from HL, three patients having received no transplant due to refractory disease, 2 having received only one autotransplant and rapid progression and 3 relapsing after the procedure (auto/allo).
Discussion: This protocol is still ongoing up to 100 patients. We confirm the poor prognosis of these patients with refractory/early relapse of HL despite intensive first-line CT. The procedure is feasible without unexpected toxicity of the procedure, the main cause of death remaining progressions, Brentuximab vedotin may help to increase the number of patients achieving CR for transplant.
[1]Morschhauser *et al*. JCO 2008.
[2]Sureda *et al* Haematologica 2011.
Disclosure of Interest: None Declared.

PH-P143

THE CLINICAL ROLE OF INTERIM PET/CT FOR PREDICTING THE OUTCOME OF FRONTLINE AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA

J.-Y. Kwak[1,], S.-H. Jung[2], D.-H. Yang [2], H.-Y. Yhim[1], J.-S. Ahn[2], Y.-K. Kim[2], J.-J. Lee[2], H.-J. Kim[2]*
[1]Department of Hematology-Oncology, Chonbuk National University Medical School, Jeonju, [2]Department of Hematology-Oncology, Chonnam National University Hwasun Hospital, Hwasun, Republic Of Korea

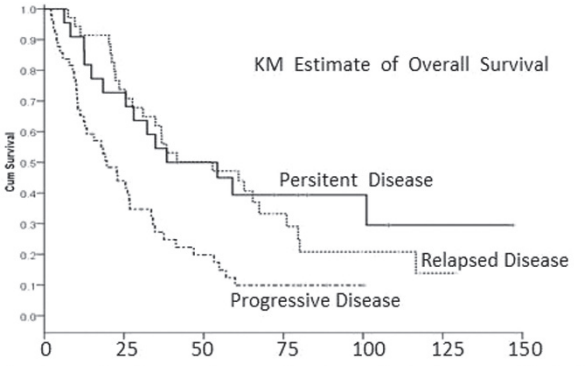
Introduction: Although several prospective studies suggested that high-dose therapy and autologous stem cell transplantation (HDT/ASCT) could improve the progression free survival in patients with poor risk diffuse large B-cell lymphoma (DLBCL), its role as an intensive consolidation remains unclear. We retrospectively investigated that interim PET/CT response could predict the clinical outcome of upfront HDT/ASCT after initial R-CHOP-21 chemotherapy in patients with high-intermediate or high-risk age-adjusted international prognostic index (aIPI).
Materials (or patients) and Methods: Sixty-six patients with newly diagnosed and high risk DLBCL were enrolled between May 2005 and January 2013. The assessment of PET/CT was performed at the time of diagnosis, the third or fourth cycle and the completion of R-CHOP-21 chemotherapy. The response evaluation of interim PET/CT was assessed based on the Deauville five-point scale (5-PS). Patients underwent ASCT with uniform conditioning regimen (busulfex, cyclophosphamide and etoposide).
Results: Over the median follow-up of 29.2 months, 8 patients in HDT/ASCT group (*n*=34) and 9 patients in non-HDT/ASCT group (*n*=32) experienced a relapse. No significant differences were observed between HDT/ASCT group and non-HDT/ASCT group for progression-free survival (PFS) and overall survival (OS). Upfront HDT/ASCT consolidation did not show survival benefits in patients (*n*=46) with favorable interim PET/CT response scoring 5-PS grade 1 or 2. However, among patients with unfavorable interim PET/CT response scoring 5-PS grade 3 or 4, the PFS was significantly high in HDT/ASCT consolidation group (*n*=13) compared to patients with R-CHOP-21 only group (*n*=7) (60.6 vs. 42.3 months, respectively) (*P*= 0.020). In addition, upfront HDT/ASCT resulted in significantly improved OS rates in patients with unfavorable interim PET/CT response (*P* = 0.022).
Discussion: Unfavorable interim PET/CT response (5-PS with grade 3 or 4) had a significant predictive potential for predicting disease progression and survival after HDT/ASCT consolidation in patients with high risk DLBCL. However, patients with favorable response (5-PS with grade 1 or 2) in interim PET/CT analysis should be considered delayed HDT/ASCT after salvage chemotherapy or another therapeutic option for preventing disease progression.
Disclosure of Interest: None Declared.

PH-P144

DIFFERENCE IN THE OUTCOMES OF HODGKIN LYMPHOMA PATIENTS WHO HAD PERSISTENT VS PROGRESSIVE VS RELAPSED DISEASE FOLLOWING HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS-SCT

S. Akhtar[1,], M. Shahzad Rauf[1], T. Ahmed M Elhassan[1], I. Maghfoor[1]*
[1]Oncology Center, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia

Introduction: Hodgkin lymphoma (HL) patients with persistent, progressive or relapsed disease following high dose chemotherapy and autologous-SCT (HDC ASCT) have dismal outcome. Some patients can still have disease control or even cure with radiation therapy (XRT) and/or chemotherapy. Limited patients are offered allogeneic-SCT. We evaluated the survival difference among patients with persistent, progressive or relapsed disease following HDC ASCT.
Materials (or patients) and Methods: Patients with relapsed or refractory HL who had HDC ASCT (1997-2012) at our institution were identified and grouped as persistent, progressive or relapsed disease after HDC ASC. Kaplan-Meier (KM) method was used to plot estimated overall survival (OS).
Results: Two hundred and seventy-eight (278) patients with HL had HDC ASCT, 106 patients had transplant failure (considered as event); grouped by (1) persistent disease (22 patients (21%)): (2) progressive disease (49 patients (46%)) and (3) relapsed disease (35 patients (33%)). Male 62%, female 38%. Median age at ASCT 23.5 years (14 - 60 years), 78% were <30 years. Stages at the time of event were I:II:III:IV:unknown % in 15:14:12:47:11 % respectively. Lung was the most common extranodal site in 31% at 1st event. Median follow-up is 80 months for alive patients (15–147 months); 4/23 alive patients had <40 months follow-up. Twenty three (22%) patients are alive and 12/23 in remission, 83 (78%) patients died (2/83 died of other cause). First post ASCT treatment offered to these patients was chemotherapy alone 31%, XRT alone 32%, chemotherapy + XRT 10%, supportive care only / no treatment 26%. Complete remission after above intervention was 21%. Curative intent treatment was planned for 53%. One patient had 2nd auto-SCT (in CR now) and 7 had mini/full allogeneic-SCT, 5 died of progressive disease and 2 in CR. For entire group, median OS from day 0 was 32 month (29% 5 year survival). OS according to the type of events is shown in the table.
Discussion: Patients with persistent disease post HDC ASCT have superior outcome as compared to other groups. This group may signify relative biologically and clinically indolent behaviour. Once relapsed post HDC ASCT, disease is rapidly progressive and frequently fatal. Allogeneic-SCT in the studied cohort failed to



Variable	Total	Alive Number (%)	Dead Number (%)	KM- OS from day 0 (months)	P-value	KM OS from Event (months)	P-value
Total patients	106	23 (22)	83 (78)	32			
Persistent	22	8 (36)	14 (64)	38		33	
Progressive	49	8 (16)	41 (84)	20	0.001	16	0.024
Relapsed	35	9 (26)	26 (74)	53		19	

produce disease control. Many patients with HDC ASCT failure were able to get palliative treatment and some of them are long term survivors.
Disclosure of Interest: None Declared.

PH-P145
EFFICACY OF HIGH-DOSE THERAPY AND AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION INTENSIFICATION AFTER RITUXIMAB-BASED FRONT-LINE TREATMENT OF AGGRESSIVE LYMPHOMAS: RESULTS OF A SYSTEMATIC REVIEW AND META-ANALYSIS

A. Kumar[1], T. Reljic[1], M. Hamadani[2], P. Castrovinci[1], T. Nishihori[3], E. Ayala[3], B. N Savani[4], B. Djulbegovic[1], M. A Kharfan-Dabaja[3,]*
[1]Evidence-Based Medicine, University of South Florida, Tampa, [2]Hematology-Oncology, Medical College of Wisconsin, Milwaukee, [3]Blood and Marrow Transplantation and Oncologic Sciences, H. Lee Moffitt Cancer Center and University of South Florida College of Medicine, Tampa, [4]Medicine, Vanderbilt Univ. Medical Center, Nashville, United States

Introduction: Uncertainty remains in regards to the role of high-dose therapy (HDT) and autologous hematopoietic cell transplantation (auto-HCT) in the front-line treatment of aggressive lymphomas. Accordingly, this systematic review/meta-analysis aims at evaluating the totality of evidence pertaining to the efficacy of HDT and auto-HCT as treatment intensification after front-line therapy with rituximab-based chemotherapy regimens in aggressive lymphomas.
Materials (or patients) and Methods: A total of 1956 references were identified through a systematic search of PUBMED/MEDLINE (*n*=1616) and abstracts from relevant conference proceedings or trial registries (*n*=340) performed on September 27, 2013. Four references were considered eligible for inclusion in our analysis; however, one of these represented an ongoing clinical trial (*Le Gouill et al. J Clin Oncol 29: 2011 (suppl; abs 8003)*). Data was meta-analyzed for benefits: complete remission (CR) rates (data from 2 studies, total *n*=485), progression-free (PFS) (data from 2 studies, total *n*=652), event-free (EFS) survival (data from 1 study, total *n*=248), and overall survival (OS) (data from 3 studies, total *n*=900); and harms: grade 3/4 adverse events (AE) (data from 1 study, total *n*=399) including infection-related AEs (data from 1 study, total *n*=253), and treatment-related mortality (TRM) (data from 3 studies, total *n*=900) using the random effects model.
Results: Three studies enrolled a total of 900 subjects in the chemoimmunotherapy-only (CIT) (group 1, *n*=455) or the CIT followed by HDT/auto-HCT (group 2, *n*=445) arm. There was no difference in CR rates [risk ratio (RR)=0.90 (95%CI=0.69, 1.18), *P*=0.45], EFS [hazard ratio (HR)=0.80 (95%CI=0.50, 1.27), *P*=0.35], and OS [HR=0.84 (95%CI=0.65, 1.08), *P*=0.17]. Group 2 had superior PFS [HR=0.55 (95%CI=0.42, 0.72), *P*<0.0001]. Only one study had extractable data to assess the impact of the IPI score, showing that subjects with high-risk IPI had better 2-year PFS [75% vs. 41%, *P*=0.001] and 2-year OS [82% vs. 64%, *P*=0.01] with HDT/auto-HCT. This was not the case for patients with intermediate-high risk IPI: 2-year PFS [66% vs. 63%, *P*=0.32] and 2-year OS [70% vs. 75%, *P*=0.48]. Moreover, group 2 had higher incidence of grade 3/4 AEs [RR=2.25 (95%CI=1.62, 3.12), *P*<0.001] or infection-related AEs [RR=3.79 (95%CI=2.36, 6.11), *P*<0.001]; but treatment intensification with HDT/auto-HCT did not result in worse TRM [RR=1.71 (95%CI=0.87, 3.37), *P*=0.12].
Discussion: Treatment intensification with HDT and auto-HCT improves PFS but not OS after rituximab-based CIT regimens in aggressive lymphomas. One study suggests a survival (PFS and OS) benefit of HDT/auto-HCT in patients with high-risk IPI but such survival benefit was not apparent in the intermediate-high risk IPI subgroup.
Disclosure of Interest: None Declared.

PH-P146
BASE-LINE QUALITY OF LIFE IMPAIRMENT AND ITS POTENTIAL PROGNOSTIC VALUE IN LYMPHOMA PATIENTS UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT)

D. A. Fedorenko[1,], T. I. Ionova[1], V. Y. Melnichenko[1], N. E. Mochkin[1], K. A. Kurbatova[2], G. Gorodokin[3], A. A. Novik[1]*
[1]National Pirogov Medical Surgical Center, Moscow, [2]Multinational Center for Quality of Life Research, St.Petersburg, Russian Federation, [3]New Jersey Center for Quality of Life and Health Outcome Research, Saddle River, United States

Introduction: The independent prognostic value of baseline QoL was reported for different types of cancer. The performance status of lymphoma patients undergoing AHSCT is an important prognostic factor, in doing so QoL assessment before AHSCT might be an informative tool for predicting treatment outcome in this patient population. The data regarding the prognostic value of baseline QoL in lymphoma patients undergoing AHSCT is lacking. We aimed to examine QoL in lymphoma patients before AHSCT and to analyze the prognostic value of pretreatment QoL.
Materials (or patients) and Methods: A total of 124 lymphoma patients: non-Hodgkin lymphomas–45 (36.3%) patients, Hodgkin lymphoma–79 (63.7%), mean age–34 years old (range 19–65); male/female–53 (42.7%)/71(57.3%). All the patients were treated by AHSCT. Mean follow-up was 19 months. QoL was assessed using generic SF-36 questionnaire. Integral QoL Index was calculated by the method of Integral Profiles. To compare patients with normative data the sample from a population norm (PN) data base adjusted for age and gender (*n*=204) was used. For comparisons t-test for independent samples or Mann-Whitney test were applied. Overall survival was calculated using the Kaplan-Meier method.
Results: Before AHSCT 38% of patients exhibited no QoL impairment–Integral QoL index was similar to the one in the population sample. 19% of patients had mild QoL impairment (<25% decrease of Integral QoL index from a PN); 12% of patients – moderate QoL impairment (25-50% decrease from a PN); 21% – severe QoL impairment (50-75% decrease from a PN), and 10% – critical QoL impairment (>75% decrease from a PN). 5-years overall survival in patients with severe and critical QoL impairment was 78% in comparison with 66.7% of patients with no or mild QoL impairment (*P*=0.02).
Discussion: One third of lymphoma patients exhibited severe or critical QoL impartment before ASCT. The baseline QoL impairment in lymphoma patients was significantly associated with overall survival after AHSCT. Thus, treatment QoL impairment may be a feasible prognostic factor in lymphoma patients undergoing AHSCT. Further studies are needed to examine prognostic value of baseline QoL impairment in lymphoma patients undergoing AHSCT.
Disclosure of Interest: None Declared.

PH-P147

BENDABEAM PLUS AUTOLOGOUS STEM CELL TRANSPLANT PRODUCE A 3-YEAR PROGRESSION-FREE SURVIVAL RATE OF 75% IN HEAVILY PRE-TREATED HODGKIN AND NON-HODGKIN LYMPHOMA

G. Visani[1,], P. M. Stefani[2], S. Capria[3], L. Malerba[1], P. Galieni[4], F. Gaudio[5], G. Specchia[6], G. Meloni[3], F. Gherlinzoni[2], C. Giardini[1], M. Gobbi[7], A. Santoro[8], F. Ferrara[9], M. Rocchi[10], E. M Ocio[11], M. D. Caballero[11], F. Loscocco[1], A. Isidori[1]*
[1]AORMN, Hematology and Stem Cell Transplant Center, Pesaro, [2]Ca'Foncello Hospital, Hematology, Treviso, [3]University "La Sapienza", Department of Biotechnologies and Hematology, Roma, [4]Hematology, Ascoli Piceno, [5]University of Bari Medical School, Division of Hematology, [6]University of Bari Medical School, Hematology, Bari, [7]San Martino Hospital, Division of Hematology, Genova, [8]Department of Oncology and Hematology, Istituto Humanitas, Rozzano, [9]Cardarelli Hospital, Hematology, Napoli, [10]University of Urbino, Insitute of Biomathematics\, Urbino, Italy, [11]Department of HematologyUniversity Hospital & Cancer Research Center (IBMCC-CSIC), University of Salamanca, Salamanca, Spain

Introduction: We demonstrated the safety of a new conditioning regimen to autologous stem cell transplant (ASCT) consisting of bendamustine, etoposide, cytarabine, and melphalan (Benda-BEAM) in resistant/relapsed lymphoma patients (EUDRACT-number2008-002736-15). The regimen was proven to be highly effective (80% CR rate). At the time of publication (Visani *et al*, Blood 2011), disease type (NHL vs HD) and disease status at transplant (chemosensitive versus chemoresistant) were significantly influencing PFS (*P*=0.01; *P*=0.007). However, median follow-up for surviving patients was short (18 months), and, therefore, it was not possible to draw final conclusions on the efficacy.
Materials (or patients) and Methods: With this aim in mind, we evaluated the efficacy of the BendaBEAM regimen in terms of disease-free (DFS) and overall survival (OS) after a median follow-up of 41 months. Forty-three patients with resistant/relapsed NHL (28) or Hodgkin lymphoma (HD, 15) were consecutively enrolled in the study. Twenty-one patients had primary refractory disease, whereas 22 had relapsed disease, 5 of whom where in second or subsequent relapse, at the time of enrolment. The primary objective of the study was to determine the 36-months event free survival rate.
Results: we updated the follow-up at 41 months after transplant. Thirty-one out of 43 patients are still in CR (72%), as documented by both PET and CT scan. Two patients with HD were refractory and rapidly died, whereas 10/43 patients (23%) relapsed after a median time of 7.5 months (range:3-23) from transplant. Five patients died (3 NHL, 2 HL), whereas 5 patients are still alive after relapse. Median PFS and OS were still not reached.
Conversely, 3-year PFS was 75%, allowing our study to met its primary end-point. Interestingly, disease type (HD versus NHL) at transplant is no longer influencing PFS (*P*=0.7), and still does not influence OS (*P*=0.1). On the other hand, disease status at transplant (chemosensitive vs chemoresistant) is still a strong predictor of both PFS and OS (*P*=0.03 and *P*=0.009, respectively). At present, one patient developed myelodisplasia after transplant. No other late effects were observed up to now.
Discussion: The new BendaBEAM regimen successfully met the primary end-point and confirmed its safety, after 41 months of follow-up. Interestingly, NHL and HD were not statistically different in terms of both PFS and OS at 41 months of observation.
Disclosure of Interest: None Declared.

PH-P148

SPLENECTOMY PRIOR TO ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION INCREASES THE RISK FOR POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE

M. Norström[1,], J. Mattsson[1], M. Remberger[1,2], M. Uhlin[1,2]*
[1]Centre for Allogeneic Stem Cell Transplantation, Karolinska University Hospital, [2]Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden

Introduction: Splenectomy before allogeneic stem cell transplantation (ASCT) is usually performed in some individuals diagnosed with lymphoma and myelofibrosis. However, much is still unknown regarding the impact of splenectomy on the outcome of ASCT. Previous work has suggested an association between acute and chronic graft versus host disease (GVHD) and splenectomy prior to ASCT. Conversely, more recent work failed to see this correlation and instead showed an association between splenectomy and relapse after chronic myeloid leukemia.
Materials (or patients) and Methods: Therefore, we performed a retrospective analysis on the impact of splenectomy on clinical outcome in patients transplanted at Karolinska University Hospital between 1995 and 2012. A total number of 1158 patients were included in this study (31 with splenectomy and 1127 without). Different clinical parameters were analyzed after ASCT.
Results: The only incidence that was significantly different between patients with or without splenectomy was the occurrence of post transplant proliferative disease (PTLD, *P*=0.001). No difference could be observed regarding transplant related mortality or overall survival. In a multivariate analysis, including parameters that were significantly different between the two patient groups, splenectomy was the only factor significantly associated to PTLD (*P*= 0.01).
Discussion: This finding might argue for a distinct role of the spleen in the control of Epstein Barr virus (EBV) associated PTLD. The results also highlight that there might be a potentially higher risk for EBV-PTLD in patient sub-categories treated with anti-thymocyte globulin (ATG) that have undergone splenectomy. Further, multi-center studies on patients treated with ATG is needed in order to verify our findings on the role of the spleen and EBV biology.
Disclosure of Interest: None Declared.

PH-P149

REDUCED INTENSITY CONDITIONING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ADULTS WITH RELAPSED AND REFRACTORY MANTLE CELL LYMPHOMA IN THE RITUXIMAB ERA

A. Mussetti[1,2,], S. M. Devlin[3], H. R. Castro-Malaspina[2], J. N. Barker[2], S. Giralt[2], C. Sauter[2], M.-A. Perales[2]*
[1]Universita' degli Studi di Milano, Milan, Italy, [2]Department of Medicine, Adult Bone Marrow Transplant Service, [3]Department of Biostatistics and Epidemiology, Memorial Sloan Kettering Cancer Center, New York City, United States

Introduction: We report the results of reduced-intensity-conditioning (RIC) allo-HCT in a retrospective cohort of patients with mantle cell lymphoma.
Materials (or patients) and Methods: Twenty-nine patients (median age 58 years, range 34-71) undergoing RIC allo-HCT from April 1999 to May 2013 are included in this retrospective analysis. The median number of previous lines of therapy was 5 (range 1-6) with 13 (45%) of patients having previously failed an autologous HCT. Twenty-six patients (90%) had chemosensitive disease at allo-HCT (CR=17, PR=9) and 3 (10%) had stable disease. The second line International Prognostic Index (sIPI) was 0 in 19 (65%) and >1 in 9 patients (31%). Data was missing in 1. RIC regimens included cyclophosphamide/ fludarabine/TBI 200cGy with (*n*=17) or without (*n*=4) peri-transplant rituximab and melphalan/ fludarabine with (*n*=6) or without (*n*=2) alemtuzumab. All patients received unmodified grafts from a matched related (*n*=12), matched unrelated (*n*=10) or mismatched unrelated (*n*=7) donor. Progression-free (PFS) and overall (OS) survival were

calculated from the time of allo-HCT. Kaplan-Meier survival curves and a permutation-based logrank test were used to compare PFS and OS based on alemtuzumab use and the sIPI.
Results: All but one patient engrafted with full donor chimerism. The cumulative incidences (CI) of grade II-IV acute GVHD at days +100 and +180 were 36% (95%CI: 19-53%) and 46% (95%CI: 27-64%), respectively. The CI of chronic GVHD at 1 and 2 years was 20% (95%CI: 7-38%) and 29% (95%CI: 12-49%), respectively. The CI of progression of disease and non-relapse mortality at 2 years were 32% (95%CI: 15-51%) and 19% (95%CI: 7-37%), respectively. With a median follow-up in survivors of 40 months (range 2-80 months), the 2-year OS and PFS are 64% (95%CI: 47-86%) and 49% (95%CI: 32-73%), respectively. *In vivo* T cell-depletion with alemtuzumab was associated with a markedly reduced 2-year PFS (0% vs 64%, *P*=0.008; Figure 1). Conversely, a sIPI at transplantation <1 was associated with a much improved 2-year PFS compared to sIPI >1 (65% vs 13%, *P*=0.021; Figure 2). Similarly, 2-year OS was also significantly reduced with alemtuzumab (33% vs 74%, *P*=0.016) and in patients with sIPI >1 (39% vs 76%, *P*=0.036).
Discussion: RIC allo-HCT is a feasible and effective strategy in patients with relapsed and refractory mantle cell lymphoma. High sIPI and use of alemtuzumab in the conditioning regimen are associated with markedly inferior PFS. Higher risk disease and the likely loss of graft-versus-lymphoma with alemtuzumab predict likelihood of failure of RIC allo-HCT in relapsed and refractory MCL patients
Disclosure of Interest: None Declared.

PH-P150
THIOTEPA-BASED HIGH-DOSE PREPARATION VS BEAM FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AUTOHSCT) IN LYMPHOMA OTHER THAN PCNSL: A RETROSPECTIVE UPDATE FROM THE EBMT

P. Dreger[1,], A. Boumendil[1]on behalf of EBMT Lymphoma Working Party, H. Finel[1], G. de Rosa[1], M. Martelli[1], V. Liso[1], R. Scime[1], M. Janvier[1], P. Mazza[1], G. Kobbe[1], D. Bunjes[1], I. Majolino[1], F. Ferrara[1], A. Rambaldi[1], A. Delmer[1], A. Sureda[1] and EBMT Lymphoma Working Party*
[1]Lymphoma Working Party, EBMT, Paris, France

Introduction: Thiotepa is an alkylating agent approved for high-dose therapy for autologous HSCT. Because of its excellent capacity to cross the blood-brain barrier, it is regularly used for autoHSCT for primary CNS lymphoma (PCNSL). However, although thiotepa-based myeloablation might have benefits over traditional BEAM preparation because of better brain availability and less pulmonary toxicity, clinical level information about thiotepa-based autoHSCT outside of the PCNSL field is sparse. The purpose of the present retrospective study was to provide information on the potential risks and benefits of thiotepa-based preparative regimens in autoHSCT for distinct subtypes of lymphoma outside of the PCNSL setting.
Materials (or patients) and Methods: **PRIMARY OBJECTIVE** was to compare the outcome of thiotepa-based autoHSCT (TT) with that of BEAM autoHSCT (BEAM) separately for diffuse large B-cell lymphoma (DLBCL; excluding PCNSL), follicular lymphoma (FL), peripheral T Cell lymphoma (PTCL) and Hodgkin's lymphoma (HL). **PRIMARY ENDPOINT** was progression-free survival (PFS), secondary endpoints were overall survival (OS), non-relapse mortality (NRM), and incidence of relapse (IR). **ELIGIBLE** were patients >=18 years from 12 European countries who were registered with the EBMT and had received TT-based myeloablation or BEAM for a first autoHSCT between 2003-2011 for FL, DLBCL, PTCL, or HL, and had Med A level information available. **STATISTICAL ANALYSIS** was based on a 1:2 matched pair comparison using stratified Cox and Fine & Gray regression models for comparison. Matching factors were age (+/– 10 years), sex, lymphoma subtype, time from diagnosis to autoHSCT, remission status at autoHSCT, performance status at autoHSCT and year of autoHSCT.
Results: 517 patients with TT fulfilled the inclusion criteria and were matched with 994 BEAM patients (40 patients had only one match). Of these 1511 patients, 67% had DLBCL, 9% FL, 2% PTCL, and 22% HL. Remission status at autoHSCT was CR/PR1 in 43%, CR/PR >1 in 30%, more advanced disease in 17%, and unknown in 10%. Because of the low numbers PTCL were excluded from endpoint analyses. The hazard ratio for PFS with TT vs BEAM in DLBCL, FL, and HL was 1.05 (0.77-1.41), 2.06 (0.87-4.89), and 0.89 (0.46-1.73). Similarly, no significant differences between TT and BEAM became evident in any lymphoma subset for OS, NRM, and IR.
Discussion: Outcome of TT-based autoHSCT was not significantly different from BEAM autoHSCT for DLBCL, FL, and HL. These results warrant further studies on TT-based high-dose therapy as alternative to BEAM in these lymphoma subtypes.
Disclosure of Interest: None Declared.

PH-P151
ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING REGIMEN IN PATIENTS WITH RELAPSED HODGKIN´S LYMPHOMA

M. Marta Rivas[1,], M. Berro[1], M. Virginia Prates[2], S. Yantorno[2], J. Milone[2], A. Lisa Basquiera[3], B. Adriana [3], J. José García[3], G. Remaggi[4], J. Martinez Rolon[5], R. Burgos[6], C. Foncuberta[6], L. Feldman[7], G. Jaimovich[7], J. Real[8], V. Milovic[9], J. Arberbide[10], M. Makiya[10], E. Dibar[10], G. Kusminsky[1] and GATMO*
[1]Hematology and Bone Marrow Transplantation, Hospital Universitario Austral, Pilar, [2]Hematology and Bone Marrow Transplantation, Hospital Italiano de La Plata, La Plata, [3]Hematology and Bone Marrow Transplantation, Hospital Privado de Córdoba, Cordoba, [4]Hematology and Bone Marrow Transplantation, Fundaleu, Ciudad A de Buenos Aires, [5]Hematology and Bone Marrow Transplantation, Fundaleu, [6]Hematology and Bone Marrow Transplantation, Instituto A Flemming, [7]Bone Marrow Transplantation, Fundación Favaloro, [8]Bone Marrow Transplantation, [9]Bone Marrow Transplantation, Hospital Aleman, [10]Hematology and Bone Marrow Transplantation, Hospital Italiano de Buenos Aires, Cuidad A de Buenos Aires, Argentina

Introduction: Hodgkin´s Lymphoma (HL) is a highly curable disease. However, there are still patients with primary refractory disease or who relapse after first-line treatment, or even after high-dose chemotherapy with hematopoietic cell rescue. Allogeneic stem cell transplant (ASCT) is therapeutic for this patients. We analyzed the experience in 8 Argentine Medical Centers with patients with refractory /relapse HL who received ASCT with reduced intensity conditioning (RIC) regimen.
Materials (or patients) and Methods: We performed a retrospective multicenter analysis from data obtained from medical records. For statistical analysis SPSS (17.0) and R (2.9.1) were used. Overall survival (OS) and disease free survival (DFS) were analyzed with Kaplan Meier curves. Relapse and non relapsed mortality were analysed by cumulative incidence analysis. For the multivariate analysis were included only variables that in the univariate analysis have had a $p \leq 0.2$. Fifty-four patients with relapsed HL who received ASCT had a median age of 26years. The relationship between male / female was 1/1. Only 3 patients (5.5%) at the time of transplant had a performance status> 1 according to ECOG. Ninety-six percent of the patients had received previously autologous transplant. Most patients 43 (80%) received an identical sibling donor transplant . All patients receiving unrelated donor transplants had *in vivo* lymphocyte depletion as prophylaxis of graft versus host disease. Forty-three patients (79.6%) received as a conditioning regimen Fludarabine + Melphalan. The disease status at transplant was: complete remission (CR) 33%, partial remission (PR) 54%, stable disease / progressed (SD / PD) 13%.
Results: With a median follow up of 2.7 years, actuarial overall survival (OS) at 1 and 5 years was 65% and 20% respectively and disease free survival (DFS) at 1 and 5 years was 35 % and 18% respectively. The incidence of acute GVHD grade II-IV was 31%. Patients in CR at the time of transplant showed significant differences compared with those who were not in CR in DFS (1-5 years 52-27% vs 19-14%, *P*=0.01), OS (1-5 years 76-38% vs 59-13%, *P*=0.02) and non relapsed mortality (NRM) (1-5 years 6-12% vs 34-39%, *P*=0.04). Age, PS, the use Fludarabine + Melphalan as

conditioning regimen, unrelated donor, aGVHD, were not variables that modified the overall survival and disease-free survival.
Discussion: The ASCT with RIC regimen is a feasible therapeutic option in patients with HL, especially in patients who can reach CR. The low rate of DFS is still an issue in this setting, may be new drugs may help in optimizing pretransplant response status to improve patients' outcome.
Disclosure of Interest: None Declared.

PH-P152

DA-EPOCH +/- R FOLLOWED BY CONSOLIDATION WITH AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN THE FIRST-LINE TREATMENT OF POOR-RISK, AGGRESSIVE NON-HODGKIN LYMPHOMA (NHL) – A SINGLE CENTER EXPERIENCE

V. Pejsa[1], Z. Prka[1,], M. Lucijanic[1], Z. Mitrovic[1], R. Ajdukovic[1], M. Pirsic[1], R. Kusec[1], M. Purgaric[2], I. Maricic[2], O. Jaksic[1]*
[1]Hematology, [2]Transfusiology, University hospital Dubrava, Zagreb, Croatia

Introduction: We hypothesized that combination of dose-adjusted (DA)-EPOCH +/- R followed by ASCT as a consolidation in the first-line therapy provide high efficacy with acceptable toxicity in patients with poor-risk, aggressive NHL.
Materials (or patients) and Methods: From May 2007 to December 2012, a total of 23 patients with poor-risk, aggressive NHL defined as IPI≥3 or T-cell NHL (except ALK+) or Ki-67+≥90%, were planned to receive total of six to eight cycles of DA-EPOCH+/–R followed by consolidation with ASCT as a part of first- line therapy. In DA-EPOCH doxorubicin, vincristine and etoposide are infused over 96 hours, cyclophosphamide and prednisone are administered on a bolus schedule, and doxorubicin, etoposide and cyclophosphamide are pharmacodynamically dose-adjusted based on the neutrophil nadir. On sixth day all patients received pegylated granulocyte colony-stimulating factor (peg G-CSF, Neulastim®). Criteria needed for transplant eligibility were: age <65, Ki-67+≥90% or IPI≥3 or T-cell NHL (except ALK+), and absence of transplant-limiting comorbidities. Myeloablation with BEAM regimen was performed in all patients. Three patients discontinued DA-EPOCH+/-R due to prolonged cytopenias, fatal reactivation of hepatitis B, and patient refusal, respectively. The remaining 20 patients proceeded to ASCT. Ten (50%) were males, median age was 42,5 years (range 21-58). There were 11 (55%) patients with DLBCL, five had T-cell NHL (3 ALCL/2 ALK-/ and 2 T-NOS), two had mantle cell lymphoma, and two had Burkitt lymphoma. A total of 14 patients (70%) had increased LDH, 15 (75%) were Ann Arbor III/IV, and 13 (65%) patients had IPI≥2. Prior to ASCT, 15 (75%) patients were in complete remission, five in partial remission.
Results: After median follow up of 36 months, 3-year OS and PFS were 73% and 75%, respectively (Table 1). According to original protocol, median dose escalation was three times per patient (172%). In 15 (75%) patients, last cycle of DA-EPOCH was successfully used for stem cell mobilization. During treatment and follow-up period there was no unexpected toxicities, cardiovascular complications or, although too early, secondary malignancies. There was no transplantation-related mortality.
Discussion: CHOP+/– R is not optimal therapy for patients with poor-risk, aggressive NHL, especially for subgroup of younger patients. Efforts to improve outcome with dose-dense regimens showed similar results, whereas dose-intensification strategies showed improvement at the cost of higher toxicity. DA-EPOCH, due to its continuous low-dose drug exposure and dose modification according to patients' hematopoietic capacity, allows us to apply highest acceptable doses of drugs. ASCT is not established as a part of first-line therapy, however, therapeutic benefit was observed in high-risk group of patients in some studies. Our results showed that ASCT as a consolidation after DA-EPOCH +/- R is acceptable and efficacious therapeutic option in the treatment of patients with poor-risk, aggressive NHL, without significant additional toxicities.
Disclosure of Interest: None Declared.

PH-P153

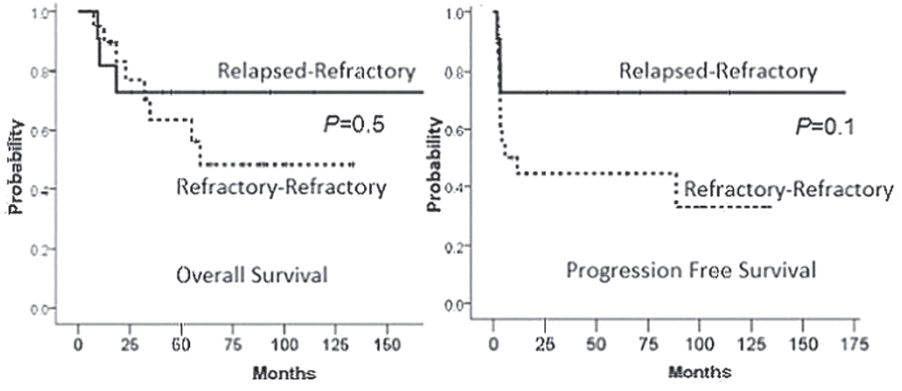
SECOND LINE SALVAGE REGIMENS PRIOR TO HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANT ARE A VALID OPTION IN PROGRESSIVE HODGKIN'S LYMPHOMA PATIENTS REFRACTORY TO FIRST LINE SALVAGE REGIMENS

M. Shahzad Rauf[1,], I. Maghfoor[1], T. Ahmed M Elhassan[1], S. Akhtar[1]*
[1]Oncology Center, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia

Introduction: Patients with relapsed and refractory Hodgkin's lymphoma (HL) unable to undergo high dose chemotherapy and autologous stem cell transplant (HDC ASCT) have a reported survival of 15-20%. Those failing to respond to first-line salvage chemotherapy (1st-salvage) may be offered second line salvage chemotherapy (2nd-salvage) and may receive HDC ASCT. This study aimed to determine the outcome of HL patients who failed to respond to 1st-salvage and received 2nd-salvage prior to HDC ASCT.

[PH-P153]

Type of Group	OS %	PFS %	PFS Median	OS Median
Whole Group	57	51	NR	NR
Relapsed-Refractory	73	73	NR	NR
Refractory-refractory	56	40	6 months	NR



Materials (or patients) and Methods: We identified 31 patients who required 2nd-salvage prior to ASCT from 1996 to 2012. Patient were grouped as (1) relapsed-refractory, defined as patients with prior CR and on relapse found refractory to 1st-salvage and required 2nd-salvage and (2) refractory-refractory, defined as patients refractory to primary treatment and also refractory to 1st-salvage and given 2nd-salvage. Kaplan-Meier (KM) method was used for overall (OS) and progression free survival (PFS).
Results: 278 patients with HL received HDC ASCT. Of these, 31 patients received both 1st-salvage and 2nd-salvage prior to HDC ASCT. Male:Female 18:13, median age at HDC ASCT was 22 years (14-44 years). Response to 2nd-salvage was complete response (CR) 16%, partial response (PR) 71%, and stable disease 13%. After HDC ASCT, CR: PR: progressive disease was observed in 16(52%): 4(12%): 9(29%) patients respectively. Overall response (CR+PR) in 20 patients (65%). 15 patients progressed within 12 months ASCT. 11/31 patients died; (3/11 died within 12 months, 8/11 from 12 to 60 months). Five years OS for entire cohort: relapsed-refractory: refractory-refractory groups is 57: 73: 56% (*P*=0.5) while PFS is 51: 73: 40% (*P*=0.1) respectively, *P*-value not significant due to small sample size. Both median OS and PFS have not yet been reached in any subgroup except refractory-refractory group whose median PFS is 6 months.
Discussion: Though long-term outcome of patients requiring two salvage regimes was inferior to patients requiring one salvage regimen, it appears better than literature reports with chemotherapy without ASCT. We conclude that HDC ASCT is valid approach for HL patients even when more than one salvage chemotherapy regimens is required for tumor reduction. relapsed-refractory seems to have better outcome than refractory-refractory in our study. Importantly, using this didn't lead to excessive toxicity such as delayed engraftment or chemotherapy related toxic deaths.
Disclosure of Interest: None Declared.

PH-P154
HEMATOPOIETIC STEM CELL TRANSPLANTATION IN REFRACTORY OR RECURRENT LYMPHOMAS OF CHILDREN AND ADOLESCENTS: A MULTICENTER SURVEY OF TURKISH PEDIATRIC BMT STUDY GROUP

V. Hazar[1,], V. Kesik[2], S. Aksoylar[3], A. Küpesiz[4], G. Öztürk[5], E. Ataş[2], M. Karakükcü[6], G. Karasu[7], S. Kansoy[3], A. Yeşilipek[4], F. Erbey[5], S. Anak[8], V. Uygun[9], E. Ünal[6], S. Yılmaz[10], A. Tanyeli[11], M. Elli[12], D. Atay[5], N. Taçyıldız[13], Z. Kaya[14], N. Kurucu[15], E. Ünal[16], Ü. Koçak[14]*
[1]Department of Pediatric Hematology&Oncology and BMT Unit, Medipol University Faculty of Medicine, İstanbul, [2]Department of Pediatric Oncology and BMT Unit, Gülhane Military Medical Academy, Ankara, [3]Department of Pediatric Oncology and BMT Unit, Ege University Faculty of Medicine, İzmir, [4]Department of Pediatric Hematology & Oncology and BMT Unit, Akdeniz University Faculty of Medicine, Antalya, [5]Pediatric BMT Unit, Medical Park Bahçelievler Hospital, İstanbul, [6]Department of Pediatric Hematology&Oncology and BMT Unit, Erciyes University Faculty of Medicine, Kayseri, [7]Pediatric BMT Unit, Medical Park Göztepe Hospital, [8]Department of Pediatric Hematology&Oncology and BMT Unit, İstanbul University İstanbul Medical Faculty, İstanbul, [9]Pediatric BMT Unit, Medical Park Antalya Hospital, Antalya, [10]Department of Pediatric Hematology and BMT Unit, Dokuz Eylül University Faculty of Medicine, İzmir, [11]Department of Pediatric Oncology and BMT Unit, Çukurova University Faculty of Medicine, Adana, [12]Department of Pediatric Oncology and BMT Unit, On Dokuz Mayıs University Faculty of Medicine, Samsun, [13]Department of Pediatric Oncology and BMT Unit, Ankara University Faculty of Medicine, [14]Department of Pediatric Hematology and BMT Unit, Gazi University Faculty of Medicine, [15]Department of Pediatric Oncology and BMT Unit, Ankara Oncology Hospital, [16]Department of Pediatric Hematology and BMT Unit, Ankara University Faculty of Medicine, Ankara, Turkey

Introduction: To examine the role of hematopoietic stem cell transplantation (HSCT) for pediatric patients ≤ 18 years old at the diagnosis with refractory or recurrent lymphomas as a multicenter survey in Turkey.
Materials (or patients) and Methods: To evaluate the role of HSCT we retrospectively analyzed the outcome of 122 patients from 14 centers in Turkey who underwent autologous (*n*=90) or allogeneic (*n*=32) HSCT because of refractory or recurrent lymphomas. The median age at the time of HSCT was 14 (range, 4-20)(42F/80M). There were 49 non-Hodgkin lymphomas (NHL) (Lymphoblastic Lymphoma, *n*=18, Burkitt's lymphoma, *n*=7; Anaplastic Large Cell Lymphoma, *n*=14 and undifferentiated NHL, *n*=2) and 73 Hodgkin Lymphoma (HL) patients. Donor source of allogeneic HSCT was HLA-matched related donor (*n*=24), matched unrelated donor (*n*=7) or mismatched related donor (*n*=1). Eighty two were chemosensitive disease (either CR or very good PR) while 42 were chemorefractory disease at the time of transplant. Risk factors (gender, conditioning regimen, transplant type, duration of the first remission and disease status at HSCT) affecting relapse free survival (RFS) were evaluated using stratified Cox regression.
Results: At the time of study, 99 of 122 patients were alive, 23 patients expired. Sixteen patients died due to progressive disease. Non-relapse mortality rate was 5.8%. Median relapse time was 5 months (range, 1-50). Overall survival (OS) and RFS were 72.6% and 64.0% with a median follow-up of 23 months (range, 1-185 months) for all patients, respectively. There was a tendency of better outcome in patients receiving allogeneic HSCT, in terms of both OS and RFS (at 5 years, OS 87.0% vs 68.1%, *P*=0.210 and RFS 70.6% vs 60.3%, *P*=0.670). Disease status (chemosensitive relapse) at HSCT and the duration of the first remission (>12 months) were predictive of RFS in multivariate analysis (*P*=0.000 and *P*=0.035, respectively). While OS and RFS for patients with HL were 66.7% and 57.6%, respectively, 81.9% and 70.5% were in patients with NHL. Disease status was the independent risk factor for RFS in patients with both HL and NHL (*P*=0.013 and *P*=0.000, respectively). Patients with lymphoblastic lymphoma and anaplastic large cell lymphoma had a RFS of 69.9% and 82.5%, respectively. Relapse free survival rate were 6/8 and 4/7 patients transplanted for diffuse large B cell lymphoma and Burkitt's lymphoma.
Discussion: HSCT gives the opportunities of relapse free survival for a significant proportion of children with relapsed or refractory lymphoma. Survival is better among patients with chemosensitive disease at HSCT.
Disclosure of Interest: None Declared.

PH-P155
ROLE OF FRONTLINE CONSOLIDATION WITH STEM CELL TRANSPLANTATION IN SYSTEMIC PERIPHERAL T-CELL LYMPHOMAS

G. Gritti[1,], C. Boschini[1], A. Rossi[1], F. Delaini[1], A. Grassi[1], A. Algarotti[1], C. Micò[1], R. Trezzi[2], A. Gianatti[2], A. Maria Barbui[1], A. Rambaldi[1]*
[1]Hematology, [2]Pathology, Ospedale Papa Giovanni XXIII, Bergamo, Italy

Introduction: Outcome of peripheral T-cell lymphomas (PTCL) is unsatisfactory and no controlled clinical study guides the therapy. Phase II studies suggest to consolidate response achieved after front-line treatment with autologous stem cell transplant (SCT) and the role of first-line allogeneic SCT is currently under investigation. However, no comparative data is available in this setting.
Materials (or patients) and Methods: We retrospectively evaluate the impact of front-line SCT consolidation in 209 patients treated from January 1990 to December 2012 at our Center. Patients treated with a palliative intent and primary cutaneous T-cell lymphomas were excluded from the study. Histologic revision was performed in all cases. Specific diagnosis were PTCL not otherwise specified (32%), anaplastic large-cell lymphoma (ALCL) anaplastic lymphoma kinase (ALK) positive (34%) and negative (17%), angioimmunoblastic T-cell lymphoma (10%), enteropathy-associated T-cell lymphoma (5%) and others (2%).
Results: Median age was 49 years (range 15-85) with a prevalence of male sex (61%), advanced stage (68%) while IPI was >2 in 44%. Primary treatment were MACOP-B (39%) CHO(E)P (39%), intensive regimens (18%) or others (4%). Complete response to primary treatment (i.e. before SCT consolidation) was 60% (5% partial remission). Outcome of primary responders was good, with a

3-year overall survival of 74% (82% in ALCL ALK+ and 69% for the other histologies). By multivariate analysis a better overall survival was significantly associated with IPI<2 (*P*=0.001), primary response (*P*=0.000), and ALCL ALK+ histology (*P*=0.012). We then focus on the 126 responding patients that either received (32%) or not (68%) a SCT consolidation. With the exception of age, the clinical characteristic of those patient were similar. In patients receiving SCT the stem cell source was mostly autologous (*N*=41/44). The multivariate analysis performed on responders, showed that only IPI was predictive of a better survival (*P*=0.006) while ALCL ALK+ histology (*P*=0.083) and performing SCT (*P*=0.303) were not.
Discussion: Response to primary treatment rather than post-remissional programs is the crucial determinant of PTCL outcome.
Disclosure of Interest: None Declared.

PH-P156

FAVORABLE OUTCOME FOR PATIENTS WITH LYMPHOID MALIGNANCIES FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION USING FLUDARABINE TREOSULFAN, COMPARED WITH OTHER REDUCED INTENSITY CONDITIONING REGIMENS

R. Yerushalmi[1,], N. Shem-Tov[1], Y. Volchek[1], I. Danylesko[1], A. Avigdor[1], A. Nagler[1], A. Shimoni[1]*
[1]Division of Hematology and Bone Marrow Transplantation, Chaim Sheba Medical Center, Tel-Hashomer, Israel

Introduction: Allogeneic SCT is potentially curative therapy for patients (pts) with lymphoid malignancies. Myeloablative conditioning is often not feasible in these pts. Reduced intensity conditioning (RIC) allows SCT by reducing non-relapse mortality (NRM) but may be associated with increased relapse rate. Fludarabine and treosulfan (FT) is a dose intense reduced toxicity regimen predominantly explored in myeloid malignancies. There is only limited data on FT in lymphoid malignancies.
Materials (or patients) and Methods: We evaluated the outcome of 144 pts with lymphoid malignancies given allogeneic SCT with FT (30-36 gr/m^2, *n*=50), fludarabine and busulfan (6.4 mg/m^2, FB2, *n*=38) or fludarabine melphalan (100-140 mg/m^2, FM, *n*=56).
Results: Median age was 52 (16-69) years. 115 pts had non Hodgkin lymphoma of various histologies and 29 had Hodgkin lymphoma. 87 pts (60%) had a prior autologous SCT (autoSCT). 13 pts (9%) had comorbidity index (HCT-CI) ≥3. 69 pts (48%) had chemosensitive disease at SCT and 75 (52%) had refractory disease. The donor was a sibling (58%) or matched unrelated (42%). The FT and FB2 groups had more pts with prior autoSCT than the FM group, 76%, 79% and 34%, respectively (*P*=0.001). There were no other differences in pt characteristics between the 3 groups. With median follow up of 39 months (4-149), 58 pts are alive and 86 died, 52 of NRM and 34 of relapse. The 3 year overall survival (OS) rate was 42% (32-49%). Disease status at SCT was the most significant predictor of OS. Pts with chemosensitive and refractory disease had OS of 62% and 20%, respectively (*P*<0.001). The 3 year OS was 54%, 43% and 29% after FT, FB2 and FM, respectively (*P*=0.02). Multivariate analysis (MVA) identified age>50 (HR=2.0, *P*=0.03), HCT-CI≥3 (HR=6.0, *P*<0.001), FM (HR=3.1, *P*=0.01) and refractory disease (HR=3.5, *P*<0.001) to be associated with shortened OS. The correlation between OS and regimen was dependant on disease status at SCT. There was no significant difference among the regimens in chemosensitive disease, 3-year OS 67%, 74% and 48% after FT, FB2 and FM, respectively (*P*=0.28). However, there was an advantage for FT in refractory disease. OS was 34%, 11% and 17%, respectively (*P*=0.03). MVA limited to pts with refractory disease showed HCT-CI≥3 (HR=5.0, *P*=0.007) and FT (HR-0.4, *P*=0.07) to be significant factors. NRM was 24%, 22% and 54% after FT, FB2 and FM, respectively (*P*=0.004). MVA identified age>50 (HR=2.1, *P*=0.07), HCT-CI≥3 (HR=6.3, *P*=0.001), FM (HR=5.1, *P*=0.007), refractory disease (HR=2.0, *P*=0.04) and sibling donor (HR=0.5, *P*=0.03) as independent factors for NRM. Relapse mortality was 22%, 35% and 18%, respectively (*P*=0.4). MVA identified HCT-CI≥3 (HR=4.2, *P*=0.04), refractory disease (HR=13.9, *P*<0.001) and prior autoSCT (HR=5.1, *P*=0.03) as adverse factors.
Discussion: FT is a promising preparative regimen for allogeneic SCT in pts with lymphoid malignancies. Dose intensity is important in lymphoid malignancies. FT and FM are both dose intensive regimens as reflected by lower relapse rates than FB2. FT is associated with lower NRM than FM, in similarity to the favorable profile of FB2. These effects result in a more favorable OS with FT, in particular in advanced disease. A subset of pts with refractory disease could also be salvaged. This regimen merits further study in larger comparative studies.
Disclosure of Interest: None Declared.

PH-P157

ALLOGENEIC STEM CELL TRANSPLANTATION AFTER AUTOLOGOUS SCT FOR PRIMARY MEDIASTINAL B CELL LYMPHOMA IN THE RITUXIMAB ERA: A RETROSPECTIVE STUDY BY THE EBMT LYMPHOMA WORKING PARTY

I. Avivi[1,], A. Boumendil[2], H. Finel[3], G. Socié[4], G. Gutiérrez García[5], R. Or[6], D. Beelen[7], L. Castagna[8], G. Wulf [9], N. Kröger[10], C. Berthou[11], F. Kroschinsky[12], J. Portratz[13], P. Dreger[14] and EBMT Lymphoma Working Party*
[1]Hematology and Bone Marrow Transplantation, Rambam Health Care Campus, Haifa, Israel, [2]EBMT Paris Study Office /, CEREST-TC, [3]EBMT Paris Study Office /, CEREST-TC, [4]Hopital St. Louis, Paris, France, [5]Hospital Clinic, Barcelona, Spain, [6]Hadassah University Hospital, Jerusalem, Israel, [7]University Hospital, Essen, Germany, [8]Istituto Clinico Humanitas, Rozzano Milano, Italy, [9]Universitätsklinikum Göttingen, Göttingen, [10]University Hospital Eppendorf, Hamburg, Germany, [11]CHRU, Brest, France, [12]Universitaetsklinikum Dresden, Dresden, [13]Diakoniekrankausen, Rotenburg, [14]University of Heidelberg, Heidelberg, Germany

Introduction: Evidence from the DLBCL setting suggests that allogeneic stem cell transplantation (alloSCT) is a reasonable salvage therapy for patients progressing despite undergoing autologous SCT (autoSCT and even as an alternative to autoSCT. Data regarding the effects of alloSCT in patients with primary mediastinal B cell lymphoma (PMBCL) are very limited. The objective of the current study was to investigate the outcome of alloSCT in patients with PMBCL who were previously treated with rituximab-based regimens and autoSCT.
Materials (or patients) and Methods: All EBMT registered patients diagnosed with PMBCL who had undergone alloSCT between 2001 and 2011 as second transplant after a previous autoSCT were eligible. Patient characteristics, disease- and transplant-related data were collected from MED-A forms. Centers with potentially eligible patients were contacted to provide additional treatment and follow-up information, including a written histopathology report.
Results: 12 patients, 5 females (42%) and 7 males with confirmed PMBCL, were included. The median age was 27 years, ranging from 21-to 61 years. Median time from diagnosis to alloSCT and from autoSCT to alloSCT were 14 (IQR 11-17 months and 7 months (IQR 4-9) respectively. Fifty-eight percent (*N*=7) had received at least 3 chemotherapeutic regimens prior allograft; 11 (92%) had been treated with rituximab-containing regimen and 67% (*N*=8) received mediastinal irradiation prior allosct. Evaluation of disease status at transplantation revealed that 42% (*n*=5) were in CR1/PR1 (alloSCT was performed here as part of a tandem transplant program), 17% (*n*=2) were in CR/PR>1 and 42% (*n*=5) were transplanted with refractory disease. 50% (*n*=6) were transplanted with residual mediastinal mass larger than 5 cm. Within a median follow up of 36 months, only 3 patients remained alive, whereas 9 patients died due to disease progression (*n*=3) or non-relapse mortality (*n*=6).
Discussion: The current preliminary study does not suggest that alloSCT for PMBCL in the rituximab era can benefit a large proportion of patients. Larger studies are warranted to further define the role of allograft in this specific indication.
Disclosure of Interest: None Declared.

PH-P158

TREATMENT RESPONSE OF EATL PATIENTS IN AMSTERDAM

P. Nijeboer[1,], O. Visser[2], L. de Baaij[1], C. J. J. Mulder[1], S. A.G.M. Cillessen[3], G. Bouma[1]*

[1]Gastroenterology, [2]Haematology, [3]Pathology, VU Medical Center Amsterdam, Amsterdam, Netherlands

Introduction: Enteropathy-associated T-cell lymphoma (EATL) is a rare T-cell Non-Hodgkin Lymphoma. Based on clinical presentation EATL can be divided into two subgroups; EATL can arise in celiac disease (CD) patients without a known history of celiac disease (EATL de novo) or EATL manifests in adult patients with previously diagnosed (refractory) celiac disease who clinically deteriorate (secondary EATL). Currently, there are no standardized treatment protocols and prognosis of EATL remains poor. We evaluate extended follow-up of EATL patients referred to our tertiary celiac disease center (1999-2013).

Materials (or patients) and Methods: A total of 61 patients with EATL were included. The diagnosis of EATL was established according to the WHO. We evaluated treatment strategies and overall survival (OS). Treatment response was assessed as complete remission (CR), stable disease (SD) or progressive disease (PD).

Results: In 31/61 patients (51%) EATL was diagnosed in patients without previous history of (refractory) celiac disease (EATL de novo). The remaining 30/61 (49%) patients suffered from secondary EATL (RCDII *n*=19. CD *n*=11). Nineteen patients (31%) were treated with chemotherapy combined with surgery. Treatment consisted of systemic chemotherapy in 12 (20%), surgery in 12 (20%), chemotherapy and resection with autologous stem-cell transplantation (auSCT) in 5 (8%) and chemotherapy and resection with allogenic stem-cell transplantation (alloSCT) in 2 (3%). CR was achieved in 23 patients (38%), SD in 5 patients (8%), and PD in 33 patients (54%). CR was mainly achieved after SCT therapy. Overall the relapse rate in CR patients was 52% with a median follow-up of 13 months after therapy (range 1-54 months). Patients treated with chemotherapy and resection with auSCT showed a relapse rate of 40%. With a median survival of 6 months (range 0 – 142 months), 50/61 patients died (82%). One, three- and five-years OS was 37 %, 16 % and 10 % respectively. Patients who were treated with chemotherapy and resection with auSCT showed a median survival of 15 months. Patients with EATL de novo showed better survival compared to patients with secondary EATL (*P* = 0.006).

Discussion: Best treatment response and the lowest relapse rate is achieved after intensive therapy (auSCT). Overall survival remains poor, although intensive chemotherapy and resection with auSCT improve survival. International cooperation is warranted to evaluate new treatment options.

Disclosure of Interest: None Declared.

PH-P159

OUTCOME ANALYSIS OF 152 PRIMARY REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PATIENTS YOUNGER THAN 60 YEARS MANAGED WITH AND WITHOUT HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION

S. Akhtar[1,], H. Soudy[1], M. Shahzad Rauf[1], A. Rehman[1], T. Ahmed M Elhassan[1], I. Maghfoor[1]*

[1]Oncology Center, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia

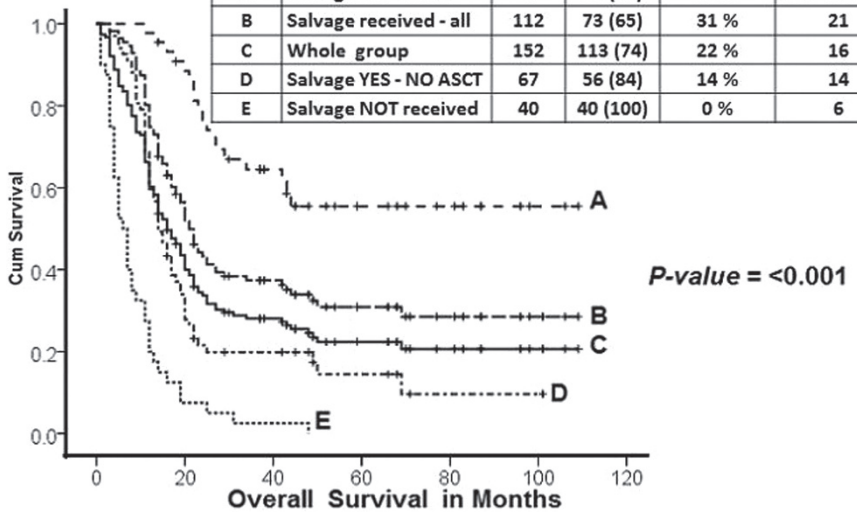
Introduction: Larger proportion of patients with diffuse large B cell lymphoma (DLBCL) refractory to anthracycline based chemotherapy (with or without rituximab) have very poor outcome due to rapidly progressive course. Only a small percentage of patients manage to complete salvage regimens and undergo HDCT. Most literature reports only patients who completed HDCT and ASCT. This report includes outcome analysis of patients with primary refractory DLBCL with and without HDC ASCT.

Materials (or patients) and Methods: Patients with DLBCL from 2002-2011 were identified from Oncology research unit/lymphoma data base. Primary refractory DLBCL is defined as patients with persistent, progressive disease on chemotherapy/chemo-immuno +/– radiation therapy or relapsed disease within three months of finishing treatment. Kaplan-Meier method (KM) was used for overall survival (OS) from the date of diagnosis.

Results: Five hundred and eighty (580) patients with DLBCL were identified during 2002-2011. A total 152 patients were identified as refractory cases. Of these, male:female 78:74, stages I-II vs III-IV 33 vs 113 (6 unknown). Median follow-up for alive patients is 52 (14 – 109 months). Median age at diagnosis was 40 years. Rituximab + chemotherapy was given to 60/152 (39%) patients. 40 /152 (26%) had rapidly progressive disease after 1-2 cycles of primary chemotherapy and were not considered fit for salvage chemotherapy. 112/152 received 1 or more cycles of salvage

[PH-P159]

Graph Line	Group	Number	No. died (%)	5 yr Survival KM	Median OS Months - KM
A	Salvage YES + ASCT	45	19 (40)	56 %	Not reached
B	Salvage received - all	112	73 (65)	31 %	21
C	Whole group	152	113 (74)	22 %	16
D	Salvage YES - NO ASCT	67	56 (84)	14 %	14
E	Salvage NOT received	40	40 (100)	0 %	6



chemotherapy, 67/112 (60%) were not eligible for HDC ASCT (58, 6 and 3 patients couldn't get this due to suboptimal response, comorbidities and patient refusal respectively). 45/112 (40%) who responded to salvage chemotherapy were able to get HDC ASCT; 27 (60%) are alive in remission, 16 (36 %) died of disease and 2 died of other causes. KM estimates for above mentioned groups are shown in table/graph.
Discussion: Substantial number of primary refractory-DLBCL failed to receive HDC ASCT and they have poor outcome. These patients are potential candidate for newer and novel treatment approaches. On the other hand, those who responded to salvage chemotherapy and received HDC ASCT enjoyed comparatively superior outcome.
Disclosure of Interest: None Declared.

PH-P160

EARLY DOSE INTENSIFICATION WITH AUTOLOGOUS STEM CELL TRANSPLANTATION RESULTS IN IMPROVED DISEASE CONTROL IN PATIENTS WITH PTCL NOS, AILD AND ALCL

K. Fritsch[1,], A. Baumgarten[1], H. Bertz[1], J. Finke[1], R. Marks[1]*
[1]University Hospital Freiburg, Germany, Freiburg, Germany

Introduction: Since no standard therapy for T cell lymphoma exists, CHOP-like therapies were used due to known effects in B-NHL. In addition, the impact of dose intensification and first line high-dose therapy with autologous stem cell transplantation (ASCT) is even less well defined. Here we present longterm follow up data from a single center using early dose intensification and first line high dose therapy/ASCT in patients with T-NHL.
Materials (or patients) and Methods: From 12/1986-07/2009 a total of 115 patients were treated at the University Hospital Freiburg. The median age was 56 years (range: 18-90), the specific diagnoses included: PTCL NOS (*n*=46), AILD (*n*=25), ALCL Alk negativ (*n*=26), and others (*n*=18). Initial chemotherapy included anthracycline based/CHOP-like regimens in most of the cases. If no complete remission (CR) could be achieved by CHOP-like induction, early intensification, mainly with VIPE/VCPE (epirubicin 50 mg/m^2, etoposide 500 mg/m^2, cisplatin 50mg/m^2, ifosfamide 4 g/m^2 or cyclophosphamide 1350 mg/m^2) or DHAP regimens, and ASCT after BEAM conditioning (66.6% of all ASCT) was initiated.
Results: After a median follow up of 13,9 years 5-year overall survival (OS) of the entire group was 49.8 %.
In detail, 5y-OS only for pts. with PTCL NOS was 56.5%. With the mentioned therapeutic approach, 34/46 (73.9%) PTCL NOS pts. experienced CR after primary therapy. Of those 34 CR pts., CR was achieved after first induction in 18 cases, and after intensification in additional 6 cases, while the remaining 10 pts. experienced CR only after ASCT. Despite the primary high-dose therapy with ASCT, 5y-OS was not significantly improved compared to pts. without ASCT (61.1% versus 53.6%). Nevertheless, cumulative 5 year relapse rate (RR) for pts. in CR was significantly worse for patients not undergoing ASCT (75% vs. 21.4%).
For pts. with ALCL Alk neg. 5y-OS with/without primary ASCT was 56.4% versus 38.5%. 14/26 (54%) experienced CR after primary therapy. Of those 14 pts, 7 pts. achieved CR after first induction, additional 3 pts. achieved CR after intensification and another 4 pts. achieved CR only after high-dose therapy with ASCT. Cumulative 5-year RR for patients with ALCL Alk neg in CR did not significantly differ for those undergoing high-dose therapy versus those who did not (11,1 versus 40 %).
In contrast, in AILD pts. treatment with ASCT resulted in a significantly improved 5-year OS (74.1% versus 21.9%). 14/25 (56%) experienced CR after primary therapy. Of those 14 pts., 7 achieved CR after induction therapy, another 2 pts achieved CR after intensification and another 5 pts. achieved CR after high-dose therapy. Of those 5 pts, one patient had allogeneic transplantation. Cumulative 5-year RR for patients in CR was 42.9% for those undergoing high-dose therapy versus 71.4% for those without.
Discussion: These longterm follow up data show that chemotherapy with primary high dose intensification in patients with T-NHL results in improved disease control. Maintenance therapy might be of interest for patients responding the therapy but not being eligible for ASCT.
Disclosure of Interest: None Declared.

PH-P161

TEAM (THIOTEPA,ETOPOSIDE,CYTARABINE,MELPHALAN) AS CONDITIONING REGIMEN FOR LYMPHOMA TREATMENT WITH AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT)

A. M. Carella[1,], G. Palumbo[2], E. Merla[1], M. Greco[1], A. Fontana[3], G. Specchia[4], G. Pisapia[5], P. Mazza[5], A. Guarini[6], A. Rana[6], P. Ditonno[7], F. Ferrara[8], P. Musto[9], D. Pastore[4], R. Scimè[10], F. Pellegrini[3], S. Capalbo[2], A. Maria Cavallaro[10], N. Cascavilla[1]*
[1]Hematology, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, [2]Hematology, Ospedali Riuniti, Foggia, [3]Biostatistics, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, [4]Hematology, Università Bari, Bari, [5]Hematology, Ospedale san Giuseppe Moscato, Taranto, [6]Hematology, Istituto Tumori Giovanni Paolo II, [7]Oncologia, Ospedale Di Venere, Bari, [8]Hematology, Ospedale Cardarelli, Napoli, [9]Hematology, CROB, Rionero in Vulture (PZ), [10]Hematology, Ospedali riuniti Villa Sofia Cervello, Palermo, Italy

Introduction: To date Hodgkin's disease (HD) and Non-Hodgkin Lymphoma (NHL) are considered as chemoresponsive-tumors, but some patients never achieve a remission or relapse after an initial response. High dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) has clearly shown to improve disease free survival in relapsing or chemorefractory patients if compared to salvage chemotherapy. From March 2007 to December 2011, 120 (80 males, 40 females) patients with refractory or relapsed aggressive Hodgkin (*n*=51) or Non Hodgkin Lymphoma (*n*=69) after first or second line chemotherapy, were included in a prospective multicenter trial to evaluate the efficacy and toxicity of a new preparative conditioning regimen based on Thiotepa, aracytin, etoposide and melphalan (TEAM) before autologous haematopietic stem cells transplantation (ASCT).
Materials (or patients) and Methods: Patients: At day -7 received 10 mg/kg thiotepa (5 mg twice every 12 hours) followed by cytarabine 200 mg/m^2 die (-5 to -3) etoposide 200 mg/m^2 die (-5 to -3) and melphalan 140 mg/ m^2 (day -2). The median age of patients was 47 years (range: 18-68 years). Median CD34+ cells doses were 5.3 x 10^6 /kg (range: 2.37-15). Before transplantation, 86% of patients had responsive disease, 45 of them (37.5%) were in complete remission and 58 (48.3%) showed a first or following partial remission. Lastly 17 patients (14.2%) developed a progression of disease.
Results: All patients achieved neutrophil and platelets recovery after a median time of 10 and 12 days, respectively. Grade III-IV mucositis occurred in 12 (10%) patients. The overall response rate was 84.8% (98 complete remissions, 82.3%; 3 partial remissions 2.5%). In particular 7/17 (41%) primary refractory patients achieved first complete remission after TEAM treatment. Eighteen patients (15%) did not respond. The cumulative treatment-related mortality was less than 1% (*n*=1). The median follow-up times for event free-survival (EFS), progression-free survival (PFS) and overall survival (OS) were: 2.3, 1.9, 2.34 years from the date of transplant, respectively. Multivariate marginal Cox regressions which included: patient's age, sex, the presence of Non Hodgkin Lymphoma, disease progression before ASCT, disease duration from the date of diagnosis to the ASCT and PEG G-CSF undertaken, showed that disease progression before ASCT was the only independent factor statistically associated to EFS (*P*=0.002), PFS (*P*<0.001) and OS (*P*=0.027). Moreover, results further suggested that the presence of Non Hodgkin Lymphoma could represent a potential independent predictor for patient's mortality risk.
Discussion: In conclusion, the novel TEAM regimen is considered to be comparable with the other preparative regimens, in particular with BEAM which is characterized by a low-risk of early treatment-related mortality.
Disclosure of Interest: None Declared.

PH-P162

LOWER RELAPSE AND BETTER PROGRESSION FREE SURVIVAL FOR CHEMOSENSITIVE HODGKIN LYMPHOMA PATIENTS UNDERGOING HAPLOIDENTICAL AS COMPARED TO HLA-IDENTICAL DONOR TRANSPLANTATION

L. Castagna[1,], R. Crocchiolo[1], S. Bramanti[1], S. Furst[2], L. Giordano[1], B. Sarina[1], J. El Cheikh[2], S. Garciaz[2], A. Granata[2], F. Broussais[2], R. Devillier[2], D. Coso[2], R. Boubdallah[2], C. Chabannon[2], C. Carlo-Stella[1], A. Santoro[2], D. Blaise[2]*

[1]Humanitas Cancer Center, Rozzano, Italy, [2]Institut Paoli Calmettes, Marseille, France

Introduction: We compared HL patients with chemosensitive disease, treated by T-cell replete haploidentical transplantation and post-infusion Cy, with a cohort of similar patients who underwent an HLA-identical transplantation from related and unrelated donor. The aim was to detect differences in survival (progression free survival PFS and overall survival, OS) and toxicity non relapse mortality NRM, acute GVHD and chronic GVHD).

Materials (or patients) and Methods: All patients received a NMAC/RIC (reduced intensity conditioning). We analyzed the results achieved in chemosensitive patients (CR and PR) (Haplo *n*= 29, HLA-id *n*= 19), undergoing to transplantation during the same period.

Results: The median time of observation for all patients was 24.8 months (1.1-49.8), not different between the 2 groups. Overall, 2-year PFS and OS were 66% and 79%, respectively. After Haplo and HLA-id HSCT, 2-year PFS was 78% vs 41% (p 0.03), and 2-year OS was 81% vs 76% (p 0.4), respectively. The relapse incidence was statistically lower in the Haplo group (11% vs 38%, p 0.019). The 2-year NRM, incidence of grade 2-4 acute GVHD and chronic GVHD were 10% vs 12% (p 0.8), 26% vs 21% (p 0.8), and 12% vs 21% (p 0.3), in the Haplo and HLA-id group, respectively. Combining disease status and donor type, we founded that 2-y PFS was better for the group CR-Haplo (86%), followed by CR-HLAid (60%) and PR-Haplo (52%), and PR HLAid (20%). The 2-y relapse rate was inversely distributed in these 4 groups (50% vs 22% vs 21% vs 8%, respectively).

Discussion: Present analysis suggested that haploidentical transplantation using T-cell replete stem cells and post-infusion cyclophosphamide, for high-risk chemosensitive HL patients, is effective and safe, challenging the question of donor choice (Haplo vs HLA-id). However, more data and prospective comparative studies are needed to draw definitive conclusions.

Disclosure of Interest: None Declared.

PH-P163

MOBILIZATION OF HEMATOPOIETIC STEM CELLS BY A BENDAMUSTINE-CONTAINING REGIMEN IN HODGKIN'S LYMPHOMA: RESULTS UPDATE

A. Anastasia[1,], C. Carlo-Stella[2], A. Pulsoni[3], F. Merli[4], C. Gabutti[5], A. Re[1], L. Castagna[2], G. Annechini[3], A. M. Liberati[6], S. Gandolfi[2], E. Brusamolino[2], A. Santoro[2]*

[1]Hematology, Spedali Civili Brescia, Brescia, [2]Hematology, Istituto Clinico Humanitas, Rozzano, [3]Hematology, University La Sapienza, Rome, [4]Hematology, Azienda Ospedaliera Arcispedale S.Maria Nuova IRCCS, Reggio Emilia, [5]Hematology, AO Niguarda, Milan, [6]Hematology, AO S.Maria Policlinico Monteluce, Terni, Italy

Introduction: Bendamustine has demonstrated efficacy as single agent in several lymphoproliferative disorders, including Hodgkin's lymphoma (HL). Despite the wide use of this compound, alone or in combination, there are no published data regarding its mobilizing activity. In 2011, we started a phase II open-label prospective study with Bendamustine, Gemcitabine and Vinorelbine (**BeGEV**) to evaluate the efficacy of this induction regimen before high dose chemotherapy plus autologus stem cell transplant (ASCT). One of the study objectives was to detect the role of Bendamustine as part of a mobilizing regimen for peripheral blood stem cell (PBSC) collection.

Materials (or patients) and Methods: Between August 2011 and September 2013, 36 consecutive patients with relapsed/refractory HL were enrolled in a Phase II open-label prospective study with BeGEV followed by ASCT. The treatment schedule was: Bendamustine (90mg/sqm, days 2-3), Gemcitabine (800mg/sqm, day 1 and 4) and Vinorelbine (25mg/sqm, day 1) plus G-CSF 10mcg/Kg beginning on day 7 continued daily until the target yield would be reached. PBSC collection was planned starting from cycle 1 or from cycle 3 in case of bone marrow involvement. Three million CD34/Kg were considered as the minimum cell dose established for a safety rescue. Other than successful rate of harvest, we evaluated the absolute number of collected CD34+ cells/Kg, the number of procedures performed per cycle, preleukapheresis circulating CD34+ cells/mcL, white blood cells (WBC) count and the day of first collection. Adverse events were also recorded. All patients provided written informed consent at the time of study inclusion.

Results: All patients were able to mobilize readily and all achieved the primary end point with at least 3.6 x 10>6 CD34+/Kg collected in a single patient. The median yield of CD 34+/Kg collected was 8.25 x 10>6 CD34+/Kg (range, 2.3-15) after a median of 1 procedure (range, 1-2). The median preleukapheresis circulating CD34+/mcL and WBC count/mcL were 94/mcL (range, 15-237) and 21750/mcL (range, 11200-87080), respectively. The median day of first collection was 12 (range, 9-15). Seventeen pts underwent leukapheresis at cycle 1, 10pts after cycle 2 and 9 after cycle 3 (due to logistic reasons in 18pts and to Cytomegalovirus reactivation in 1). Hematologic and non-hematologic side effects were acceptable and no toxic deaths occurred. One patient developed blood-pressure decrement during the apheresis, but she was able to complete the procedure without sequelae. To date, 21 patients (58%) underwent ASCT with prompt engraftment. Data about neutrophils and platelets engraftment will be presented in the final analysis added to comparison with historical IGEV published data (Magagnoli *et al*, BMT 2007).

Discussion: In our knowledge this is the first biggest prospective study evaluating Bendamustine as mobilizing agent in resistant HL pts before ASCT. These results confirm that BeGEV regimen, combined with G-CSF support, can be successfully and safely used to mobilize PBSC.

Disclosure of Interest: None Declared.

PH-P164

THE LYMPHOCYTES TO MONOCYTES RATIO IDENTIFIES A PATIENT SUBGROUP AMONG HIGH RISK DLBCL THAT MAY BENEFIT FROM UPFRONT INTENSIVE TREATMENT WITH AUTOGRAFT

A. M. Barbui[1,], A. Masciulli[2], M. Scarano[2], F. Delaini[1], A. Rossi[1], M. Ladetto[3], P. Corradini[4], M. Di Nicola[5], C. Patty[6], A. Mulè[6], M. Zanni[3], V. Zoli[7], A. Billio[8], A. Gallamini[9], F. Di Raimondo[10], A. J. M. Ferreri[11], F. Benedetti[12], P. Leoni[13], G. Semenzato[14], M. Frezzato[15], L. Flenghi[16], R. Marchioli[2], T. Barbui[17], A. M Gianni[5], C. Tarella[18], S. Cortelazzo[8], A. Rambaldi[1]*

[1]Hematology, A.O. Papa Giovanni XXIII Bergamo, Bergamo, [2]Epidemiology, Istituto Mario Negri, S. Maria Imbaro, [3]Hematology, Università di Torino, Torino, [4]Hematology, Istituto Nazionale dei Tumori di Milano, [5]Oncology, Istituto Nazionale dei Tumori di Milano, Milano, [6]Hematology, Ospedale V. Cervello, Palermo, [7]Hematology, Ospedale San Camillo Forlanini, Roma, [8]Hematology, San Maurizio Regional Hospital, Bolzano, [9]Hematology, Ospedale S.Croce e Carle, Cuneo, [10]Hematology, Ferrarotto Hospital, Catania, [11]Hematology, Istituto Scientifico San Raffaele, Milano, [12]Hematology, Università di Verona, Verona, [13]Hematology, Azienda Ospedaliera Universitaria Ospedali Riuniti, Ancona, [14]Hematology, Università di Padova, Padova, [15]Hematology, Ospedale San Bortolo, Vicenza, [16]Hematology, Ospedale Santa Maria della Misericordia, Perugia, [17] Research Foundation, A.O. Papa Giovanni XXIII Bergamo, Bergamo, [18]Hematology, Molecular Biotechnology Center, Torino, Italy

Introduction: At diagnosis, a peripheral blood lymphocytes to monocytes ratio (LMR) lower than 2.6, identifies a group of DLBCL patients with a poor prognosis when treated with R-CHOP (Rambaldi *et al*, ASH 2012). No data are available for patients treated upfront with Rituximab containing high dose sequential

chemotherapy programs (R-HDS) and autologous stem cell transplantation (AST). Aim of this study was to investigate whether LMR ratio may identify a high-risk patient subgroup that benefits from a primary high-dose program with ASCT.
Materials (or patients) and Methods: We analysed LMR ratio at diagnosis in a series of DLBCL patients enrolled into a trial comparing R-CHOP 14 with R-HDS associated with AST (R-HDS 0305, Clinical Trials.gov.number NCT00355199 by GITIL). Patients characteristics: DLBCL without CNS involvement, with an age between 18–60 years and an High IPI (stage > II B-bulk with ECOG-PS=0-3 and age adjusted IPI (aaIPI) 2–3 or age 61–65 years with ECOG-PS = 0–2 and IPI > 3). R-CHOP 14 (8 cycles) or R-HDS regimen and AST were carried out as previously reported(Tarella C *et al*, Leukemia 2007).
Results: LMR data were collected in 216 evaluable DLBCL patients enrolled into this trial. We identified two groups of patients according to baseline LMR: 144 patients (67%) had a low LMR (<2.6), while 72 patients (33%) had a high (>2.6) LMR. The two groups were comparable for age, gender, stage, ECOG, extranodal sites, bone marrow infiltration while high LDH level was associated with a low LMR (*P*= 0.009). In multivariate analysis OS and EFS corrected by age, gender, stage, ECOG, LDH, extranodal sites, BM involvement and treatment arm, resulted significantly improved by R- HDS and AST only in the low LMR group with an hazard ratio (HR) of 0.41 (95% IC 0.2-0.81), *P*=0.011. In the same patient population with a low LMR, a high ECOG was associated with a two times higher risk of events (HR 2.55, 95% IC 1.26-5.16, *P*=0.009). After a median observation of 35.4 months (0.3–89.2), the OS and EFS of patients treated by R- CHOP 14 or R-HDS and AST were 71% versus 86% (*P*=0.022) and 63% versus 83% (*P*=0.008) respectively.
Discussion: The analysis performed among high-risk DLBCL patients enrolled in a prospective, randomized study, confirms the negative impact of a low LMR at diagnosis in patients treated with R-CHOP. R-HDS and AST improved OS and EFS and overcome the prognostic value of LMR.
Disclosure of Interest: None Declared.

PH-P165
MANAGEMENT OF POSTRANSPLANT RELAPSING OR PERSISTENT DISEASE IN LYMPHOID MALIGNANCIES: EXPLORING GRAFT VERSUS LYMPHOMA EFFECT.

M. Cabrero[1,], O. Lopez-Godino[1], L. Lopez-Corral[1], A. Martin[1], R. Garcia-Sanz[1], M. V. Mateos[1], F. Sanchez-Guijo[1], L. Vazquez[1], C. del Cañizo[1], D. Caballero[1]*
[1]Hematology Department, Hospital Universitario de Salamanca-IBSAL, Salamanca, Spain

Introduction: Allogeneic hematopoietic stem cell transplantation (AlloSCT) represents a potential curative option for relapse or refractory lymphoproliferative disorders, based in part on "graft versus lymphoma effect" (GVLE). However the role of GVLE enhancement, and the adequate way to implement it in relapse or persistent disease after AlloSCT remains unclear. Our goal was to evaluate how the GVLE enhancement, through tapering immunosuppressive treatment (IST) and/or DLI, is able to control the disease in patients with lymphoid malignancies who relapse after AlloSCT or have disease persistence at day +100.
Materials (or patients) and Methods: Twenty-six patients with post-AlloSCT relapse or persistent disease were retrospective analyzed from a series of 112 patients who consecutively underwent alloSCT in our centre.
Results: In 19/26 (73%) patients, GVLE was enhanced by tapering immunosuppressive treatment (IST) and/or donor lymphocyte infusion (DLI), achieving response in 13 (68%), with 11 complete remissions (CR). Median of days from immune-manipulation to response was 105 (39-343), and 9/19 (47%) of patients were alive and disease-free at last follow-up. Concerning histologies, immune-mediated response was observed in 6/6 patients with non-Hodgkin lymphoma (NHL), 5/7 with chronic lymphoid leukemia (CLL) and 2/6 with Hodgkin lymphoma (HL). Graft versus host disease appeared in 16/19 (84%) with a median of 36 days (-3 to 114) from IST withdrawal or DLI; only one death due to GVHD was observed.
The remaining 7 patients in whom GVLE enhancement was not possible due to active or previous GVHD received conventional chemotherapy +/– radiotherapy, achieving 2 CR and 1 PR. Among them, 2 patients were alive in CR at last follow-up.
With a median follow up 56 months (range 11-138), estimated four years progression free survival (PFS) and overall survival (OS) for the whole series of 26 patients were 32% and 45% respectively. In those patients who achieved CR after GVLE appeared, 4 years PFS and OS were 40% and 50% respectively. Main cause of death whatever the histologic diagnosis or treatment group was progression or relapse. On the univariate analysis the development of cGVHD after immune manipulation (4 years PFS 32% vs 0%; *P*=0.017), severe cGVHD (4 years PFS 51% vs 25%; *P*=0.05) and diagnosis different of HL (4 years PFS 35% vs 17% months; *P*=0.016) were the variables with significant influence on PFS between patients with immune manipulation.
Discussion: Disease response to immune manipulation proves the existence of GVLE, which appear in all histological subtypes, although it seems less frequent in HL. Trough GVLE enhancement we are able to achieve maintained responses, achieving PFS and OS similar to those described in non-relapsing patients. Therefore, immune approach should always be considered as treatment in relapse/refractory patients.
Disclosure of Interest: None Declared.

PH-P166
TREATMENT OF PATIENTS WITH HODGKIN LYMPHOMA RELAPSING AFTER AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION- A POLISH LYMPHOMA RESEARCH GROUP (PLRG) MULTICENTER RETROSPECTIVE ANALYSIS OF PROGNOSTIC FACTORS AND LONG-TERM OUTCOME

A. Czyz[1,], J. Romejko-Jarosinska[2], J. Manko[3], E. Drab[3], B. Nasilowska-Adamska[4], T. Czerw[5], A. Giza[6], W. Knopinska-Posluszny[7], J. Drozd-Sokolowska[8], P. Stelmach[9], J. Dybko[10], A. Lojko-Dankowska[1], M. Hus[3], J. Walewski[2], M. Komarnicki[1]*
[1]Poznan University of Medical Sciences, Poznan, [2]Maria Sklodowska-Curie Institute and Oncology Centre, Warsaw, [3]Medical University of Lublin, Lublin, [4]Institute of Hematology and Transfusion Medicine, Warsaw, [5]Maria Sklodowska-Curie Institute and Oncology Centre, Gliwice, [6]University Hospital, Krakow, [7]Independent Public Hospital of Ministry of the Interior with Warmia and Mazury Oncology Centre, Olsztyn, [8]Medical University of Warsaw, Warsaw, [9]Medical University of Lodz, Lodz, [10]Wroclaw Medical University, Wroclaw, Poland

Introduction: This report is a retrospective analysis of patients with Hodgkin lymphoma (HL) who relapsed or progressed after first autologous stem cell transplant (autoSCT). The aim of the study was to determine the response rate to conventional salvage chemo- and/or radiotherapy and prognostic factors influencing treatment outcome. We also intended to analyze overall survival (OS), and progression-free survival (PFS) for patients treated with conventional salvage therapy alone, and for those who proceeded to second autoSCT or to allogeneic stem cell transplant (alloSCT).
Materials (or patients) and Methods: The study group consisted of 103 patients who relapsed at the median of 9 months (1-138) after autoSCT. The median age of patients was 30 years (20-68) at relapse. Four patients died due to rapid disease progression before salvage treatment. Ninety nine patients received conventional salvage chemotherapy +/– radiotherapy. Following salvage therapy 29 patients proceeded to allogeneic and 24 to second autoSCT.
Results: The overall (complete or partial) response rate to salvage therapy was 64%. On multivariate logistic regression the variables that were associated with lower response rate were age >= 40 years (OR 13,3; 95% 1.9-90.9; *P*=0.008), and clinical stage 4 at relapse (OR 8.2; 95% 2.4-28.1; *P*=0.001). After the median follow-up time of 36 months (1-113), the 3-year OS and PFS was 44% and 26%, respectively. Within a group of patients with complete or partial response (PR) after conventional salvage therapy, those patients who proceeded to second autoSCT showed significantly

better OS than those who proceeded to alloSCT or who did not undergo second transplant. The respective 3-year OS was 72%, 45% and 58% (*P*=0.006). The median survival time for patients who underwent second autoSCT was not reached in comparison to 23 months (95% CI 3-44) for patients who proceeded to alloSCT, and 49 months (95% CI 34-63) for those who did not undergo second transplant. Within the group of patients with response worse than PR after salvage therapy the respective median survival time was 41 months (95% CI 16-66), 17 months (95% CI 12-23) and 11 months (95% CI 7-15) (*P*=0.006). In univariate analysis age (>= 40 years vs < 40 years), B symptoms, and bulky disease at relapse (>= 5cm vs < 5 cm), time to progression after first autoSCT (=< 12 vs > 12 months), the best response to conventional salvage therapy (worse than PR vs at least PR), and second autoSCT were significant for OS (*P* < 0.05). In the multivariate analysis only response to salvage therapy (HR 4.0; 95% CI 2.3- 7.0; *P*<0.001), second autoSCT (HR 0.4; 95% CI 0.2-0.8; *P*=0.01) and time to progression after autoSCT (HR 1.8; 95% CI 1.0-3.2; *P*=0.051) remained significant for OS.
Discussion: Our results indicate that age and clinical stage are independent predictors of response to salvage chemo- and/or radiotherapy for patients with HL who relapsed after first autoSCT. Second autoSCT following salvage therapy seems to provide longer survival than salvage therapy alone or followed by alloSCT. The outcome of second autoSCT for patients with at least partial response after salvage therapy is satisfactory. The treatment strategy for patients who did not respond to conventional salvage regimens remains an area for further studies.
Disclosure of Interest: None Declared.

PH-P167
SERUM TARC LEVEL MONITORING MAY PREDICT DISEASE RELAPSE DETECTED BY PET SCAN AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION IN HODGKIN'S LYMPHOMA PATIENTS

L. Farina[1,], F. Rezzonico[1], F. Spina[1], A. Dodero[1], A. Mazzocchi[1], F. Crippa[2], A. Alessi[2], S. Dalto[1], S. Viviani[3], P. Corradini[1,4]*
[1]Hematology, [2]Nuclear Medicine, [3]Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, [4]Chair of Hematology, Università degli Studi di Milano, Milan, Italy

Introduction: Relapsed and refractory Hodgkin's lymphoma (HL) patients may experience long-term survival after allogeneic transplant (alloSCT), but disease recurrence represents the main cause of treatment failure. PET (positron-emission tomography) -positive patients after alloSCT have a dismal outcome. Serum TARC (thymus and activation-regulated chemokine) is produced by Reed-Sternberg cells and may be a marker of disease. Our study had the following aims: i) to assess whether serum TARC levels were correlated to disease status; ii) to correlate TARC levels with PET results after alloSCT; iii) to evaluate whether the combined results could increase the ability to assess or predict relapse.
Materials (or patients) and Methods: Twenty-two patients (M/F=14/8) were evaluated in a prospective observational study. The median age was 31,5 (range, 18-45). Twenty patients had nodular sclerosis HL. Disease status before alloSCT was complete remission *n*=8, partial remission *n*=9, stable/progressive disease *n*=5. Patients received reduced intensity conditioning regimen and peripheral stem cells from a sibling (matched *n*=7, haploidentical *n*=2) or unrelated (*n*=13) donor. Median follow-up was 28 months (range, 2-48). TARC was assessed before and after alloSCT with a median time interval of 47 days (range, 7-700). PET was performed every 3-6 months after alloSCT.
Results: Before alloSCT, the median TARC level was 720,6 pg/mL (range, 208,6-1332) in PET-negative patients, and 2542,5 pg/mL (range 94-13870) in PET-positive patients. After alloSCT, TARC was 1218 pg/mL (range, 31-4388) in persistently PET-negative patients compared to 22397 pg/mL (range, 602-106578) in PET-positive ones (*P* < 0.0001). In 6 patients who relapsed after alloSCT TARC increased progressively before PET became positive, with a median fold increase of 2,93 (range, 1,5-7,11) at the time of relapse. Considering TARC values done on the day of PET scans, the ROC curve showed that the cut-off value of 1726 pg/mL had a sensitivity of 93% and specificity of 84%.
Discussion: TARC was correlated with HL tumor burden as detected by PET after alloSCT. Patients with disease after alloSCT had elevated TARC levels compared to those in CR. TARC monitoring may be able to predict PET positivity, thus potentially allowing immune manipulation before clinical relapse.
Disclosure of Interest: None Declared.

PH-P168
RITUXIMAB MAINTENANCE THERAPY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION PROLONGS PROGRESSION FREE SURVIVAL IN PATIENTS WITH MANTLE CELL LYMPHOMA

S. Dietrich[1,], J. Weidle[1], J. Meissner[1], A. Radujkovic[1], A. Ho[1], P. Dreger[1], M. Witzens-Harig[1]*
[1]Department of Hematology, University Hospital of Heidelberg, Heidelberg, Germany

Introduction: Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma which is characterized by early dissemination and an unfavorable clinical course. A recent report by the European MCL-Network suggests that the poor prognosis of elderly patients with MCL could be significantly improved by rituximab maintenance treatment. However, it is not clear if rituximab maintenance treatment can further improve the prognosis of younger patients after autologous stem cell transplantation (autoSCT). To address this question, we compared patients with MCL who have received rituximab maintenance treatment after autoSCT with those who were followed after autoSCT without any further treatment until progression in a single-centre retrospective study.
Materials (or patients) and Methods: Eligible for this analysis were all patients who underwent autoSCT for MCL at our institution between 2000 and 2012. Patients with MCL who received rituximab maintenance therapy after autoSCT within a prospective phase II trail on the value of rituximab maintenance in B cell lymphoma (every 3 months for 2 years, NCT number 01933711) were compared with patients who were transplanted during the same time period within or outside the aforementioned trial but did not receive rituximab maintenance therapy. The impact of maintenance therapy on PFS and OS was analysed by multivariate cox regression. Rituximab maintenance was considered as a time-dependent event.
Results: A total of 72 patients met the inclusion criteria. Median age was 60 years (30-74). Prior to autoSCT all patients had been exposed to rituximab-based induction and/or salvage therapy, and 45 patients had been treated with high dose cytarabine (HD-ARA-C). AutoSCT was performed after first line treatment in 51 patients. A complete response (CR) before autoSCT was achieved by 27 patients. Twenty-two patients from the phase-2 trial were randomized to receive post-transplant rituximab maintenance for two years, whilst the 50 remaining patients were followed with observation only. Patients with and without rituximab maintenance therapy did not significantly differ with regard to age, upfront autoSCT, remission status and HD-ARA-C exposure prior to autoSCT. However, there was a trend that patients who received rituximab maintenance therapy were transplanted more recently (control group: 2000-2012, rituximab maintenance group: 2002-2012, *P*=0.06).
Median observation time after autoSCT was 56 months. Two-year PFS and OS from autoSCT of the control group were 65% and 84%, respectively, as compared to 90% and 90%, respectively, of the rituximab maintenance group. By univariate landmark analysis rituximab maintenance therapy was associated with significantly better PFS (HR 0.21, *P*=0.014) but so far not OS. Multivariate adjustment for age, year of transplant, achievement of CR prior autoSCT, upfront autoSCT and high dose ARA-C treatment confirmed the beneficial impact of rituximab maintenance therapy (*P*=0.02 HR 0.23).
Discussion: Our observation that rituximab maintenance therapy improves PFS in MCL patients after autoSCT extends the findings of a recent report that showed a benefit for rituximab maintenance after R-CHOP in elderly MCL patients and provide a rationale for

studying maintenance approaches for eligible MCL patients after autoSCT in a comparative prospective trial.
Disclosure of Interest: None Declared.

PH-P169
EBMT SCORE ONLY PREDICTS DAY 100 OVERALL SURVIVAL AND OVERALL SURVIVAL AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULT T-CELL LEUKEMIA/LYMPHOMA PATIENTS

S. Takeuchi[1,], N. Nakano[1], A. Kubota[1], M. Tokunaga[1], Y. Takatsuka[1], A. Utsunomiya[1]*
[1]Hematology, Imamura Bun-in Hospital, Kagoshima, Japan

Introduction: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been used as a curative option for adult T-cell leukemia/lymphoma (ATLL). However the suitable pre-transplant prognostic index is not well known. Here we intend to clarify the relation between pre-transplant prognostic index and day 100 overall survival (OS) and OS after allo-HSCT in our single institute located in an endemic area of ATLL.
Materials (or patients) and Methods: There were 87 ATLL patients undergone allo-HSCT at Imamura Bun-in Hospital from June 1998 to July 2013. We analyzed the relation between pre-transplant prognostic indices such as HCT-CI, EBMTscore, the prognostic index for acute- and lymphoma-type adult T-cell leukemia/lymphoma (ATL-PI) and day100 OS and OS after allo-HSCT retrospectively. OS was analyzed with Kaplan-Meier method. Cox regression analysis about OS was also performed. Statistical significance defined $P<0.05$ in log-rank test. All statistical analysis was performed with EZR (R commander).
Results: In 87 patients (52 male, 35 female), median age was 52 (range: 32-69) years. Performance status (PS) at HSCT was 0-1 in 66 patients and 2 in 11 patients. Disease status at HSCT was 28 complete remission (CR) and 59 non-CR (15 partial remission (PR), 15 stable disease (SD), 29 progressive disease (PD)). Fifty-one patients received BMT, 20 PBSCT, and 16 CBT, respectively. Fifty-five patients had received myeloablative conditioning (MAC) and 32 reduced intensity conditioning (RIC). HCT-CI was 0 in 19 patients, 1 and 2 in 55 patients, and over 2 in 13 patients, respectively. EBMT score was 1 and 2 in 4 patients, 3 in 20 patients, 4 in 34 patients and over 4 in 29 patients, respectively. ATL-PI at HSCT was 0 and 1 in 23 patients, 2 in 42 patients, 3 in 13 patients and over 3 in 9 patients, respectively. In univariate analysis, HCT-CI ≥3 ($P<0.01$), EBMT score ≥4 ($P<0.01$), and ATL-PI at HSCT ≥3 ($P<0.01$) contributed to inferior OS. EBMT score ≥4 ($P<0.01$) also contributed to inferior day 100 OS, however, HCT-CI ≥3 ($P=0.31$) and ATL-PI at HSCT ≥3 ($P=0.12$) did not contribute to inferior day 100 OS. In multivariate analysis, EBMT score ≥4 contributed to inferior day 100 OS and OS (hazard ratio :2.11 and 8.76 respectively).
Discussion: Among pre-transplant prognostic indices, EBMT score ≥4 only contributed to inferior day 100 OS and OS after HSCT in ATLL patients. The fact that 1/3 of mortality happened within 100 days after HSCT takes into consideration, our results suggest that EBMT score would be most important pre-transplant prognostic index when planning to undergo allo-HSCT for ATLL patients.
Disclosure of Interest: None Declared.

Minimal Residual Disease

PH-P170
A SIGNIFICANT EARLY DETECTION OF POOR OUTCOME IN ACUTE LEUKEMIA PATIENTS HAVING A MINIMAL RESIDUAL DISEASE USING MULTIPARAMETER FLOW CYTOMERTY COMBINED TO MIXED CHIMERISM AT THREE MONTHS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

F. Beckerich[1,], M. Sobh[1], A. Plesa[2], V. Dubois[3], I. Mollet[3], H. Labussière[1], L. Gilis[1], S. Ducastelle[1], F. Barraco[1], X. Thomas[1], F. Emmanuel Nicolini[1], M. Michallet[1]*
[1]Hematology, [2]Immunology, Centre Hospitalier Lyon Sud, PIERRE BENITE CEDEX, [3]HLA laboratory, EFS, Lyon, France

Introduction: The aim of our study was to evaluate the impact of positive minimal residual disease (MRD) by Multiparameter Flow Cytometry (MFC) associated to chimerism documentation at 3 months in AML patients after allo-HSCT on overall and progression-free survival (OS, PFS).
Materials (or patients) and Methods: We evaluated 137 AML patients who received allo-HSCT between January 2005 and October 2012. There were 71 (52%) males and 66 females with a median age of 47 years (range: 19-66), 77% had de novo AML, 20% had secondary AML and 3% had biphenotypic AML. According to cytogenetics, 40% were normal, 51% were unfavorable (9% classified as failure). According to molecular markers, 9% were favorable, 31% intermediate, 44% unfavourable and 16% had no molecular markers. At allo-HSCT, 46% of patients were in first complete remission (CR1), 25% were in CR 2 and 29% had active disease; 40% received a full intensity conditioning and 60% got reduced intensity one. As cell source, 35% were bone marrow, 53% peripheral blood and 12% cord blood cells. Donors were related in 53% of the cases (45% were 10/10 HLA matched) and unrelated in 47% of cases (20% were 10/10 HLA matched). MFC was performed using BM samples with a sensitivity of 0.01%. Chimerism analysis was performed on marrow and/or blood samples using PCR with an accuracy of ± 5%, a mixed chimerism was defined by having 5% or more of recipient cells.
Results: After transplantation, all patients engrafted, the cumulative incidence of acute GVHD at 3 months was 19.9% (95% CI: 16.2-20.6) while the cumulative incidence of chronic GVHD reached 26.7% (95% CI: 22.9-30.5) at 1 year. After a median follow-up of 16 months (range: 3-77), the median OS was 66 months (65-NR) with a 3 years probability of 64% (95% CI: 56-73), the median PFS was 32 months (13-NR) with a 3 years probability of 50% (95% CI: 37-58) while the transplant related mortality rate reached 13.6% (95% CI: 10-16) at 2 years. The 3 months chimerism evaluation ($n=137$) showed a mixed chimerism in 12 (9%) patients, while the MFC ($n= 62$) detected 15 patients with leukemic cells. Sixty eight patients showed morphological relapse after a median time of 4.8 months (1-34.7); the correlation study between MRD positivity, mixed chimerism detection and morphological relapse showed a higher correlation for both chimerism and MFC (correlation=0.69, $P<0.001$) than if we consider chimerism or MFC alone. Multivariate analysis showed a significant worse OS for patients with 3 months positive MFC [1 year OS of 20% vs. 80%, HR= 4 (95% CI: 1.4-11.7), $P=0.01$] and patients with mixed chimerism [1 year OS of 21% vs. 70%, HR= 4 (95%CI: 1.3-12.1), $P=0.01$]; these results were still valid even after stratification on disease status at transplantation. These results applied also in terms of PFS for positive MFC [1 year PFS of 13% vs. 76%, HR= 3.6, $P=0.02$], and mixed chimerism [1 year PFS of 0% vs. 70%, HR= 7, $P=0.001$].
Discussion: The 3 months MRD evaluation using MFC combined to chimerism documentation seems to be an independent prognostic factor on overall and progression-free survival for AML patients undergoing allo-HSCT. The standardisation of this evaluation may lead to the identification of patients with high relapse risk suggesting the need of early therapeutic intervention.
Disclosure of Interest: None Declared.

PH-P171

EFFICACY OF AZACITIDINE TO RESTORE FULL DONOR CHIMERISM IN MDS OR AML PATIENTS AFTER ALLOGENEIC HSCT: A THERAPEUTIC OPTION FOR TREATMENT OF EARLY RELAPSE

A. Gozzini[1,], S. Guidi[1], C. Nozzoli[1], R. Saccardi[1], V. Santini[2], I. Donnini[1], R. Angarano[1], R. Boncompagni[1], A. Sanna[1], A. Bosi[2]*

[1]Hematology, A.O.U. CAREGGI, [2]Hematology, university of florence, Firenze, Italy

Introduction: In young patients with high risk myelodysplastic sindrome (MDS) or high risk acute myeloid leukemias (AML), allogeneic stem cell transplantation (HSCT) is the only potentially curative therapeutic strategy. In patients who are candidate to undergo to this procedure, disease relapse represents the major reason of failure. The current options for relapsed disease after allogeneic HSCT are salvage chemotherapy, donor lymphocyte infusion (DLI) or a second allogeneic transplant. Unfortunately the majority of patients who relapsed after allogeneic HSCT shows a very short survival. DLI can induce a second remission for this subgroup of patients, but a severe graft versus host disease (GvHD) can occur, resulting in significant mortality. In recent years, the monitoring of chimerism and minimal residual disease (MRD) by flow cytometry analysis facilitated the detection of residual burden of disease, even though the kinetics of disease recurrence are very rapid. Recent studies documented as Azacitidine, a nucleoside analog of cytidine, induces FOXP3 expression in naïve T-cells, which in turn induces a regulatory T-cell population that mitigates GvHD effects, while preserving a graft-versus-leukemia (GvL) effects. Here we report the results of the efficacy of azacitidine in the setting of MDS or AML patients who showed a drop in donor chimerism and/or MRD positive after allogeneic HSCT to prevent relapse of disease.

Materials (or patients) and Methods: We analyzed data from 10 patients (5 MDS and 5 AML) who received azacitidine 75 mg/m^2 every 28 (Vidaza, Celgene Corporation, Summit, NJ, USA) subcutaneously on days 1-7, every 28 days. A limited number of azacitidine cycles were administered (range 1-6) because of possible induction of GvHD. The median age of patients was 49 years (range 38-55). Three patients received HSCT from Cord Blood (mm 4/6), 3 from sibling donor, and 4 from MUD 8/8. Nine patients received myeloablative conditioning regimen, only one RIC received. Three patients were also treated with infusions of DLI after 3 cycles of azacitidine, 2 of them in partial response (mixed chimerism) and in absence of GvhD and one in complete remission and full donor chimerism, in absence of GvHD.

Results: The PFS is 40% after azacitidine initial treatment with a maximum follow up of 30 months. Four patients died because of relapse of disease, 2 of them are actually in complete remission, MRD negative and full donor chimerism. Two patients are in mixed chimerism with MRD positive. We also compare data analyzed from an historic cohort of 12 patients (median age 46 years) affected by AML who received DLI infusions because of drop of chimerism, and/or appearance of positivity of MRD to delay the relapse. Six patients received HSCT from sibling donor and 6 from MUD source, 5 were treated with RIC regimen, while 7 myeloablative conditioning regimen received. Only two patients are alive with a follow up of 48 months form DLI infusion.

Discussion: In conclusion these data demonstrated that MRD treatment with azacitidine seems to be effective to prevent or to delay the relapse of disease with an acceptable profile of safety in patients with high risk MDS and high risk AML after allogeneic HSCT. These data provide the rationale for the combination of azacitidine and DLI, using more and continuous cycles to maintain the response.

Disclosure of Interest: None Declared.

PH-P172

IMMUNOPHENOTYPIC REMISSION AFTER ALLOGRAFTING IN MULTIPLE MYELOMA

L. Giaccone[1,], L. Brunello[1], M. Festuccia[1], M. Gilestro[1], E. Maffini[1], F. Ferrando[1], E. Talamo[1], R. Passera[2], M. Boccadoro[1], P. Omedè[1], B. Bruno[1]*

[1]Division of Hematology - A.O.Città della Salute e della Scienza di Torino, [2]Division of Nuclear Medicine-A.O.Città della Salute e della Scienza di Torino, Torino, Italy

Introduction: Recent studies suggest that immunophenotypic remission (IR) may be a relevant prognostic factor in patients with multiple myeloma (MM), however data after allografting are lacking.

Materials (or patients) and Methods: At our center, between January 2000 and December 2011, 80 consecutive multiple myeloma patients underwent an allograft. Sixtynine/80, with a follow-up of at least 3 months were included in this study. Three of them were further excluded because of incomplete data or death before disease restaging. Thus, 66 patients, median age 54 years (35-66), were evaluated for IR compare to conventional complete remission (CR). Bone marrow aspirates had to contain at least 13000 cells/microL for flow cytometry studies with high-sensitivity immunophenotyping for IR investigation. Plasmacells quantification was obtained by 4 to 6-colour staining with the following monoclonal antibodies: CD38, CD138, CD56, CD19, CD45, cyKappa, cyLambda. IR was defined as less than 0.01% monoclonal plasmacells in bone marrow aspirate detected by multiparameter flow cytometry, and CR according to standard criteria. The times of observation were censored on 01/10/2013.

Results: Conditioning regimen was non-myeloablative in 55 patients, reduced intensity in 10 and myeloablative in 1 patient. Post-grafting immunosuppression consisted of cyclosporine with mycophenolate mofetil or methotrexate. Donors were HLA identical siblings in 58 patients and unrelated in 8. Only 1 patient received bone marrow as source of stem cells. Allograft was part of the first line treatment in 35/66 (53%) patients. All patients included had adequate bone marrow samples for IR evalutation. After a median follow-up of 85 months (range 31-158), the incidence of acute and chronic graft-versus-host disease was 45% and 52%. Three-year treatment related mortality was 14% in the overall population (N=80) and 9.1% among patients who survived at least 3 months and included in the analysis (N=66). At follow-up, 24 patients achieved CR and IR (CR/IR group), 21 achieved IR but not CR because of persistence of urine/serum M-component (noCR/IR group), and 21 did not achieve either CR or IR (noCR/noIR group). Median overall survival (OS) and event-free survival (EFS) in patients who achieved IR were 96 and 41 months versus 36 and 6 months in those who did not (P<0.001). In details, median OS and EFS were not reached and 59 months in the CR/IR group, 64 and 16 months in the noCR/IR, and 36 and 6 months in the noCR/noIR, respectively (P<0.001 for both EFS and OS). In univariate analysis, being in the CR/IR group was the only significant predictor for prolonged OS and EFS (P<0.001). Of note, cumulative incidence of extra-medullary disease at first disease relapse after the allograft was 4% in the CR/IR, 38% in the noCR/IR and 14% in the noCR/noIR groups (P<0.001) at 4 years.

Discussion: The achievement of IR showed a significant impact on clinical outcomes, also among patients not in CR. Discrepancies between IR and CR and a higher incidence of extramedullary relapse observed in the noCR/IR group, suggest that myeloma cells may escape immune control outside the bone marrow. In this group, imaging studies such as positron emission tomography may be indicated to allow detection of early relapse.

Disclosure of Interest: None Declared.

PH-P173

PREDICTIVE ROLE OF MINIMAL RESIDUAL DISEASE BEFORE AND AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A COMPARISON BETWEEN MULTIPARAMETER FLOW CYTOMETRY AND WILM'S TUMOR 1 EXPRESSION

G. Rossi[1,], A. Michele Carella[1], M. Marta Minervini[1], L. Savino[1], F. di Nardo[2], C. de Waure[2], M. Mario Greco[1], E. Merla[1], A. Melpignano[3], A. Spirito[1], S. Capalbo[4], G. Palumbo[4], G. Perla[1], G. Pio De Cillis[1], N. Cascavilla[1]*

[1]Department of Hematology and Stem Cell Transplantation Unit, IRCCS " Casa Sollievo della Sofferenza", San Giovanni Rotondo (FG), [2]Department of Hygiene, Catholic University of Sacred Heart, Rome, [3]Department of Hematology, "Perrino" Hospital, Brindisi, [4]Department of Hematology, "Riuniti" Hospital, Foggia, Italy

Introduction: Relapse represents the main cause of treatment failure in patients with acute myeloid leukemia(AML) undergoing allogeneic stem cell transplantation (allo-SCT). The identification of minimal residual disease (MRD) prior to allo-SCT strongly predicted the recurrence while results came from post-transplant monitoring did not show the same. Anyway, only few studies were reported after allo-SCT and MRD was evaluated in small cohorts of patients using different techniques as well as different timing of assessment, cut-offs and methods of analysis. In our study we investigated MRD before and after allo-SCT by multiparameter flow cytometry (MFC) and Wilm's tumor 1 levels (WT1). Our purpose was firstly to compare the diagnostic performance of MFC and WT1 in the identification of relapse, secondarily to evaluate the impact of MRD on survival when it was studied by both methods at the optimal cut-off values and timing of assessment.

Materials (or patients) and Methods: Fresh BM samples from 42 pts (20 males, 47.6%;mean age 45 ys, SD: 14). were investigated before and one month after the allo-SCT by six-color MFC and RQ-PCR WT1 mRNA. MRD was evaluated in pts achieving a complete remission (CR). Thirty-two out of 42 pts were in their 1st or 2nd CR before the transplant whereas 40 pts were in CR after this procedure (2 0f 42 died for transplant related mortality). At least 250.000 events were acquired for MRD analysis by MFC while samples containing less of 1000 copies of ABL were evaluated as degraded and inadequate for analysis by WT1.

Results: Area under curve of the ROC curves were analyzed . Only MRD evaluated after allo-SCT by both MFC and WT1 achieved a fair accuracy (AUC≥0.700) in predicting the relapse. Indeed, post-transplant was chosen as the optimal timing of assessment and 0.05% as well as 101.4 x 10^4 ABL copies were considered the most predictive cut-offs for MFC and WT1, respectively. Despite the greater sensitivity of MFC compared to WT1 (80.0% vs 75.0%), less specificity (66.7% vs 87.5%) and positive predictive value (44.4% vs 66.7%) were showed.Pts with positive MRD by MFC had significantly higher risk to relapse compared to pts with a negative test (disease free survival (DFS): 86% vs. 40%; Cox regression crude P = 0.013). Similarly, patients with positive MRD by WT1 showed a lower DFS compared to negative ones (DFS: 87% vs. 29%; Cox regression crude P = 0.003).No significant differences were observed in overall survival. At multivariate analysis, post transplant positive MRD identified by both MFC and WT1 was significantly related to a shorter DFS after adjusting for age, gender and cytogenetics (P = 0.016 and P=0.017, respectively).

Discussion: In this study, pre and post transplant MRD were evaluated by MFC and WT1. Although both methods showed a moderate sensitivity to identify the relapse, a lower specificity characterized the MFC. These data confirmed concerns recently reported on some leukemia –associated phenotypes (LAP). These pitfalls should increase the risk of false –positive results. On the other hand, the choice of post-transplant as the most predictive timing of assessment may reflect a major uniformity of BM status at this time. Finally, the strong impact of post-transplant positive MRD on DFS, independently of method used, should encourage a wider use of MRD in this setting.

Disclosure of Interest: None Declared.

PH-P174

MRD MONITORING EARLY AFTER ALLOGENEIC TRANSPLANTATION WAS MORE PREDICTIVE THAN KIT MUTATION IN ADULT T (8; 21) AML AND ALLOW FURTHER RISK STRATIFICATION: RESULTS FROM A MULTI-CENTER STUDY

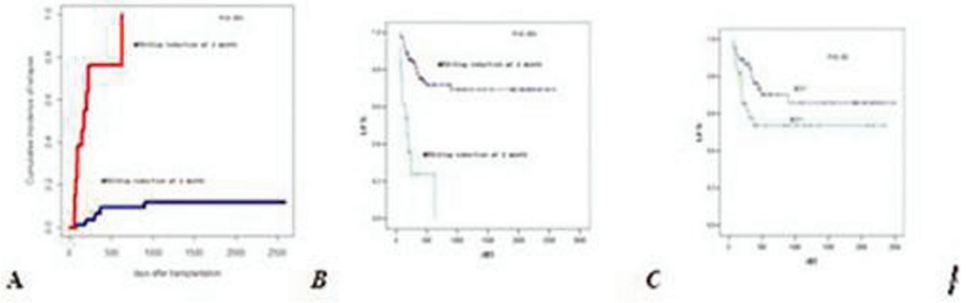
Y. wang[1,], Y.-Z. qin[1], D.-P. wu[2], Q.-F. liu[3], J.-B. wang[4], D.-H. liu[1], L.-P. xu[1], Y.-R. liu[1], J. chen[2], M. dai[3], H.-Y. cheng[4], X.-J. huang[1]on behalf of XJH*

[1]Peking University People's Hospital, Peking University Institute of Hematology, beijing, [2]The First Affiliated Hospital of Soochow University, suzhou, [3]Nanfang Hospital, Nanfang Medical University, guangzhou, [4]aero center space hospital, beijing, China

Introduction: Recent results from our prospective multi-center study identified patients with t (8; 21) AML as high-risk according to their MRD status after the second consolidation chemotherapy and allo-HSCT can benefit this part of patients; however, the relapse rate was reported to be 22% even after allo-HSCT for those high-risk patients. In addition, allo-HSCT tended to lower the relapse rates of KIT-mutated patients. To date, the respective value of MRD and KIT mutation to further distinguish between patients with low and high risks of relapse in allo-HSCT setting has not been assessed.

[PH-P174]

Figure 1. *relapse rate and LFS after allo-HSCT in adult t (8; 21) patients. (A) Comparison of relapse rate at 2 month post HSCT between different MRD levels. (B) Comparison of LFS at 2 month post HSCT between different MRD levels. (C). Comparison of LFS between KIT + and KIT- patients.*



Materials (or patients) and Methods: one hundred consecutive adult AML patients with t (8; 21) receiving allo-HSCT in CR were enrolled between January 2006 and June 2013. KIT mutation was screened at diagnosis in 80 patients. Serial MRD monitoring by RQ-PCR post HSCT was done for all patients. The impact of MRD monitoring and KIT mutation on transplant outcomes was assessed.
Results: Achieving > 3 log reduction at 1, 2 or 3 month after HSCT in transcripts from diagnosis, is associated with a significant lower cumulative incidence of relapse (CIR) (20% vs 34%, *P*=0.037; 12% vs 100%, *P*<.001; and 10% vs 43%, *P*<0.001, respectively) and higher probability of LFS at 2 or 3 month (67% vs 0%, *P*<.001; and 72% vs 0%, *P*<.001, respectively). KIT mutation at diagnosis, is associated with a higher relapse rate (32% vs 14% for KIT+ vs KIT-, *P*=0.022; 32% vs 14% vs 26% for KIT+ vs KIT- vs KIT unknown, *P*=0.074) while there was no statistically significant difference in LFS with respect to KIT mutation (64% vs 50% for KIT+ vs KIT-, *P*=0.11). Furthermore, in multivariate analysis, MRD remained the sole prognostic factor for CIR. In addition, the serial monitoring as well as the combination of MRD and KIT also allowed identification of relapse risk after HSCT.
Discussion: this study was the first one to evaluate the impact of MRD monitoring and KIT mutation and their relative importance on transplant outcomes. Results showed that MRD monitoring by RQ-PCR at regular early time points post HSCT had more prominent significance in identification of patients at high risk of relapse, as compared to KIT mutation among adult t (8; 21) AML after allo-HSCT and could now be incorporated in clinical trials to evaluate the role of risk directed prophylactic/preemptive therapy.
Disclosure of Interest: None Declared.

PH-P175
MONITORING MLL EXPRESSION MAY HELP IDENTIFY MLL–REARRANGED AL PATIENTS AT HIGH RISK OF RELAPSE AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

J. Liu[1,], Y. Wang[1], Y. Qin[1], L. Xu[1], D. Liu[1], K. Liu[1], X. Huang[1]*
[1]Peking University Institute of Haematology, People's Hospital, Beijing, China

Introduction: Mixed-lineage-leukaemia (MLL) gene is a recurrent chromosome change in acute leukaemia. In a prospective, multi-centre cohort study conducted in 2012, we demonstrated that allo-HSCT could be a valuable treatment choice for patients with MLL+ acute leukaemia. To date, no studies have focused on the impact of monitoring MLL expression before and after HSCT. Therefore, we performed a prospective cohort study to evaluate the prognostic value of MLL gene expression for predicting relapse in patients with MLL–rearranged acute leukaemia following allogeneic haematopoietic stem cell transplantation (allo-HSCT).
Materials (or patients) and Methods: From Oct 2007 to Oct 2012, forty consecutive patients diagnosed with MLL+ acute leukaemia undergoing allo-HSCT were enrolled. These patients were part of a multi-centre clinical trial, registered at www.chictr.org as # ChiCTR-ONC-12002739, whose clinical outcome was previously reported in 2013. Bone marrow (BM) MLL transcript levels were monitored serially by real-time quantitative polymerase chain reaction (RQ-PCR) at designated time points in 40 MLL–rearranged acute leukaemia patients who were treated with allo-HSCT. The patients were followed up for a median of 24.5 months (range: 12-60 months). A total of 236 BM samples were collected and analysed. Of these, 230 were concurrently monitored for MRD by FCM for leukaemia-associated aberrant immune phenotypes (LAIPs) and by RQ-PCR to assess WT1 gene expression. Detectable MLL expression at any level (MLL >0.0000%) was defined as MLL positive. MRD positivity (MRD+) was defined as being MLL positive at any time during the first year post-HSCT, while the absence of MLL positivity was defined as MRD negativity (MRD-).
Results: The 3-year cumulative incidence of relapse of patients who experienced MRD+ (*n*=9) post-HSCT was 93.5% (CI: 87%>100%), compared to 12.5% (CI: 5.6%>19.4%) for MRD- patients (*n*=31) (*P*<0.001). For these patient groups, the 3-year overall survival was 12.5% (CI: 0.8%>24.2%) and 77.8% (CI: 68.4%>87.2%), respectively (*P*<0.001), and the 3-year LFS was 0% and 72.2% (CI: 61.1%>83.3%) (*P*<0.001), respectively. MLL positivity was associated with a higher relapse rate (HR=18.643, 95% CI: 3.449-100.757, *P*=0.001) and lower DFS (HR=11.05, 95% CI: 3.169-38.533, *P*<0.001) and OS (HR=14.438, 95% CI: 3.638-57.297, *P* <0.001), as determined by Cox multivariate analysis. A good correlation was found between MLL and WT1 expression. MLL gene expression had a higher specificity and sensitivity than WT1 or MRD monitored by FCM for predicting relapse in these MLL+ AL patients undergoing allo-HSCT.
Discussion: Our data indicate that MLL expression is a valuable and essential marker for MRD monitoring in MLL+AL patients following HSCT. The MLL expression during follow-up is highly predictive of leukaemia relapse and has better specificity and sensitivity than WT1 and MRD monitored by FCM. Although our results need to be confirmed in a large-scale, prospective study, we consider the current qualitative assessment of MLL expression to be a very useful test for further risk stratification to guide the prevention and management of early relapse in MLL+AL patients following HSCT. Further research should focus on suitable MRD monitoring-directed interventions after HSCT, which could improve the clinical outcomes of these patients.
Disclosure of Interest: None Declared.

Myelodysplasia

PH-P176
A SINGLE-CENTRE EXPERIENCE OF ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN MYELOFIBROSIS

D. J. M. Routledge[1,], F. Dignan[1], E. Tholouli[1]*
[1]Clinical Haematology, Central Manchester NHS Foundation Trust, Manchester, United Kingdom

Introduction: Myelofibrosis is a rare bone marrow disorder characterised by increased megakaryocyte numbers leading to bone marrow fibrosis. It is a heterogeneous disorder with some patients requiring no treatment while others can have life threatening cytopaenias or even transformation to Acute Myeloid Leukaemia (AML). The optimum time for Haematopoietic Stem Cell Transplantation (HSCT) is not known. Here we report our single centre experience at Manchester Royal Infirmary.
Materials (or patients) and Methods: A retrospective case analysis was performed for 14 consecutive patients (8 male, 6 female) who had undergone allogeneic HSCT for Primary Myelofibrosis (*n*=7) or transformed from Primary Polycythaemia (*n*=4) or Essential Thrombocythaemia (*n*=3). Patients received an allogeneic marrow (*n*=2) or peripheral blood stem cells (*n*=12) from HLA-matched siblings (*n*=6), matched voluntary unrelated donor (VUD, *n*=6) and single mismatched VUD (*n*=2). These were conducted between July 2008 and November 2011. The median age was 52 years (range 24-64). At transplantation 9 patients had a DIPSS-Plus score of INT-2 or High and 5 had INT-1 (one patient had developed AML and another MDS RAEB-2). 6 out of 14 patients received a full intensity conditioning regimen, 1 patient had total body irradiation/Cyclophosphamide (CY) and 5 patients Busulphan (BU)/CY. 8 patients received a reduced-intensity conditioning regimen with Fludarabine (FLU)/BU. RIC with ALG (VUD, *n*=4 and Sibling, *n*=2) or alemtuzumab (mismatched VUD, *n*=2) were used. These patients also received cyclosporin. All other patients received cyclosporin and methotrexate as GvHD prophylaxis. Splenectomy was performed in 3 patients prior to HSCT. 12 patients had a Karnoskfy score of 80% or greater and 2 a HCT-CI score of 2 or greater.
Results: The median time to neutrophil engraftment was 17 days (Range 11-27 days). Acute GvHD Grades 2 to 4 was observed in 8 patients. Thrombosis was the most common post transplant complication (*n*=4). 5 patients have died, 4 from infection and one from transformation to AML. 9 patients are still alive with a median survival of 30 months (range 13 to 68 months). 2 of these

patients have been alive for over 60 months since transplantation. Both had PMF and were transplanted over the age of 50 years with minimal splenomegaly, and a DIPPS-Plus of INT-1 and INT-2. 3/6 patients who received myeloablative conditioning have died. One had MDS RAEB-2 pre-transplant, one had transformed to AML pre-transplant and the other developed AML post transplant.
Discussion: Our centre experience shows that HSCT still has an important role in the management of Myelofibrosis, with potential long term disease remission.
Disclosure of Interest: None Declared.

PH-P177
DISEASE-FREE SURVIVAL OF MYELODYSPLASTIC SYNDROME AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IS NOT ASSOCIATED WITH DISEASE STATUS AND DONOR SOURCES

Y. Lu[1,], T. Wu[1], Y. Xing Cao[1], L. Yan Zhao[1], Y. De Liu[2], R. Jia Zhou[1], P. Jian Zhang[1], M. Xiong[1], D. Lu[1], J. Rui Sun[1], J. Zhi Wei[1], Q. Shen Ji[1]*
[1]Ludaopei Hematology & Oncology Center, [2]Yanda International Hospital, lang fang, China

Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) is an only curative modality currently for myelodysplastic syndrome (MDS). High-risk MDS usually has lower complete remission (CR) rate and higher chemotherapy-related mortality compared with *de novo* acute myeloid leukemia (AML). To examine whether CR before HSCT has survival benefit for MDS treated by HSCT, we retrospectively analyzed the data during 11 years from our center. The clinical outcomes of MDS after HSCT from different donor sources have also been evaluated.
Objective: In present clinical study, the effects of disease status and donor sources on disease-free survival (DFS) of MDS after HSCT were studied.
Materials (or patients) and Methods: From August 2001 to May 2013, total 125 patients with MDS that underwent HSCT in our center were enrolled. Male to Female was 78: 47. The median age was 35 (8 to 57) years old. The median blasts in bone marrow (BM) before conditioning were 10% (1% to 65%). According to 2008 WHO classification, the patients were diagnosed as refractory cytopenias with unilineage dysplasia (RCUD) in 12, refractory anemia with ring sideroblasts (RARS) in 2, 5q- in 1, refractory cytopenias with multilineage dysplasia (RCMD) in 15, refractory anemia with excess blasts (RAEB) -1/RAEB-2 in 36 and transformed AML in 59. For International Prognostic Scoring System (IPSS), 12 patients were in low-risk, 27 in intermediate-1, 25 in intermediate-2, and 61 in high-risk. Based on BM blast percentage pre-conditioning, 49cases were less than 5%, 43 patients were between 5% to 20%, and 33 cases were more than 20%. The stem cells were from identical siblings (46) or unrelated donor (25) or haploidentical family members (54). Conditioning regimens were BUCY/BUFLU for identical sibling HSCT, and BUCY/BUFLU plus ATG (Thymoglobuline, 8-10mg/kg) for unrelated or haploidentical transplants. Graft-versus-host disease prophylaxis was employed by Cyclosporin A, Methotrexate and Mycophenolate mofetil as reported previously (DP Lu *et al.*, Blood 2006; 107:3065).
Results: With median follow-up 33(1-144) months, DFS was 74.7%. Fourteen patients (8%) relapsed. Transplant-related mortality was 17.6%. No significant differences on DFS were found among RCUD/RARS/5q- (87.5%), RCMD (85.7%), RAEB-1/RAEB-2 (74.0%) and transformed AML (67.7%) (*P*=0.661). A similar DFS was seen in different risk categories (86.7% in low-risk, 78.1% in intermediate-1, 76.7% in intermediate-2 and 68.6% in high-risk; *P*=0.748). Moreover, CR or not before HSCT has no remarkable effect on DFS (blasts <5%, 80.0%; blasts 5% to 20%, 64.6%; blasts > 20%, 74.1%; *P*=0.336). Donor sources have also no significant effects on DFS (identical sibling 73.3%, unrelated donor 80.9%, haploidentical family member 70.0%; *P*=0.801).
Discussion: Our clinical results have shown that under current protocol, DFS of MDS after allogeneic HSCT is quite encouraging no matter the disease status and stem cell donor sources. Therefore, it is not necessary that complete remission is achieved by chemotherapy before transplant. Haploidentical family member is an important alternative donor for patients with MDS when matched either identical sibling or unrelated donor is not available.
Disclosure of Interest: None Declared.

PH-P178
COMPARISON OF DIFFERENT PRETRANSPLANT STRATEGIES PRIOR ALLOGENEIC TRANSPLANTATION IN PATIENTS WITH MDS: UPFRONT TRANSPLANTATION VS. INDUCTION CHEMOTHERAPY

N. Wegener[1,], A. Groten[1], C. Saure[1], A. Dienst[1], E. Rachlis[1], M. Kondakci[1], U. Germing[1], R. Haas[1], T. Schroeder[1], G. Kobbe[1]*
[1]University of Duesseldorf, Department of Hematology, Oncology and Clinical Immunology, Duesseldorf, Germany

Introduction: Allogeneic hematopoietic stem cell transplantation (allo-SCT) is the only curative option for patients with myelodysplastic syndromes (MDS). The role of treatment prior allo-SCT and in particular induction chemotherapy is a matter of an ongoing debate, since it might on the one hand reduce the likelihood of relapse by remission induction, but is also potentially associated with several toxicities.
Materials (or patients) and Methods: We compared the outcome of a group of 110 patients suffering from MDS (*n*= 89) or secondary AML, (*n*=21) who had been transplanted between 2001 and 2012 at our center, in accordance with their pretransplant treatment strategies. Of those, 54 patients were directly transplanted (upfront group) without any prior therapy to transplantation including 74% of them receiving a sequential conditioning regimen using the so-called FLAMSA protocol. Of 44 patients having received induction chemotherapy prior to transplantation 20 were in complete remission at the time of transplantation, while 24 had still active disease. The chemotherapy group and the upfront group were well balanced with regard to disease parameters and transplant characteristics with the exception, that there were more therapy-related MDS in the upfront group. (16% vs. 5%; *P*=0.002) In addition, we also compared the results of 12 patients having received disease modifying drugs like Valproat or 5-Azazytidine prior to transplantation.
Results: The 5-year overall survival (OS) of the entire group was 43%. The 5-year OS of the patients in the upfront group was significantly higher in comparison to the chemotherapy group (67 % vs. 43%; *P*=0,032). This inferior outcome of the chemotherapy group was mainly attributed to those patients who were refractory after induction chemotherapy (5-year OS 33%; *P*=0.006), while the OS of those patients who achieved CR (55%, *P*=0,462) was comparable with the upfront group. Relapse-free survival after 5 years was significantly lower in the chemotherapy group in comparison to the upfront group (34% vs. 56%; *P*=0.034), which was related to a low RFS in the group of patients refractory to induction chemotherapy (5-year RFS 17%). The RFS of patients in CR after induction chemotherapy (55%) was similar to that of the upfront group. Non-relapse mortality (NRM) was 25% for the entire group with no differences between the different subgroups.
Discussion: Despite the limitations of retrospective analyses our data suggest that upfront transplantation, for example using the sequential FLAMSA approach, results in promising results and might be a relevant alternative for patients with advanced MDS.
Disclosure of Interest: None Declared.

PH-P179
ALLOGENEIC STEM CELL TRANSPLANTATION FOR HYPOPLASTIC MDS IS COMPLICATED BY INCREASED REJECTION, WHEREAS DISEASE RELAPSE IS THE PREDOMINANT CAUSE OF TREATMENT FAILURE IN ACQUIRED HYPOPLASIA IN MDS

J. Clay[1,], K. Raj[2], D. McLornan[1], H. De Lavallade[1], V. Potter[1], A. Kulasekararaj[1], M. Kenyon[1], J. Marsh[1], A. Pagliuca[1], G. Mufti[1]*
[1]Haematology, [2]Kings College Hospital, London, United Kingdom

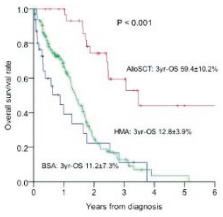
Introduction: Allogeneic stem cell transplant is performed in the treatment of patients with both hypoplastic MDS as well as to

rescue those high risk MDS-AML patients rendered hypoplastic due to induction chemotherapy. Whether the risks of rejection, disease progression or relapse differ in these patient groups and consequently the appropriate duration of immunosuppression post-transplant remains to be determined.
Materials (or patients) and Methods: Retrospective data including diagnostic and pre-transplant histology and chimerism for all sequential patients with MDS/AML transplanted between 2008-2013 with a hypocellular bone marrow in pre-transplant bone marrow biopsies were analysed. The patients were divided into those who presented with a hypocellular marrow or had a cytogenetic abnormality associated with hypocellular MDS (13q14) (hypo MDS) and those who were normocellular/hypercellular on presentation, but became persistently hypocellular following treatment (acquired hypoplasia). Clinical information including dates of taper of immunosuppressive therapy (IST), GvHD, survival status and cause of death were also collected.
Results: Thirty one patients (20 with hypo MDS, 11 with acquired hypoplasia) were transplanted (36 transplants, 1st transplant *n*= 31, 2nd transplant *n*=4, 3rd transplant *n*=1), 34 were t-cell depleted reduced intensity conditioned (25 with Alemtuzumab, 9 with ATG), and 2 with ric haplo peripheral blood stem cells with post-transplant Cyclophosphamide. In the hypo MDS group mean cellularity at presentation was 19% (range 5-30%), falling to 12% pre-transplant (the exception was a patient with 13q deletion who had an original cellularity of 60%, falling to 5% post Azacytidine and pre-transplant). There were 7 episodes of graft failure/non-engraftment, with no episodes in the acquired hypoplasia group (*P*=0.0756). The mean time to death or last follow up was 682 days (59-1692), with overall survival of 68.4%, and 1 episode of disease relapse.
In the acquired hypoplasia group mean cellularity at presentation was 70% (35-90%), falling to 15% (0-25%) pre-transplant. The mean follow up was 609 days (177-1937), with overall survival of 46%. There were 4 (31%) episodes of disease relapse.
In both groups the initiation of IST weaning was very variable (day 28-1852). During the first month following transplants which resulted in non-engraftment or graft failure 73% of tests performed had cyclosporine levels <300ug/l or tacrolimus levels <10µg/l.
Discussion: Our data suggest that whilst patients with hypoplastic MDS have very low rates of relapse post-transplant and excellent overall survival their rate of graft failure is high. In contrast for patients with acquired hypoplasia disease relapse limits overall survival. We suggest that hypoplastic MDS patients may benefit from intensive (dose/duration) immune suppression akin to that in HSCT for aplastic anaemia. In contrast for patients with acquired hypoplasia, the higher risk of relapse may be attenuated by early withdrawal of immunosuppression.
Disclosure of Interest: None Declared.

PH-P180
IMPROVED SURVIVAL WITH ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME OF HIGH AND VERY-HIGH RISK BY IPSS-R

J. H. Moon[1], S. K. Sohn[1,], J. S. Ahn[2], H. J. Kim[3], I. C. Song[4], D. Y. Jo[2], H. S. Lee[5], Y. S. Kim[5], S. H. Bae[6]*
[1]Hematology/Oncology, Kyungpook National University Hospital, Deagu, [2]Hematology/Oncology, [3]Hematology, Cheonnam National University Hwasun Hospital, Cheonnam, [4]Hematology/Oncology, Chungnam National University Hospital, Chungnam, [5]Hematology/Oncology, Kosin University Gospel Hospital, Busan, [6]Hematology/Oncology, Daegu Catholic University Medical Center, Deagu, Republic of Korea

Introduction: The revised International Prognostic Scoring System (IPSS-R) has been recently adopted for classifying risk groups in patients with myelodysplastic syndrome (MDS). The current study evaluated the role of allogeneic stem cell transplantation (allo-SCT) and hypomethylating agent (HMA) in high risk MDS patients classified by IPSS-R system.
Materials (or patients) and Methods: A total of 410 MDS patients between July 1999 and April 2013 were revised using IPSS-R.



Among 410 patients, Treatment outcome were retrospectively analyzed in 198 patients with high and very-high (H/VH) risk groups. The factors affecting the long-term outcomes were evaluated for H/VH risk patients. Overall survival (OS) rate was analyzed with Kaplan-Meyer test and each treatment groups were compared with log-rank test. Cox-proportional hazard model was used to define the prognostic factors affecting OS.
Results: Median age of the patients were 67 years (range 18-86 years) and 88 patients (44.4%) were under age 65 years. ECOG performance status (PS) were 0 to 1 in 151 patients (76.3%) and 2 to 4 in 47 (23.7%). The IPSS-R H/VH risk groups were 88 patients (44.4%) and 110 (55.6%), respectively. HMA was used for 129 patients (65.2%), allo-SCT for 34 (17.2%), and best supportive care (BSC) for 35 (17.7%). Among 34 patients with allo-SCT, 29 patient were treated with HMA before allo-SCT. The response (CR/PR/HI) rate of HMA before allo-SCT were 26.5%. Median survival time were 1266±288 days in allo-SCT, 504±41 days in HMA group, and 320±107 days in BSC group. The 3-year OS rate was 59.4±10.2% in allo-SCT, 12.8±3.9% in HMA group, and 11.2±7.3% in BSC (*P*<0.001), respectively. In the multivariate analysis, allo-SCT were only related with favorable OS (HR 0.152, *P*<0.001) in patients with H/VH risk. While ECOG-PS 2-4 (HR 3.354, *P*>0.001) and no response to HMA (HR 2.794, *P*=0.001) adversely affected to OS.
Discussion: Allo-SCT was the only factor predicting favorable long-term outcome in MDS patients with H/VH risk based on IPSS-R system. No benefit of HMA was observed in terms of OS compared to BSC in H/VH risk group. The role of the pretransplant HMA for higher risk MDS patients needs to be elucidated in the future.
Disclosure of Interest: None Declared.

PH-P181
MYELOABLATIVE CONDITIONING WITH FLUDARABINE AND (IV) BUSULFAN (F-BU4) FOR ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MYELODYSPLASIC SYNDROMES

A. Talbi[1,], R. Ahmed Nacer[1], M. Benakli[1], F. Mehdid[1], R. Belhadj[1], N. Rahmoune[1], M. Baazizi[1], D. Ait Ouali[1], H. Bouarab[1], F. Harieche[1], R. M. Hamladji[1]*
[1]Pierre and Marie Curie Center, Algiers, Algeria

Introduction: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders associated with worsening cytopenias and a variable risk of progression to acute leukemia, these disorders also leading to reduced survival and a compromised quality of life, especially in transfusion-dependent patients. Allogeneic BMT is considered the only curative approach for patients with MDS, while bone marrow transplantation clearly has a role in the treatment of MDS, the decision to proceed to transplantation is not always easy and the optimal approach has not been clearly defined.
Materials (or patients) and Methods: From February 2008 to December 2012, twenty three pts with a diagnosis of de novo MDS according to the WHO classification (RA: 6, RAEB1:7, RAEB 2: 10) received an HSCT from an HLA identical sibling donor. The median age of the series is 38 years old (25-52), the sex ratio is 1,5. Twenty pts (86, 9%) were stable on their cytopenias, their percentage of blasts on the bone marrow and their transfusion requirements, three of them were in progression. All of them received only blood and platelets transfusions and none received others

therapy as growth factors, hypomethylating agents or chemotherapy. The median duration of the disease before the transplant is 18 months (9-39). The conditioning regimen used was an association of Fludarabine (200mg/m^2) and Busilvex (12.8 mg/Kg) in a daily injection, for 4days for twice. GVHD prophylaxis consists in the association of Ciclosporin (CSA) and Methotrexate (short course Seattle), All the patients received peripheral stem cell transplant with a median rate of CD34:5 X10^6/Kg (3, 2-9).
Results: At the 15th of August 2013, the median follow up was 25 months (9-60). Median time of neutropenia is 10d (3-21). All pts have both, blood and platelet needs (3 blood units/pt) and (2 platelet units/pt).Twenty two pts (95, 6%) are still alive, 20 pts in CR (86.9%) and two pts in relapse (8.6%). Transplant related mortality is about 4.3%. Two pts (9%) have an Acute GVHD (II-IV) and 15 pts (68.1%) developed a Chronic GVHD witch is extensive in 36% pts. The overall survival (OS) and the Disease free survival are respectively 89% and 86%.
Discussion: According to our good results in TRM (4.3%), in relapse (8.6%) and OS 89% and DFS 86%, this conditioning regimen F-BU4 seems a good option in MDS.
Disclosure of Interest: None Declared.

PH-P182
ALLOGENEIC HAEMATOPOIETIC CELL TRANSPLANTATION IN MDS-PATIENTS: A RETROSPECTIVE, SINGLE INSTITUTION STUDY COMPARING NMA VS. MA CONDITIONING.

L. Smidstrup Friis[1,], L. Vindeloev[2], D. Hovgaard[2], I. M Schjoedt[2], N. Smedegaard Andersen[2], S. L. Petersen[2], H. Sengeloev[2]*
[1]Haematology, National University Hospital, Copenhagen, [2]Haematology, National University Hospital, Copenhagen, Denmark

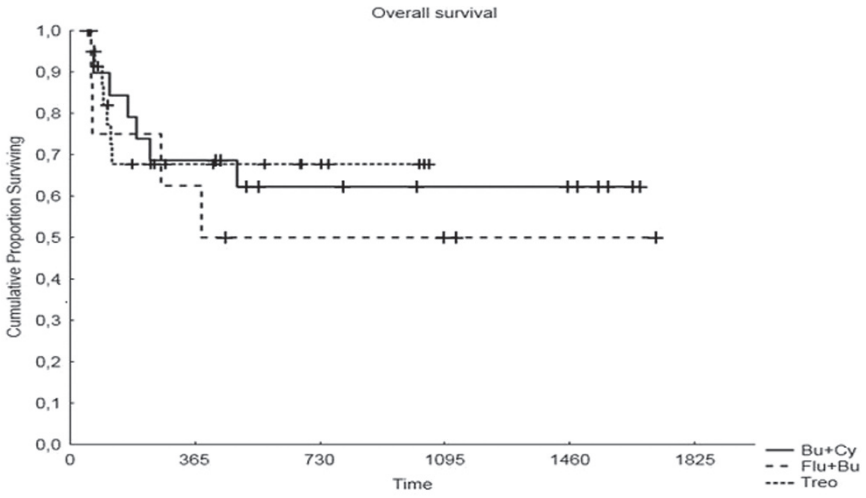
Introduction: Allogeneic haematopoietic cell transplantation (HCT) is the only potential curative treatment for patients suffering from myelodysplasia (MDS). So far, only a minor subgroup of these patients has been candidates to allogeneic HCT because of high age and comorbidity. However, due to a raising life expectancy and introduction of non-myeloablative (NMA) conditioning regimens the number of MDS patients undergoing allogeneic HCT has been increasing.
Materials (or patients) and Methods: A retrospective study of 99 patients with MDS who underwent allogeneic HCT between January 2000 and August 2013 at the department of Haematology in Copenhagen was performed. The patients had the following WHO-diagnoses: RAEB-2: 30, RAEB-1: 10, t-MDS: 16, RA/RCMD: 23, PNH: 3, MDS with fibrosis: 6, CMML: 10, Unspecific: 1. Sixty-three patients were transplanted with NMA conditioning: (Fludarabine 90 mg/m^2 and TBI 2 Gy) and 36 patients with a myeloablative (MAC) regimen (Cyclophosphamide/TBI 12 Gy or Cyclophosphamide/Busilvex).
The patients in the MAC group were significantly younger than in the NMA-group with a mean age of respectively: 39 years and 58 years. Significant more patients in the MAC group had ≥ 5% blasts in the bone marrow at the time of transplantation, 33 % vs. 11 % of the patients in the NMA-group. Otherwise, there was no difference in the two groups regarding sex, donor type, the time of transplantation (before or after 2009) or the incidence of t-MDS and CMML.
Results: The median survival for the whole MDS-group was 4,4 years. In univariate analyses there was no significant difference of the survival in the MAC and NMA-groups with a median survival of 3,6 years and 4,4 years, respectively. Surprisingly, there was also no significant difference between the relapse rates. Likewise, no differences in OS were found between patients with a sibling or unrelated donor. This was also the case when the survival of patients with an age below 50 and ≥ 50 years was compared. Marrow blast % before SCT (under vs. above 5%) had no influence on OS. CMML patients had a significant worse OS than other MDS-patients with a median survival of only 5,1 months post transplantation, *P*= 0.006. Half of the patients were transplanted in the late period after 2009. There was, however, no difference in OS in the two time periods.
Discussion: Despite a difference in median age at almost 20 years, we observed comparable overall survival in MAC and NMA patients. Overall survival was also comparable in sibling and unrelated donor transplants. CMML continues to confer a very dismal prognosis after SCT. The rather disappointing outcome of SCT in MDS patients, calls for investigations of improved transplant protocols.
Disclosure of Interest: None Declared.

PH-P183
FLUDARABINE AND TREOSULPHAN IS AN EFFECTIVE CONDITIONING REGIMEN FOR ADULT PATIENTS WITH MDS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

P. Ljungman[1,], K. LeBlanc[2], M. Engström[3], M. Jädersten[1], S. Reykdal[4], J. Mattsson[3], M. Remberger[3], E. Hellström-Lindberg[1]*
[1]Department of Hematology and Section of Hematology, Department of Medicine Huddinge, Karolinska University Hospital, Karolinska Institutet, [2]Department of Hematology, Dept. of Laboratory Medicine, Karolinska University Hospital and Karolinska Institutet, [3]Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden, [4]Department of Hematology, Landspitali University Hospital, Reykjavik, Iceland

Introduction: The only curative option for MDS is allogeneic HSCT. New therapeutic options for controlling MDS for example the use of demethylating agents such as azacytidine have been introduced during recent years. Various conditioning regimens have been used. At Karolinska University Hospital, fludarabine + treosulphan (flu+treo) as conditioning regimen has been used mainly in elderly patients while busulphan (po) + cyclophosphamide (bu+cy) has been used in younger patients. Fludarabine + busulphan (po; flu+bu) has been used in patients >60 ys either with low risk disease or co-morbidities. The aim of this analysis was to analyze outcome using the current strategy for selecting conditioning regimen.
Materials (or patients) and Methods: 53 patients with MDS were transplanted since 2009. The median age was 58 (36-68) ys. The IPSS risk scores were: INT-1 = 12; INT-2 = 16; CMML = 10; High = 5, MDS-AML = 7. Thirty patients were treated with azacytidine prior to HSCT, 20 received induction chemotherapy (of whom 9 also had received azacytidine), 4 patients got cytokines only (epo+G-CSF), while 7 patients were untreated before HSCT. Bu+cy was used in 20 patients; flu+bu in 10; and flu+treo in 23 patients, with ATG given to patients receiving unrelated donor grafts. The median age in the three groups was 50, 64, and 60 ys, respectively. The IPSS scores were comparable with the exception that no patient with MDS-AML received flu+bu. 13 patients received grafts from sibling donors and 40 from unrelated donors. The stem cell source was bone marrow in two patients, PBSC in 49, and double cord blood units in two patients.
Results: The estimated overall survival (OS) for the entire cohort at two years is 72% with a DFS of 62%. Nine patients have died from transplant related causes and 8 from disease relapse. The 2-year OS was 69% for bu+cy, 62% for flu+bu, and 80% for flu+treo. The corresponding 2-year DFS were 63% for bu+cy, 52% for flu+bu, and 68% for flu+treo. DFS was 66% for int-1, 74% for int-2, 38% for CMML, and 59% for high/AML.
Discussion: We conclude that flu+treo +/- ATG is an effective and safe preparative regimen for MDS comparable to bu+cy also in higher-risk patients. New strategies are needed to improve outcome of allogeneic HSCT in CMML.
Disclosure of Interest: None Declared.

[PH-P183]



PH-P184

IMPACT OF R-IPSS CYTOGENETICS ON OUTCOME AFTER ALLO-SCT FROM HLA-IDENTICAL SIBLINGS OR HLA-MATCHED UNRELATED DONORS FOR MYELODYSPLASTIC SYNDROMES: A STUDY OF THE SOCIÉTÉ FRANÇAISE DE GREFFE DE MOELLE ET THÉRAPIE CELLULAIRE (SFGM-TC)

J. Gauthier[1,], G. Damaj[2], A. Duhamel[3], M. Robin[4], M. Michallet[5], P. Chevallier[6], Y. Beguin[7], S. N'Guyen[8], P. Bories[9], D. Blaise[10], J. Cornillon[11], A. Clavert[12], M. Mohty[13], A. Huynh[14], A. Thiébault-Bertrand[15], S. Vigouroux[16], I. Yakoub-Agha[3] and Société Française de Greffe de Moelle et de Thérapie Cellulaire - SFGM-TC*

[1]Service des Maladies du Sang, CHRU Lille, Lille, [2]CHU Amiens, Amiens, [3]CHRU Lille, Lille, [4]Hôpital Saint Louis, Paris, [5]Centre Hospitalier Lyon-Sud, Lyon, [6]CHU Nantes, Nantes, France, [7]CH-Liège, Liège, Belgium, [8]G.H. Pitié-Salpêtrière, Paris, [9]CHU Strasbourg, Strasbourg, [10]Unité de Transplantation Médullaire, Institut Paoli Calmettes, Marseille, [11]Haematology, ICL, Saint Priest en Jarez, [12]Haematology, CH Nantes, Nantes, [13]Hôpital Saint Antoine, Paris, [14]CHU Toulouse, Toulouse, [15]CHU Grenoble, Grenoble, [16]CHU Bordeaux, Bordeaux, France

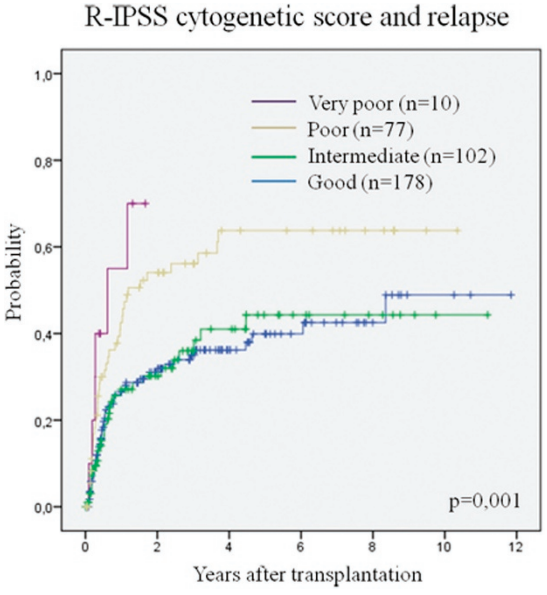
Introduction: The prognosis of myelodysplastic syndromes (MDS) is critically influenced by cytogenetic abnormalities, which can be associated with a higher risk of relapse after hematopoietic stem cell (HSC) transplantation. In this multicenter, retrospective study, we assessed the impact of the R-IPSS cytogenetic score on the outcome of MDS patients transplanted from HLA-identical siblings or HLA-matched unrelated donors.

Materials (or patients) and Methods: We analysed 367 patients who underwent allogeneic HSC transplantation between January 1999 and December 2009 at 23 centres in France and Belgium. 229 patients (62%) were transplanted from a sibling while 138 patients (38%) had an allelic HLA-matched unrelated donor (10/10). Patients received myeloablative (*n*=141, 38%) or reduced-intensity conditioning (*n*=226, 62%). Median time from diagnosis to transplantation was 54 months. At transplantation, median age was 54 years and 159 patients (43%) had a marrow blast percentage superior or equal to 5%. When classified according to the R-IPSS cytogenetic classification, 178 patients (48 %) fell in the good-risk, 102 (28%) in the intermediate-risk, 77 (21%) in the poor-risk and 10 (3%) in the very poor-risk group.

Results: We observed a dramatic impact of the R-IPSS cytogenetic classification on overall survival and relapse rates after allo-SCT. In multivariate analysis, the poor and very-poor risk categories significantly correlated with poorer overall survival (HR=1,52, *P*=0.019 and HR=2.69, *P*=0.005, respectively) and higher relapse rates (HR=1,85, *P*=0.003 and HR=2,68, *P*=0.026, respectively). The R-IPSS cytogenetic classification separated into three groups (intermediate, poor and very poor risk) patients categorised as poor risk using IPSS cytogenetics. Relapse rates were significantly different between the intermediate and the poor/ very-poor categories (17% and 55%, respectively, *P*=0,036, *n*=95 patients).

Discussion: The R-IPSS cytogenetic classification predicts the outcome of MDS patients after bone marrow transplantation and is more discriminating than IPSS cytogenetics. Forthcoming research should focus on preventive strategies in patients at high risk of relapse.

Disclosure of Interest: None Declared.



PH-P185

THIOTEPA-BASED CONDITIONING FOLLOWED BY ALLOGENEIC SCT IN PATIENTS WITH MDS A SURVEY OF THE CHRONIC MALIGNANCIES WORKING PARTY OF EBMT

N. Kröger[1,], L. de Wreede[2], A. van Biezen[2], T. de Witte[3] on behalf of CMWP (EBMT) and Chronic Malignancies Working Party*

[1]University Hospital, Hamburg, Germany, [2]University Medical Center, Leiden, [3]Radboud University Medical Center, Nijmegen, Netherlands

Introduction: To investigate the use and outcome of thiotepa in combination with fludarabine or other drugs we screened 4852 patients in EBMT database and found 225 patients with a median age of 56 years (range 18 - 71) who received thiotepa-based regimen.

Materials (or patients) and Methods: Male/female distribution was138/87. Diagnoses were RA/RARS in 29 (16%), RCMD in 16 (9%), RAEB in 76 (41%), RAEB-t in 28 (15%), and sAML/sMDS in 35 (19%). At transplantation 51 (25%) were in CR1, 105 (51%) did not respond to chemotherapy (no CR), and 49 (24%) were transplanted without prior treatment.

Reduced intensity conditioning was used in 144 (64%) and MAC in 81 (36%) of the patients prior to allogeneic stem cell transplantation from HLA-identical sibling (39%), matched unrelated (54%), and mismatched related or unrelated (6%) donor or other relatives (1%). Bone marrow or peripheral blood were used in 35% and 65%, respectively.

Results: The median time to leukocyte engraftment was 17 days (9 – 46). The non-relapse mortality at 1 year was 36% and CI of relapse at 3 years was 21%. The 3-year relapse-free and overall survival were 40% and 42%, respectively.

We further analysed a comparison between thiotepa plus fludarabine (n = 42) vs. thiotepa/fludarabine plus other drugs such as busulfan, cyclophosphamide, or melphalan (n = 86). A non-significant (P = 0.63) trend for more NRM at 1 year (39% vs. 31%) and a trend for higher risk of relapse at 3 years (25% vs. 14%) was seen for the thiotepa/fludarabine plus other drugs group resulting in a favorable trend in survival at 3 years for the thiotepa/fludarabine combination (51% vs. 32%, P = 0.28).

Within the thiotepa group significant factors for improved survival were age less than 60 years (P = 0.01), and RA/RARS vs. others ($p < 0.001$). The survival of 33 patients with RA/RARS, del 5q, and RCMD-RS was excellent with 75% survival at 3 years.

In a multivariate analysis for overall survival including all 4852 patients significant factors were age (HR 1.015, 95% CI: 1.011 – 1.1019, $p < 0.001$), non CR at transplantation (HR 1.316, 95% CI: 1.174 – 1.474, $p < 0.001$), RAEB (HR 1.389, 95% CI: 1.202 – 1.6044, $p < 0.001$), and sAML (HR 1.507, 95% CI: 1.302 – 1.74, $p < 0.001$), whereas thiotepa-containing regimens vs. other regimen did not influence survival significantly (HR 1.178, 95% CI: 0.893 – 1.341, P = 0.126).

Discussion: We conclude thiotepa alone or in combination can be used as conditioning regimen for patients with MDS who undergo allogeneic stem cell transplantation and deserves further investigations.

Disclosure of Interest: N. Kröger Conflict with: Research funding from Riemser, L. de Wreede: None Declared, A. van Biezen: None Declared, T. de Witte: None Declared.

PH-P186

ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR JAPANESE FANCONI ANEMIA PATIENTS WITH MYELOID MALIGNANCIES

M. Yabe[1,], T. Morimoto[2], T. Shimizu[2], T. Koike[2], H. Takakura[2], K. Ohtsubo[2], A. Fukumura[2], M. Itosu[2], K. Muroi[3], K. Koh[4], S. Kato[1], H. Yabe[1]*

[1]Department of Cell Transplantation and Regenerative Medicine, Tokai University Hospital, [2]Department of Pediatrics, TOKAI UNIVERSITY HOSPITAL, Isehara, [3]Division of Cell Transplantation and transfusion, Jichi Medical University Hospital, Shimotsuke, [4]Department of Hematology/Oncology, Saitama Children's Medical Center, Saitama, Japan

Introduction: The only cure for the hematological abnormalities of Fanconi anemia (FA) remains allogeneic hematopoietic cell transplantation (HCT). Few reports are available on outcomes after HCT in FA patients (pts) with myeloid malignancies. We analyzed data of the outcome of 33 Japanese FA patients with myeloid malignancies.

Materials (or patients) and Methods: Between 11/1991and 11/2013, 33 FA pts received HCT. These included 16 males and 17 females aged 1.1-37.4 (median age 11.2 years). Twenty-three pts had myelodysplastic syndrome (MDS) in refractory anemia (RA) (N=12), refractory anemia with excess blasts (RAEB) (N=11), while ten pts had acute myeloid leukemia (AML). All pts with RAEB or AML and 8 pts with RA had cytogenetic abnormalities involving: chromosome (Chr) 1(N=13), Chr 3 (N= 10) and Chr 7 (N=12), with 13pts having complex abnormalities. Donors were related for 16 pts: matched (N=7) or mismatched (N=9) and unrelated for 17 pts: matched (N=8) or mismatched (N=9). Twenty-five pts received bone marrow cells (BM), two received peripheral blood stem cells (PB), three received BM + PB cells, and three received cord blood grafts. Nine pts received radiation-cyclophosphamide (CY) based regimens, and 24 received fludarabine-based regimens. Three pts received cyclosporine-based GVHD prophylaxis, six received CD34 positive selection or T-cell depleted grafts, and 24 received tacrolimus-based GVHD prophylaxis.

Results: Twenty-eight pts had neutrophil recovery by day 28. Two pts received CD34 positive selected grafts had rejection, and three pts died of infection or bleeding by day 28. Two pts had secondary graft failure by one year after engraftment. With a median follow-up of 3.8 years (range 0.4-19.6), 21 pts are alive with leukemia free, and 12 pts died; causes of death were relapse (N=4), graft failure (N=1), infection (N=2), bleeding (N=1), multiple organ failure (N=1), lymph proliferative disorder after HCT (N=1), donor-type leukemia (N-1) and secondary cancer (N-1). Acute graft-versus-host disease (GVHD) of grades II or more developed in five pts and chronic GVHD developed in 12 pts. Survival probabilities at 3 years of RA, RAEB and AML were 91.7% (95% CI, 76% to 100%), 80.8% (95% CI, 57% to 100%), and 26.7% (95% CI, 0% to 56%), respectively.

Discussion: Our study indicates that long-term survival for FA patients with myeloid malignancies is achievable, and HCT may be necessary before developing of advanced MDS (RAEB) or AML. Cytogenetic abnormalities involving chromosome 1, 3, 7 and complex karyotype may be associated with increased risk of MDS and AML development for FA patients.

Disclosure of Interest: None Declared.

PH-P187

IMPACT OF ACUTE GVHD ON OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL IN CHILDREN AND ADOLESCENTS SUFFERING FROM MYELODYSPLASTIC SYNDROME

A. Alekseevna Ratc[1,], A. Svytoslavna Borovkova[1], N. Vasilevna Stancheva[1], P. Valerevna Kozhokar[1], S. Viktorovna Razumova[1], T. Alexsandrovna Bykova[1], E. Vladimirovna Semenova[1], O. Vladimirovna Paina[1], I. Viktorovna Marcova[1], M. Mixailovna Smirennikova[1], K. Alexsandrovich Ekushov[1], Y. Gennadevna Fedukova[1], E. Vladislavna Morozova[1], S. Nilolaevich Bondarenko[1], A. Vadimovich Kozlov[1], L. Stepanovna Zubarovskay[1], B. Vladimirovich Afanasyev[1]*

[1]R.GORBACHEVA MEMORIAL INSTITUTE OF CHILDREN ONCOLOGY, HEMATOLOGY AND TRANSPLANTATION, FIRST STATE PAVLOV MEDICAL UNIVERSITY, Saint-Petersburg, Russian Federation

Introduction: Myelodisplastic syndrome (MDS) is a group of clonal and heterogeneous diseases, characterized by ineffective hematopoesis 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 childhood MDS, but long-term survival depends on a lot of factors. Among them impact of acute graft-versus-host disease (aGVHD) on overall survival is controversial.

To analyze the influence of aGVHD on the outcome of childhood MDS after allo-HSCT.

Materials (or patients) and Methods: Allo-HSCT were performed in 36 patients (pts) (19 boys; 17 girls) with following diseases: refractory cytopenia of childhood -9 pts (25%), refractory anemia with excess blasts -11 pts (30,5%), refractory anemia with excess blasts in transformation-14 pts (39%), juvenile myelomonocytic leukemia in 2 pts (5,5%). Cytogenetic analysis showed: normal karyotype in 8 pts; monosomy 7-11 pts; structurally complex abnormalities of karyotype- 6 pts; other aberrations–11 pts. The median of age was 10 years (1-19 years). Unrelated allo-HSCT was done in 26 pts (72 %), related – in 6 pts (17 %), haplo- in 4 pts (11%). Myeloablative conditioning regimens (MAC) were used in 18 pts (50%); reduced-intensity conditioning (RIC) in 18 pts (50 %). MAC consisted of Busulfan (Bu) 16 mg/kg + Cyclophosphamide 120 mg/kg. RIC included Fludarabine (Flu) 150 mg/m^2 + Melphalan (Mel) 140 mg/m^2, Flu 150 mg/m^2 + Bu 8mg/kg. The bone marrow (BM) was used in 22 pts (61,1%), peripheral blood stem cells (PBSC) in 12 pts (33,3%), combination of BM and PBSC in 2 pts (5,6%).
Results: 5-year overall survival (OS) was 55%. OS after MAC allo-HSCT -64%, after RIC allo-HSCT – 45% (*P*=0,25). Engraftment was on day + 19 (range 11-43). In group of patients who achieved engraftment (*n*=36) aGVHD I-III grade developed in 19 pts (55%) (gr I – 6 pts,gr. II -4 pts.,gr.III- 9pts). 12 pts had no signs of GVHD. 5- years OS in group of pts with grade I-III aGVHD (*n*=19) was 76% (MAC – 72%, RIC –52%; *P*=0,214). 5- years OS in group of pts without signs of aGVHD (*n*=12) – 20% (*P*=0,003). The main reason of pts mortality in aGVHD group – 4 pts gr IV aGVHD, 2 pts infectious complications, 2 pts progression of disease, without aGVHD–3 pts infectious complications, 4 pts progression of disease.
Discussion: Allogeneic HSCT – effective treatment for children and adolescence with MDS.
Our data demonstrate that aGVHD reliably influence on OS of pts with childhood MDS and further studies should be conducted.
Disclosure of Interest: None Declared.

PH-P188
ESSENTIAL THROMBOCYTOSIS PATIENTS HAVE ACCUMULATION OF CD8+ CELLS IN THE MARROW AND THE PROPORTION OF MARROW CELLS PRODUCED IL-17

M. Sedzimirska[1], D. Dlubek[1,2], A. Klimczak[2], E. Jaskula[2], A. Lange[1,2,]*
[1]Lower Silesian Center for Cellular Transplantation with National Bone Marrow Donor Registry, [2]L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Introduction: Thirty-one patients with essential thrombocytosis (ET, F/M: 20/11 age 19-86, median 60 years, platelets: 499-1724x10^3/µl (median: 652x10^3), JAK2 V617F mutation: 26 positive, marrow cellularity: 6.1–184x10^3/µl (median: 31x10^3/µl)) were studied at the time of the diagnostic procedure for the presence in the marrow (i) cells and (ii) some transcripts associated with the immune response.
Materials (or patients) and Methods: Five techniques were employed: (i) marrow smears analysis, (ii) four color cytometric (BD, San Jose; e-biosciences, San Diego, CA, USA) analysis, (iii) RT-PCR relative quantification of IL-17, ROR gamma t, IFN gamma and CXCL10 mRNA against four housekeeping genes (ABL, HPRT, b-actin, beta 2 microglobulin), (iv) trephine biopsies specimens were in addition to the routine analysis (PAS, HE and reticulin silver stain) stained for CD34, CD15, CD68, CD3, CD4, CD8, IL-17 and (v) immunofluorescence double staining (IL-17/CD15). For transcripts study the ET patients results were compared with those of CML patients (6 patients at diagnosis). Nine healthy volunteers served as a control group for the peripheral blood mononuclear cells IL-17 study.
Results: (i) The marrow lymphocyte population (gated according to the physical parameters and CD45 positivity) differed as compared to *blood* with respect to: lower CD4+/CD8+ ratio (1.30±0.115 vs *2.125±0.202*, *P*<0.001), lower CD45RO+CCR7+/CD45RO+CCR7- ratio (0.243±0.032 vs *0.322±0.036*, *P*=0.007).
(ii) IL-17+ blood mononuclear cells (subjected to 4 hours of Ionomycin, Brefeldin A, and PMA stimulation) were present in higher proportions in ET patients in both CD4+ and CD4- lymphocyte subpopulations as compared to healthy volunteers (CD4+IL-17+: 0.412±0.129% vs 0.063±0.022%, *P*=0.002; CD4-IL-17+: 0.627±0.290% vs 0.034±0.007%, *P*=0.029).
(iii) IL-17 (4.19E-04±2.44E-04 vs 6.0E-07±3.0 E-07, *P*=0.027) and ROR gamma t (3.32E-02±1.22E-02 vs 2.60 E-03±3.57E-04, *P*=0.003) transcripts levels in marrow cells population enriched in mononuclear cells with the use of density gradient separation were significantly higher in ET patients as compared to those found in CML patients (at diagnosis).
(iv) IFN gamma (8.60E-01±6.48E-01 vs 1.95E-02±1.04E-02, *P*=0.018) and CXCL10 (5.76E-01±4.01E-01 vs 1.59E-02±5.56E-03, *P*=0.054) transcripts also prevailed in marrow of ET patients as compared to CML group.
(v) Trephine biopsies staining revealed: high numbers of CD8+ cells in 22 out of 30 patients (> 40 CD8+ cells/HPF); high proportions of cells positive for IL-17 in 25 patients out of 30 (30 to 180 IL-17+ cells/HPF). Double staining for IL-17 and CD15 documented that IL-17+ cells were in the majority also CD15 positive cells.
Discussion: We conclude:
• in the lymphocyte marrow population of ET patients there is an increase of cytotoxic lymphocytes and those of effector/memory cells phenotype what may suggest the presence of an active immune response at the marrow site in at early stage of the disease prior to the treatment,
• IL-17 engagement in ET associated processes is suggested by an increase of IL-17+ lymphocytes in blood and high proportions of IL-17 producing cells in the marrow the majority of which are CD15 positive. The latter observation suggests that the innate immunity is involved in initiation of the immune system recognition of ET associated factors.
Supported by the National Centre for Research grant NN402430039.
Disclosure of Interest: None Declared.

Non-hematopoietic Stem Cells

PH-P189
CHARACTERIZATION OF SPLEEN-DERIVED MESENCHYMAL STEM CELLS IN PH-NEG MPN PATIENTS

M. Mantelli[1], M. antonietta Avanzini[1,], D. Maria Ingo[1], E. Lenta[1], A. Aronica[1], E. Bonetti[1], L. Cobianchi[1], S. Zonta[1], S. Baleri[1], G. Acquafredda[1], L. Catenacci[1], A. Moretta[1], R. Maccario[1], G. Barosi[1], V. Rosti[1], M. Zecca[1], M. Ester Bernardo[2]*
[1]Fondazione IRCCS Policlinico S. Matteo, Pavia, [2]Ospedale Pediatrico Bambino Gesù, Roma, Italy

Introduction: Extramedullary hematopoiesis, as consequence of bone marrow microenvironment dysregulation, is a key feature of advanced stage disease in Philadelphia negative myeloproliferative neoplasms (Ph-neg MPN), and in particular of myelofibrosis. In patients with myelofibrosis, extramedullary hematopoiesis occurs mainly in the spleen, where a microenvironment that provides a residence for circulating hematopoietic progenitor/stem cells (HSCs/HPCs) may be derived from endogenous splenic cells and/or from the mobilization of bone marrow mesenchymal stromal cells (MSCs).
Materials (or patients) and Methods: In order to investigate Ph-neg MPN splenic microenvironment, we evaluated the possibility to *in vitro* isolate MSCs from the spleen of 23 patients with myelofibrosis undergoing therapeutic splenectomy for progressive splenomegaly and of 7 healthy donors (HDs) undergoing splenectomy for trauma surgery. Written informed consent was obtained from both patients and HD. Following the standard procedure for BM-MSC expansion, we were able to isolate and *in vitro* expand MSCs from the spleen of 9 patients with myelofibrosis (39%) and of 3 HDs (43%). MSCs were characterized for morphology, clonogenic efficiency (CFU-F), proliferative capacity (population doubling, PD), immunophenotype (flow-cytometry),

osteogenic and adipogenic differentiation potential (histological staining), and ability to reach senescence. Moreover, the capability to support hematopoiesis of HD-derived CD34+ cells by co-cultures on feeder layers of irradiated spleen MSCs from both patients and HDs will be evaluated
Results: Preliminary data suggest that: *i*. spleen MSCs from both patients and HDs show a morphology typical of aged cells, and they enter senescence phase at earlier passages (p) (median value: p4, range: p2-p10 and p4, range: p4-p10, respectively), in comparison with BM-MSC; *ii*. CFU-F number is higher in patients than in HDs (median: 0.07, range: 0.03-0.1 and median: 0.03, range. 0.03-0.04/10^6 seeded cells, respectively) showing that higher number of MSC precursors are present in myelofibrosis splenic tissues; *iii*. patients MSC proliferative capacity is lower than that of HDs.
Discussion: Experiments aimed to assess the differentiation potential and the support to *in vitro* hematopoiesis are in progress. How these characteristics of spleen-derived MSCs from patients may affect local microenvironment and support extramedullary hematopoiesis need further investigations.
Disclosure of Interest: None Declared.

PH-P190
ADIPOCYTE HYPERPLASIA INDUCED BY ARA-C IS INHIBITED BY NAC-INDUCED DECREASE OF INTRACELLULAR REACTIVE OXYGEN SPECIES (ROS) LEVEL

W. Wang[1,], R. Zhu[2], Y. Zhang[1], W. Lu[1], K. Liu[1]*
[1]Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Institute of Hematology, Peking University People's Hospital, [2]Peking Union Medical College Hospital, Beijing, China

Introduction: This study was designed to investigate the involvement of intracellular ROS in adipocyte hyperplasia induced by Ara-c.
Materials (or patients) and Methods: C57BL/6J female mice (6-8 weeks, 20g, *n*=80) were divided into 4 groups. Ara-C group animals were administered 0.5 g/(kgμd) Ara-C (Sigma, USA) via intraperitoneal injection for four consecutive days to induce hematopoietic stress. NAC group animals were administered 0.1 g/(kgμd) N-acetyl-L-cysteine (NAC, Sigma, USA) via intraperitoneal injection for four consecutive days and 1g/(Lμd) NAC drinking water were given for 28 days. Ara-C+NAC group animals were given both reagents as described above. Control group animals were injected with the same volume PBS. Intracellular ROS levels were measured using an H2DCFDA probe (Molecular Probe, USA) 10mM. To observe the changes of adipocyte in bone marrow, tibias were collected and detected by histopathology once a week. PPARγ and adiponectin protein levels were assessed by western blotting and mRNA levels were assessed by qRT-PCR. Data were presented as mean± S.D. Statistical differences between two groups were evaluated by Student's t test. For multiple group comparisons, data were analyzed by one-way analysis of variance (ANOVA).
Results: We found that adipocyte hyperplasia could be induced by Ara-C treatment. Compared to control group, the sinuses of Ara-C treated mice were widely dilated, hyperemic and composed of discontinuous endothelial cells. A significant increase of adipocyte counts was also observed in the tibias of Ara-C treated mice. Moreover, the gene expression and protein levels of major adipogenic transcription factor PPARγ and its target gene adiponectin were significantly increased.
Next, we investigated whether ROS is involved in the adipogenesis induced by chemotherapy. The flow cytometry analysis on bone-marrow derived mesenchymal stem cells revealed that Ara-C was able to induce ROS generation with a significant increase compared to control group whereas NAC reduced the production of ROS. In addition, adipogenesis in long bones following Ara-C treatment is successfully inhibited by NAC. Decreased numbers of adipocyte were observed and the expression of PPARγ and adiponectin was suppressed by treatment with NAC.
Discussion: Recent studies reveal that adipocyte may play a negative role in hematopoiesis. However, the cause of adipocyte hyperplasia after chemotherapy remains unknown. Mesenchymal stem cells (MSCs) could differentiate into adipocyte. ROS plays an important role during the early stage of adipocyte differentiation of MSCs *in vitro*. However, whether ROS involves in the adipogenesis induced by chemotherapy *in vivo* is unclear. In the present study, we demonstrated that Ara-C treatment is able to induce ROS generation that in turn influences key factors involved in adipocyte differentiation of MSCs *in vivo*. We show that adipocyte hyperplasia is diminished in the presence of ROS scavenger NAC. The use of antioxidant may be a potential way to inhibit the generation of adipocyte induced by chemotherapy that in turn improves the hematopoietic recovery.
In addition, several reports demonstrate the role of ROS in other kinds of stem cells. Future studies will shed light to whether an increase in ROS is a general requirement for stem cells differentiation and the common mechanisms by which ROS initiate stem cell differentiation.
Disclosure of Interest: None Declared.

PH-P191
GENETIC SIGNATURE OF MESENCHYMAL STROMAL CELLS DERIVED FROM HUMAN BONE MARROW CD271+ MONONUCLEAR CELLS

S. Kuci[1,], Z. Kuci[1], J. Kollet[2], H. Kreyenberg[1], H. Boenig[3], T. Klingebiel[4], P. Bader[1]*
[1]Hematology/Oncology, University Children's Hospital, Frankfurt am Main, [2]Bioinformatics, Miltenyi Biotec, Bergisch Gladbach, [3]Cell Processing, Institute for blood transfusion and immunohematology, [4]Hematology/Oncology, University Childlren's Hospital, Frankfurt am Main, Germany

Introduction: As the number of reports on the gene expression profile of the mesenchymal stromal cells (MSCs) derived from $CD271^+$ BM-MNCs (CD271-MSCs) is scanty, determination of the genetic signature of these MSCs was the main focus of this study.
Materials (or patients) and Methods: For this purpose, we expanded CD271-MSCs and MSCs generated through plastic adherence (PA-MSCs) as a control group until passage 3. Both MSC types (*n*=3) were compared against each other in gene expression microarray experiments. Raw intensity data were extracted from Feature Extraction output files for Agilent Whole Human Genome Oligo Microarrays 8X60K (Agilent Technologies, Inc) using Rosetta Resolver software (Rosetta, Inpharmatics, LLC.). The set of up- or down-regulated genes between CD271-MSCs and PA-MSCs were hierarchically clustered and displayed in heatmap images using Multiple Experiment Viewer software. Reporters identified in the discriminatory genes analysis were annotated with information from Gene Ontology (GO), which provides information on molecular function, as well as various pathway resources for information on involvement in biological signalling pathways. Differences between the sample group means of CD271-MSCs and PA-MSCs were assessed with Student's t-test (two-tailed, equal variance). Reporters were considered as differentially expressed when they passed the filtering criteria of an uncorrected *P*-value of 0.05 or less, and fold change difference of at least 1.5-fold up- or down-regulation of the mean of the CD271-MSC sample group compared to the PA-MSC sample group.
Results: In CD271-MSCs were identified 287 genes with higher expression and 204 genes with lower expression compared to PA-MSCs. Preliminary results using Functional Grouping Analysis showed that the most prominent associations of upregulated genes were related to immune response. The majority of associated genes with 'T-cell immunity' overlap with partially redundant categories of 'Innate immunity' (34 genes, *P*=3.9e-07), 'Response to toxins' (52 genes, *P*=9.7e-06) and 'Receptor signaling' (52 genes, *P*=5.5e-05). Interestingly, many of these genes are involved in antigen presentation and cell-mediated immune responses: HLA-Class II, CIITA, invariant chain (CD74), alpha-2-microglobulin and genes important in peroxisome function. In addition, genes of innate immunity were also upregulated e.g. several members of the defensin gene family, complement factors and iNOS. Genes identified with 'Receptor signalling' were mainly related to developmental processes including cell proliferation and

differentiation. Only the set of down-regulated genes involved members of significant pathways. Consistent with the findings of the functional processes, signalling pathways such as that of TGF-beta and Wnt-pathway were affected which may correlate with more alterations in cytoskeleton and proliferation potential. In addition, cytokine/ chemokine signalling pathways were significantly enriched, confirming the aforementioned expression of immunoregulatory molecules by these MSCs.
Discussion: Taken together, these results may explain the genetic basis for the functional differences between CD271-MSCs and PA-MSCs concerning their proliferative, differentiation and engraftment-promoting properties.
Disclosure of Interest: None Declared.

PH-P192

MESENCHYMAL STROMAL CELLS INHIBIT PROLIFERATION OF CMV-SPECIFIC CD8+ T CELLS

G. Malcherek[1], N. Jin[1], P. Wuchter[1], A. Diehlmann[1], A. Schmitt[1], P. Dreger[1], A. D. Ho[1], S. Michael[1,]*
[1]University Clinic Heidelberg, Heidelberg, Germany

Introduction: Mesenchymal stem cells (MSCs) are multipotential cells which are capable of differentiating into a variety of cell types. Due to their immunomodulatory properties, MSCs have been used as cell-based therapy to reduce grafts-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). As reactivation of cytomegalovirus (CMV) constitutes a frequent problem after HSCT, we studied whether MSCs may also have an influence on CMV-specific T cell responses.
Materials (or patients) and Methods: To study the effect of MSCs on alloantigen-induced proliferation a mixed lymphocyte reaction (MLR) was conducted. CD8 negative peripheral blood mononuclear cells (PBMCs) from donor A were co-incubated with CD8 positive cells from donor B (APC: T cell ratio=8:1) for 6 days. MACS®-purified CD8 positive T cells from buffy coats of HLA-A2/CMV seropositive healthy volunteers were stimulated with immunodominant CMVpp65- and influenza-derived peptides in a mixed lymphocyte-peptide culture (MLPC). Bone-marrow derived MSCs from human platelet lysate (PL) or fetal calf serum (FCS) based medium were co-cultured with the MLR and MLPC. Flow cytometry and enzyme-linked immunospot (ELISPOT) assays were used to study the immunomodulatory effect of MSCs on virus-specific CD8 positive T cells.
Results: We confirmed in a MLR that third party MSCs suppress alloantigen-induced proliferation. We demonstrated that MSCs do also inhibit proliferation of CMVpp65 (495-503)-specific CD8 positive T cells. Inhibition was strictly dependent on the number of MSCs but independent of the medium. We could corroborate our findings with other immunodominant T cell specificity towards CMVpp65 (417-26) and influenza matrix protein. Thus, our data are not in line with the notion that MSCs have a differential effect on alloantigen- and virus-specific T cells.
Discussion: MSCs have strong immunosuppressive effects on alloreactive T cells and infusion of MSCs could be a promising immunotherapy for GVHD. However, MSCs can also inhibit CMV specific CD8 positive T cells. Therefore attention must be paid to early detection and pre-emptive treatment of CMV reactivation when patients are undergoing therapy with MSC.
Disclosure of Interest: None Declared.

PH-P193

PHENOTYPICAL AND FUNCTIONAL CHARACTERIZATION OF *IN VITRO* EXPANDED BONE MARROW MESENCHYMAL STEM CELLS FROM PATIENTS WITH CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

B. Wang[1,], Y. Hu[1], R. Tie[1], Y. He[1], S. Fu[1], L. Sheng[1], X. Yu[1], H. Huang[1]*
[1]Zhejiang University School of Medicine, Hangzhou, China

Introduction: Mesenchymal stem cells (MSCs) have been applied to treat refractory chronic graft-versus-host disease (cGVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT) because of their potent immunomodulatory effects. However, the influence of cGVHD on MSCs is unknown. Here we analyzed the characteristics of MSCs derived from patients with cGVHD and without cGVHD.
Materials (or patients) and Methods: Bone marrow MSCs were isolated from 22 patients with cGVHD (median age 29, range 15-47) and 18 patients without cGVHD (median age 26, range 18-43). Chimerism was analyzed by short tandem repeat (STR)-PCR. MSC frequency was evaluated by counting colony forming unit fibroblasts (CFU-F) in 10^6 bone marrow mononuclear cells. Senescence-associated β-galactosidase (SAβ-gal) assay and real time PCR were used for evaluating senescence of MSCs. Apoptosis and immunoregulatory functions were assessed by flow cytometry, and supernatant cytokines was detected by ELISA. The migration potential was evaluated using transwell chambers.
Results: Both MSCs from patients with or without cGVHD were of host origin, and positive for CD73, CD90, CD105; negative for CD11b, CD19, CD34, CD45, HLA-DR. The frequency of MSCs did not differ significantly between patients with cGVHD (19.35±4.16) and without cGVHD (23.77±10.21) (P=0.635). They showed similar morphology, population doubling times and differentiation capacity. SAβ-gal assay revealed that the percentage of senescenct cells was 8.82±1.17% in patients with cGVHD and 11.64±3.17% in patients without cGVHD (P=0.415). The expression of senescence-associated genes such as p53, p21, p16 were also comparable. No remarkable differences of apoptotic cells were observed between two groups. Importantly, the immunoregulatory functions of MSCs were not affected by cGVHD. They strongly inhibited the proliferation of phytohemagglutinin-activated peripheral blood mononuclear cells (PBMCs). Cocultured with $CD4^+$ T cells, they significantly increased the percentage of $CD4^+CD25^{hi}CD127^{lo/-}$ Treg cells compared with $CD4^+$ control cells (7.81±0.53% versus 1.64±0.19% P<0.001), and no significant differences compared with MSCs from patients without cGVHD (8.59±0.88% P=0.788). In addition, secretion of immunosuppressive cytokines IL-10, TGF-β, HGF, PGE2 was similar between two groups. To test whether migration of MSCs was influenced by cGVHD, we used transwell assay and results showed that this function was slightly impaired but did not reach significance.
Discussion: These results suggest that MSCs of patients with cGVHD preserve similar characteristics and functionality as those from no cGVHD controls. Hence, they could be considered in an autologous setting for those patients whose MSCs were not impaired before hematopoietic stem cell transplantation.
Disclosure of Interest: None Declared.

PH-P194

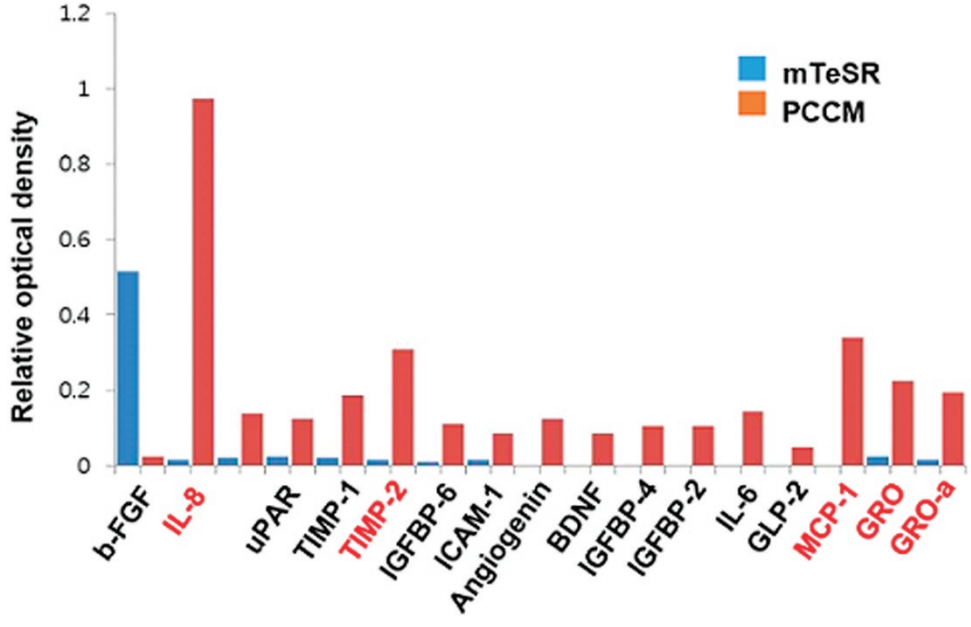
HUMAN PLACENTAL MESENCHYMAL STEM CELLS (PMSCS)-CONDITIONED MEDIA SHOWED THE LONG-TERM EFFICACY FOR SUPPORTING STEMNESS OF HUMAN INDUCED PLURIPOTENT STEM CELLS *EX VIVO*

Y. Park[1,], J.-H. Jung[1], J. H. Kim[1], H. J. Sung[1], S. C. Hong[2], B. S. Kim[1]*
[1]Korea University School of Medicine, Institute of Stem Cell research, Seoul, Korea, Republic Of, [2]Department of Obsterics and Gynecology, Korea University School of Medicine, Institute of Stem Cell research, Seoul, Republic Of Korea

Introduction: For clinical application of cells derived from human pluripotent stem cells (hiPSCs), the safety and cost-effectiveness of culture system for hiPSCs should be guaranteed. Current *ex vivo* feeder-free culture system for human pluripotent stem cells requires the gelatin conditioned with mouse embryonic fibroblast (Matrigel®) and continuous supplementation of basic FGF. In this culture system, Very high cost is required and contamination of animal product is unavoidable. In this study we evaluate the long-term efficacy of newly developed hiPSCs culture system using human pMSCs-conditioned media (PCCM) without bFGF and Matrigel®.
Materials (or patients) and Methods: Surgically isolated placental chorionic plates from healthy women who had undergone abortion at 6-8 weeks of gestation were minced and incubated. At

approximately 2 weeks after inoculation, colonies of fibroblast-like cells were collected. For purification of pMSCs, CD44+CD34- cells was obtained by flowcytometric isolation. For generation of PCCM, These cells were cultured by chemically defined culture media for 48 hours and then culture media (PCCM) was collected. bFGF-supplemented feeder-free culture media(mTeSR1®) was used as control. The hiPSCs were maintained on culture plate coated only by gelatin using PCCM with or without bFGF. Stemness of cultured hiPSCs was identified by immunostaining for alkaline phosphatase (ALP), flowcytometry for stage specific embryonic antigen (SSEA)-1, SSEA-4, tumor rejection antigen (TRA)-60, TRA-81, and RT-PCR for Oct-4, Nanog, and Rex-1 at every 10th passage. Embryoid body (EB) formation was induced from cultured hiPSCs at every 10th passage. For detection of the presence of three germ layers within the formed EB, RT-PCR analysis were performed on day 21 (Desmin for mesoderm, AFP for endoderm, TUJ1 for ectoderm). To define the composition of PCCM, cytokine array and ELISA was performed.
Results: Over the 26 passages for 6 months, the undifferentiated morphology of colonies of hiPSCs was observed only in condition using PCCM without bFGF. Stemness markers of morphologically undifferentiated colony were well expressed in this condition. EB formation from this colonies was successful and expression of three germ layer specific markers were well observed. Cytokine array and ELISA identified higher concentration of IL-8, MCP-1, GRO, and GRO-a and lower concentration of bFGF in PCCM compared with mTeSR1®.
Discussion: This newly developed culture system using PCCM without exogenous bFGF supplementation and gelatin coating plate showed to be effective in long-term *ex vivo* maintaining stemness of hiPSCs. The mechanism by which PCCM supports stemness of hiPSCs is considered to be bFGF-independent. However, exact pathway should be investigated. Based on the results of cytokine array, IL-8 is considered to be most likely associated with supporting stemness of hiPSCs in PCCM-based culture system. [This research was supported by Bio-Health care Technology Development Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (R1211902)].
Disclosure of Interest: None Declared.

[PH-P194]



Reduced-intensity Conditioning

PH-P195
COMBINATION OF FLUDARABINE, AMSACRINE AND CYTARABINE FOLLOWED BY REDUCED-INTENSITY CONDITIONING AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN 15 PATIENTS WITH MYELOFIBROSIS

M. Krejci[1,], M. Doubek[1], Y. Brychtova[1], M. Tomiska[1], O. Horky[1], V. Sandecka[1], J. Mayer[1]*
[1]Department of Internal Medicine, Hematology and Oncology, University Hospital, Brno, Czech Republic Brno

Introduction: Patients (pts) with myelofibrosis with intermediate-2 or high-risk disease according to the Dynamic International Prognostic Scoring System (DIPSS) plus prognostic model can be treated by allogeneic hematopoetic stem cell transplantation (SCT). Different variants of conditioning regimens have been used for pts with myelofibrosis. It is not yet clear whether some of these regimens are better than others. Reduced-intensity conditioning (RIC) regimens have been advocated to reduce transplant related toxicity in older pts with comorbidities. Sequential use of chemotherapy and reduced-intensity conditioning (RIC) with allogeneic stem cell transplantation (SCT) has been proposed to improve the treatment outcomes predominantly in pts with high-risk myeloid malignancies. Here we present our experience with this therapeutic strategy in cohort of 15 pts with high risk or intermediate-2 risk myelofibrosis (MF).
Materials (or patients) and Methods: The DIPSS plus prognostic model was used to define intermediate-2 and high risk groups of pts with MF. We analyzed 15 pts (10 with primary myelofibrosis, 5 with post-polycytemia vera/essential trombocythemia MF; intermediate-2 risk in 6 cases, high risk in 9 cases) undergoing chemotherapy and RIC SCT in our centre from May 2008 to October 2013. Fludarabine (30 mg/m^2), cytarabine (2 g/m^2), and amsacrine (100 mg/m^2) for 4 days (FLAMSA) were used for cytoreduction. After 3 days of rest, RIC consisting of 4 Gy TBI, anti-thymocyte globulin (ATG-Fresenius) 10-20 mg/kg/day for 3 days, and cyclophosphamide 40-60 mg/kg/day for 2 days followed. Median age was 43 years (range 27-58). Types of donors and used grafts were as

follows: HLA identical sibling, *n*=4; unrelated donor, *n*=11; PBSCs, *n*=15.
Results: The median time of neutrophil engraftment (above 0.5x10E9/L) was 16 days, all pts engrafted. The most frequent toxicities were grade III/IV infections according to common toxicity criteria in 11 of 15 pts and gastrointestinal toxicities (grade III in 8 of 15 pts). Incidence of acute GVHD was evaluated in 14 pts: 50% (7/14) of pts had GVHD (grade I+II in 4 pts, grade III in 3 pts). Incidence of chronic GVHD was evaluated in 13 pts, 46% (6/13) of pts had GVHD (limited in 3 pts, extensive in 3 pts). Nonrelapse mortality (NRM) after 1 year and 2 years was 7% and 13%. Causes of death were refractory GVHD (*n*=1) and infection (*n*=1). Complete remission was achieved in 13 of 15 pts (87%), progression was presented in 2 pts (13%). Complete chimerism was achieved in 67% of pts (10/15). With median follow-up from SCT 30 months (range 6-65), 83% of all pts (11/15) were alive (9 pts in remission, 2 pts with relapse), 4 pts died (2 deaths from NRM, 2 deaths from progression of MF). Two relapses (13%; 2/15) occurred in intervals of 6 and 18 months after SCT.
Discussion: FLAMSA-RIC protocol represents a promising approach to the treatment of high-risk or intermediate-2 risk myelofibrosis with high response rate (87%); progression-free survival and overall survival at 2 years from SCT were 62% and 83%, respectively. It provides a combination of effective disease control, low non-relapse mortality, and acceptable toxicity. Other prospective clinical trials are needed to confirm the results of this novel therapeutic strategy.
Disclosure of Interest: None Declared.

PH-P196

PHASE II PROSPECTIVE MULTICENTRE STUDY TESTING THE EFFICACY AND SAFETY OF A CLOFARABINE (CLO), I.V. BUSULFAN (BU) AND ANTITHYMOCYTE GLOBULINS (ATG)-BASED RIC REGIMEN BEFORE ALLO-SCT FOR HIGH-RISK MDS OR ACUTE LEUKEMIA: THE CLORIC TRIAL

P. Chevallier[1,], M. Labopin[2], G. Socie[3], S. Vigouroux[4], S. Furst[5], M. Detrait[6], B. Lioure[7], T. Guillaume[1], J. Delaunay[1], R. Peffault de La Tour[3], N. Milpied[4], J. El-Cheikh[5], D. Blaise[5], M. Michallet[6], K. Bilger[7], P. Moreau[1], M. Mohty[8] and on behalf of the SFGM-TC*
[1]Hematology, CHU Hotel-Dieu, NANTES, [2]Hematologie et Therapie Cellulaire, Hopital Saint-Antoine, [3]Hematology, Hopital Saint-Louis, Paris, [4]Hematology, CHU, Bordeaux, [5]Hematology, Institut Paoli-Calmette, Marseille, [6]Hematology, CHU, Lyon, [7]Hematology, CHU, Starsbourg, [8]Hematology, Hopital Saint-Antoine, Paris, France

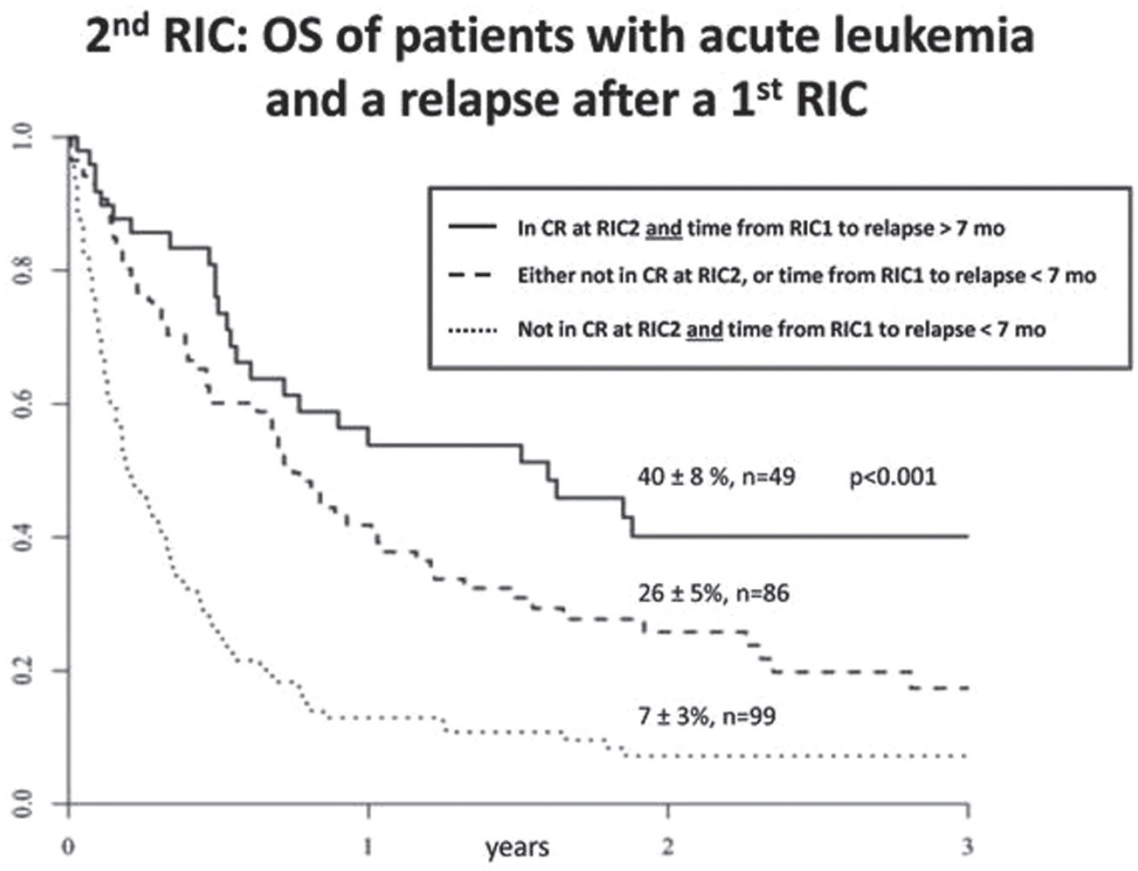
Introduction: Clofarabine (Clo) is a purine analogue which has higher antileukemic activity compared to fludarabine and cladribine. Thus, can one exploit the antileukemic effect of Clo for further improving outcome after RIC allo-SCT for patients with high-risk myelodysplastic syndrome (MDS) or acute leukemia. Here we report the results of a prospective multicentre trial testing the use of Clo in replacement of fludarabine in combination with i.v. Busulfan (Bu) and ATG in 30 patients with high-risk MDS/acute leukemia (clinicaltrials no. NCT00863148).
Materials (or patients) and Methods: Thirty patients (male *n*=18, female *n*=12) were included in this study between October 2009 and August 2012. Sixteen patients were diagnosed with high-risk MDS (*n*=5) or acute myeloid leukemia (AML, *n*=11), while 13 patients had high-risk acute lymphoblastic leukemia (ALL, Ph+ *n*= 2, Ph- *n*=11) and 1 patient a biphenotypic leukemia. All patients were in first (AML/MDS, *n*= 10; ALL/biphenotypic *n*=10) or second (AML *n*= 3; ALL *n*= 4) complete remission, or in response (MDS *n*=3) at time of transplant. Median age at transplant was 59 years (range: 20.5-64.5). The median interval between diagnosis and transplant was 6 months (range: 3.8-124). The RIC regimen consisted of: i.v. Clo 30 mg/m^2/day for 4 days (day-8 to day-5), i.v. Bu 3.2 mg/Kg/day for 2 days (day-4 and day-3) and ATG (Thymoglobuline) 2.5 mg/kg/day for 2 days (day -2 and day-1). All patients received G-CSF-mobilized PBSCs and cyclosporine alone for GVHD prophylaxis, irrespective of the type of donor (sibling donors *n*=14; 10/10 MUD, *n*=16). For the purpose of this study, the single case of binephotypic leukemia was considered as ALL for comparison between AML/MDS and ALL patients. The primary endpoint of the trial was the assessment of leukemia-free survival (LFS) at one year after allo-SCT.
Results: Engraftment was observed in all patients (100%). Median time for neutrophils (>500/µL) and platelets (>50.000/µL) recoveries were 18 (range: 14-26) and 12 (range: 0-23) days, respectively. With a median follow-up of 23 months (range: 14-48), the 2-year OS, LFS, RI and NRM rates were 58+–10%, 53+–9%, 43+–9%, and 3.3±3%, respectively. 2-year OS and LFS were higher for AML/MDS patients compared to ALL/bi-phenotypic patients (75±10% vs 38±14%, *P*=0.07; and 69±10% vs 36±14%, *P*=0.08). The RI was significantly higher for ALL/bi-phenotypic patients (64±14% vs 25±11%, *P*=0.05). Finally, 2-year NRM were similar between both groups (AML/MDS: 6±6% vs 0%, *P*=0.36). Thirteen patients relapsed (43%) at a median time of 3.5 months (range: 2.3-13.1) after allo-SCT. Overall, 14 patients died, including 13 patients already in relapse. The causes of death were mainly relapses in 11, then GVHD in 2 and sepsis in 1.
Discussion: This phase 2 prospective multicentre trial shows that a Clo-i.v. Bu-ATG RIC regimen prior to allo-SCT in high-risk MDS/leukemia is feasible allowing for full engraftment and very low toxicity. Disease control appears to be satisfactorily, especially in AML/MDS, warranting a prospective comparison with other widely used fludarabine-based RIC regimens.
Disclosure of Interest: None Declared.

PH-P197

SECOND RIC ALLOGENEIC TRANSPLANT AS A RESCUE STRATEGY FOR ACUTE LEUKAEMIA PATIENTS WHO RELAPSE AFTER AN INITIAL RIC ALLOGENEIC TRANSPLANTATION: ANALYSIS OF RISK FACTORS AND TREATMENT OUTCOMES

R. Vrhovac[1,], M. Labopin[2], F. Ciceri[3], J. Finke[4], E. Holler[5], J. Tischer[6], B. Lioure[7], J. Gribben[8], L. Kanz[9], D. Blaise[10], P. Dreger[11], A. Nagler[12], G. Held[13], R. Arnold[14], M. Mohty[2] and Acute Leukemia Working Party of the EBMT*
[1]Hematology, Bone Marrow Transplant Unit, University Hospital Centre Zagreb, Zagreb, Croatia, [2]Hematology and Cellular Therapy, Saint-Antoine Hospital and University Pierre & Marie Curie, Paris, France, [3]Hematology and Bone Marrow Transplantation, San Raffaele Scientific Institute, Milano, Italy, [4]Medicine, Hematology and Oncology, University of Freiburg, Freiburg, [5]Hematology and Oncology, University of Regensburg, Regensburg, [6]Med Klinik III, Klinikum Grosshadern, Munich, Germany, [7]Hematology, Oncology and Bone Marrow Transplantation, CHU Hautepierre, Strasbourg, France, [8]St. Bartholomew`s and The Royal London NHS Trust, London, United Kingdom, [9]Medicine, Tuebingen University, Tuebingen, Germany, [10]Transplantation and Cellular Therapy, Institut Paoli Calmettes, Marseille, France, [11]Medicine, University of Heidelberg, Heidelberg, Germany, [12]Chaim Sheba Medical Center, Tel-Hashomer, Israel, [13]Internal Medicine, BMT Unit, University of Saarland, Homburg, [14]Hematology and Oncology, Charite Universitaetsmedizin, Berlin, Germany

Introduction: Limited therapeutic options are available after relapse of acute leukemia following RIC hematopoietic stem cell transplantation (RIC1). A retrospective study on EBMT registry data was performed to evaluate second RIC transplantation (RIC2) as rescue strategy in this setting and to idenfity prognostic factors that determine the outcome.
Materials (or patients) and Methods: A total of 234 patients (121 males) with acute myelogenous (205), lymphoblastic (24), biphenotypic (4) or undifferentiated (1) leukemia have received RIC2 from 2000 to 2012 as a salvage treatment for relapse following RIC1. The median age at time of RIC2 was 53 (range 20-74) years, the median time from RIC1 to relapse was 220 (33-4254) days, and the median time from RIC1 to RIC2 was 359 (60-4402) days. At the time of RIC2 transplant 49 patients (20,9%) were in second CR, 18 (7,7%) were in third or higher CR, while 166 (70,9%) had advanced disease. Stem cells originated from HLA-identical siblings in 43,3% of cases and from unrelated donors in 56,7% of cases. The vast majority of patients (94%) received PBSC.

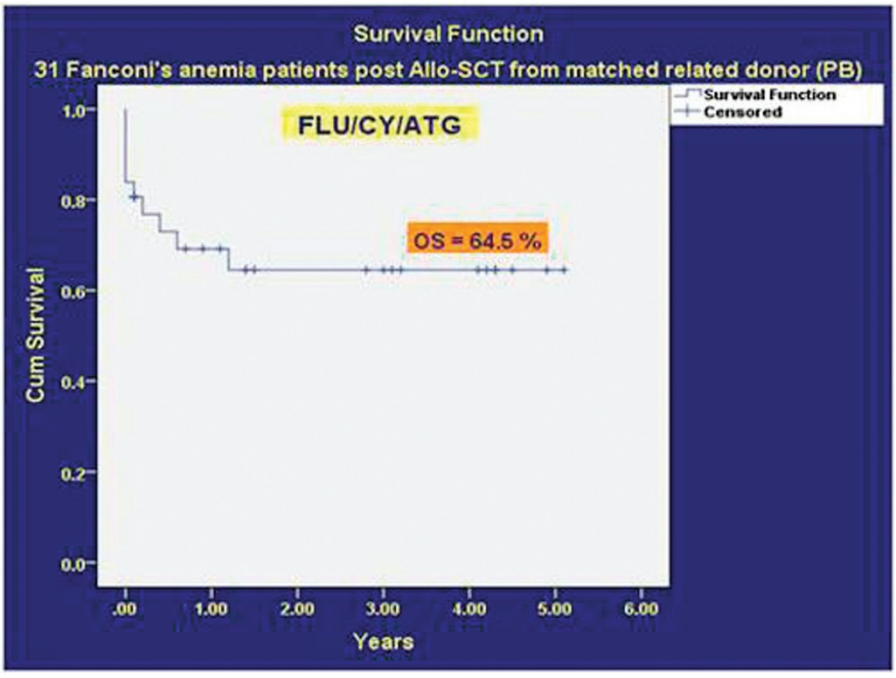


Results: After RIC2, 201 patients (88,5%) engrafted with the median time to ANC>500/μL of 15 (range, 1-40) days. Grade II-IV acute GVHD after RIC2 occurred in 31,9% of patients. With a median follow-up of 21 (range 1,5-79) months after RIC2, 56 patients were still alive. At 2 years, the rates of OS, LFS, NRM and RI were 21 +/–3%, 14+/–2%, 22+/–3%, and 64+/–3%, respectively. Duration of remission following RIC1 and disease status at RIC2 were found to be strongest predictors of patients' outcome – 3 distinct groups were identified: those who relapsed more than 7 months after RIC1 and were in CR at time of RIC2, those with either early relapse after RIC1 or no CR at RIC2, and those with early relapse after RIC1 and no CR at RIC2. The overall survival rates in those groups were 40+/-8 vs. 26+/-5% and 7+/-3%, respectively.
Discussion: Second reduced intensity conditioning allogeneic transplant is feasible in acute leukemia patients who relapse after RIC1. Due to the lack of other effective therapeutic options, this approach remains a valid option for patients with delayed relapses following RIC1 as well as for those achieving CR prior to RIC2.
Disclosure of Interest: None Declared.

PH-P198
A SINGLE CENTER EXPERIENCE OF MATCHED SIBLING DONORS PBSCS BONE MARROW TRANSPLANTATION FOR 31 CASES OF FANCONI ANEMIA USING LOW DOSE CYCLOPHOSPHAMIDE

G. Mohamed Fathy[1,], R. Abdelfattah[2], A. Sobhy[2], M. Abdel-Mooti[1], A. El-Haddad[1], O. Fahmy[1], H. k Mahmoud[1], M. Elsayed[2]*
[1]Bone Marrow Transplantation, [2]Nasser Institute Hospital, cairo, Egypt

Introduction: Fanconi anemia is a genetic disorder associated with diverse congenital abnormalities, progressive bone marrow failure, and increased risk of leukemia and other cancers. Affected persons often die before 30 years of age. Bone marrow transplantation is an effective treatment. Since 1997, when we started our transplant program at Nasser Institute (Cairo, Egypt), Fanconi anemia patients, subjected to BMT using high dose cyclophosphamide had an inferior outcome with overall survival near to 16%. Thus, in this study, we investigated the use of lower dose cyclophosphamide and its impact on the outcome and overall survival.
Materials (or patients) and Methods: In the time period between December 2007 and February 2012, thirty one patients with Fanconi anemia (FA) with evidence of bone marrow (BM) aplasia underwent allogeneic BM transplants (BMT) from matched sibling donors using PBSCs at the BMT unit of Nasser institute, Cairo, Egypt. Median age at BMT was 11.7 years. Conditioning consisted of low-dose cyclophosphamide (CY; 5 mg/kg x 4 days) and a total dose of fludarabine of 120/m^2. Graft-versus-host disease (GVHD) prophylaxis using cyclosporine-A starting dose 3 mg/kg then tailored according to trough level. In addition anti-thymocyte globulin (ATG) was administered in the pre-transplant period to promote engraftment and in the post-transplant period for additional GVHD prophylaxis (30 mg/kg total dose).
Results: Thirty one Fanconi anemia patients with median observation time 1.9 years, the overall survival was 64.5%. Engraftment occurred rapidly (mean, 13.6 days for an absolute neutrophil count > or = 0.5 x 10^9/L; mean, 16.1 days for platelet count > or = 50 x 10^9/L). Twenty patients have sustained engraftment and are transfusion-independent . Two patients developed secondary graft failure after initial engraftment and one had primary graft failure. Five patients developed acute GVHD (15.6%),and the incidence of chronic GVHD did not exceed 3.2%.
Discussion: PBSCs transplantation is a feasible option for FA patients having matched sibling donors and should be performed



early in the course of the disease, before the development of complications. We believe that the use of low-dose CY in addition to ATG in the conditioning regimen was responsible for improvement in the survival of FA patients undergoing BMT. The regimen was well tolerated and was associated with a low incidence of complications including GVHD.
Disclosure of Interest: None Declared.

PH-P199
SEQUENTIAL CHEMOTHERAPY FOLLOWED BY REDUCED INTENSITY CONDITIONING AND ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION FOR HIGH RISK ACUTE MYELOID LEUKEMIA PATIENTS IN FIRST COMPLETE REMISSION: A PROSPECTIVE PILOT STUDY

M. Michallet[1,], M. Sobh[1], H. Labussière[1], S. Hayette[2], I. Tigaud[2], M. El Hamri[1], L. Gilis[1], F. Barraco[1], S. Ducastelle[1], F. Emmanuel Nicolini[1], X. Thomas[1]*
[1]Hematology, [2]Cytogenetics and molecular biology laboratory, Centre Hospitalier Lyon Sud, PIERRE BENITE CEDEX, France

Introduction: With the aim to improve the effect of allo-HSCT by sequential use of chemotherapy followed by reduced intensity conditioning (RIC), we conducted a prospective pilot study in high-risk AML patients in first complete remission (CR1).
Materials (or patients) and Methods: The high-risk population included intermediate II and unfavourable patients (Dohner *et al*. Blood 2010), secondary AML, and patients requiring 2 induction courses to obtain CR. The chemotherapy sequential regimen consisted in fludarabine 30 mg/m^2, high-dose cytarabine 2 g/m^2, and amsacrine 100 mg/m^2 from days -12 to -9 (FLAMSA). After 3 days of rest, RIC consisted of 4 Gy TBI on day -5, cyclophosphamide (40 mg/kg with HLA-identical sibling, 60 mg/kg for unrelated or mismatched donors) on days -4 and -3, and rabbit antithymocyte globulin (ATG, Genzyme) (5 mg/kg total dose) from day -3 to day -1. As a new experimental approach, we replaced TBI by iv. busulfan (BU) 3.2 mg/kg/d during either 4 or 2 days according to patient age (>55 years) (from day -7 to -4 or from day -5 to -4). GvHD prophylaxis consisted in ciclosporine from day −1, and mycophenolate mofetil (15 mg/kg bid), starting from day 0. Except for cord blood transplantation, patients received 3 prophylactic increased doses of donor lymphocyte infusions (DLI) if they were in CR and GvHD-free at day +120 or 30 days after discontinuation of immunosuppressive agents starting at 1x10^6 CD3+ cells/kg.
Results: Between August 2010 and March 2013, 26 consecutive AML patients in CR1 were included; 11 males and 15 females with a median age at allo-HSCT of 55 years (range: 24-67), 19 (73%) were *de novo* AML and 7 (27%) secondary AML. According to cytogenetics and molecular markers, 22 (85%) were unfavourable and 4 (15%) were in intermediate II category. Before allo-HSCT, to reach CR1, 20 (77%) patients received one induction chemotherapy and 6 (23%) needed 2 inductions. Stem cell source was PBSC for 23 (88%) patients, CB for 2 and BM for 1 patient. Donors were 10/10 HLA matched siblings in 9 (35%) patients, 10/10 HLA matched unrelated in 8 (31%) patients and HLA mismatched for the rest of patients [unrelated 9/10 (*n*=7), CB 4/6 (*n*=2)]. For ABO compatibility, 13 (50%) were compatible, 5 (19%) had minor incompatibility and 8 (31%) had major incompatibility. For conditioning, 6 (23%) patients received TBI, 13 (50%) received 4 days BU and 7 (27%) received 2 days BU. After transplantation, 23 (88%) patients engrafted. At day 90 post-allo-HSCT, 18 (78%) showed total donor chimerism and 5 (22%) had mixed chimerism and all patients were in CR. There were 6/23 patients with acute GvHD [2 gr I, 2 gr II and 2 gr III] and 5/23 chronic GvHD [4 limited and 1 extensive], all before DLI. After a median follow-up of 9 months (range: 0.03-35), the 2-years probability of overall survival (OS) for the whole population was 58% (47-69) and the 2 years cumulative incidence of relapse was 18% (17-19). No statistical difference in terms of OS and relapse incidence was found between the 3 types of conditioning.
Discussion: FLAMSA-RIC regimen followed by allo-HSCT showed promising results in high-risk CR1 AML patients. Because of some early severe infections, an efficient prophylactic anti-infectious strategy is recommended. The use of BU instead of TBI does not impact on transplant outcomes.
Disclosure of Interest: None Declared.

PH-P200

ALLOGENEIC STEM CELL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES: 4GY TBI TO INTENSIFY TREOSULFAN AND FLUDARABINE-BASED CONDITIONING

F. Giglio[1,], C. Zuanelli Brambilla[1], F. Lorentino[1], S. Piemontese[1], L. Crucitti[1], R. Greco[1], M. Morelli[1], C. Messina[1], A. Forcina[1], M. T. Lupo Stanghellini[1], L. Vago[1], S. Marktel[1], M. Marcatti[1], A. Assanelli[1], C. Corti[1], M. Bernardi[1], C. Bonini[2], F. Ciceri[1], J. Peccatori[1]*

[1]Hematology and bone marrow transplantation unit, [2]Experimental hematology unit, San Raffaele Scientific Institute, Milan, Italy

Introduction: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative option for patients with aggressive hematologic malignancies. Since many patients cannot find a suitable HLA-identical (related or unrelated) donor, it is essential to analyze the safety of alternative graft sources, such as haploidentical donors. The use of reduced-intensity conditioning regimens in patients with an advanced disease did not improve the outcome because of a higher incidence of relapse. Here are analyzed preliminary results from a phase II prospective clinical trial (TrRaMM4Gy; EudraCT# 2011-001534-42) that investigates the effects of intensification of a Fludarabine and Treosulfan-based conditioning regimen by 4 Gy TBI. Aim of the study is to maintain an acceptable toxicity profile while reducing the incidence of relapse in high-risk patients.

Materials (or patients) and Methods: At interim analysis evaluation, 59 patients underwent haploidentical allo-HSCT for AML (*n*=35), and other myeloid (*n*=7) and lymphoid (*n*=17) malignancies. The median age was 45 years (range 17-67). At the time of the transplantation most patients (76%) were beyond CR2 or in active disease phase, while the remaining 24% were in CR1, CR2 or upfront. Thirty-six percent of the patients were enrolled for relapse after a previous allogeneic HSCT. Median comorbidity index score (according to Sorror criteria) was 2 (range 0-5); median disease risk index (DRI) was 3 (high). Median number of $CD34^+$ and $CD3^+$ cells/kg were 7 millions and 223 millions respectively. Conditioning regimen included Treosulfan (14 g/m^2 for 3 days), Fludarabine (30 mg/m^2 for 5 days) and 4 Gy TBI split in 2 fractions. GvHD prophylaxis consisted of *in vivo* T and B cell depletion with ATG-Fresenius (10 mg/kg for 3 days) and Rituximab (200 mg/m^2 in single dose), Sirolimus (target concentration 8-15 ng/ml, till day +60) and Mycophenolate Mofetil (MMF; 10 mg/kg tid till day +30). Preliminary results from these patients are compared with those from 121 patients, with comparable characteristics, previously enrolled in TrRaMM trial (EudraCT# 2007-5477-54).

Results: Neutrophil and platelet engraftment was rapid and robust in both TrRaMM and TrRaMM4Gy trials. After a median follow-up of 398 days for TrRaMM4Gy trial, results (and projections at 2 years) are the following.

Discussion: The investigated protocol resulted feasible for HSCT in patients with high-risk hematologic malignancies. GvHD incidence and NRM, especially in the first 6 months after HSCT, were high, but still in a range comparable to mismatched unrelated donor setting. However, further ante- and post-transplant strategies for relapse prevention should be investigated and applied. New less toxic modalities of irradiation (Total marrow Irradiation, TMI) are under investigation at our Center.

Disclosure of Interest: None Declared.

TrRaMM		TrRaMM4Gy
25% / 34%	NRM: day +100 / +365	28% / 38%
38% / 25%	aGvHD: grade II-IV / III-IV	41% / 30%
50%	cGvHD	58%
48% / 64%	Relapse: 1 / 2-year	41% / 50%
47% / 30%	OS: 1 / 2-year	41% / 41%
34% / 22%	PFS: 1 / 2-year	37% / 31%

PH-P201

ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR PRIMARY AND SECONDARY MYELOFIBROSIS: RESULTS OF A SINGLE-CENTER RETROSPECTIVE ANALYSIS

C. Dorn[1,], S. P. Haen[1], C. Faul[1], W. Vogel[1], L. Kanz[1], W. Bethge[1]*

[1]Universitaetsklinik Tuebingen, Tuebingen, Germany

Introduction: Allogeneic hematopoietic cell transplantation (HCT) is the only curative therapy for patients with myelofibrosis (MF). Reduced intensity conditioning (RIC) regimens allow increasingly successful application of this curative therapy even in comorbid and older patients. Here, we report our single centre experience with allogeneic HCT in patients with MF.

Materials (or patients) and Methods: Between 1997 and 2013, a total of 59 patients with primary or secondary MF were treated with allogeneic HCT. Patient data were analysed retrospectively.

Results: A total of 59 consecutive patients (34 male, 25 female) were transplanted for MF with a median age of 55 years (range 28-75). 32% (*n*=19/59) received a myeloablative conditioning regimen (MAC, median age 47 years (range 28-60)), 68% (*n*=40/59) were treated with RIC (median age 58 years, range 45-75). Peripheral blood stem cells (*n*= 50/59) were mainly used as graft source, 9 patients received bone marrow. Donors were either matched related (MRD, *n*=17, 29%), matched unrelated (MUD, *n*=22, 37%) or mismatched unrelated (MMUD, *n*=20, 34%). As MAC regimen, either a combination of 12 Gy total body irradiation and high-dose cyclophosphamide (TBI/Cy, 74%) or high-dose busulfan and cyclophosphamide (Bu/Cy, 26%) were used. The RIC regimens consisted most frequently of fludarabine and busulfan (Flu/Bu, *n*=31, 85%), fludarabine in combination with TBI (*n*=1, 3%), treosulfan-containing regimens (*n*=3, 8%) and in 2 patients a FLAMSA/BuCy regimen. As prophylaxis against GVHD, 80% (*n*=44) of the patients received antithymocyte globuline prior transplantation, followed by postgrafting immunosuppression with calcineurine inhibitors and methotrexate or mycophenolate mofetil.

The median follow-up was 36 months (range 1-151) with a Kaplan Meier estimated overall survival (OS) at 3 years of 64% for all patients and 68% after RIC compared to 59% after MAC (*P*= 0.45). Patients with a low and intermediate-1 DIPSS-Score had a significant better OS than patients with an intermediate-2 or high DIPSS-Score (*P*=0.0231). There was no significant difference in OS of patients with MRD, MUD and MMUD (60%, 62% and 58%). Cumulative incidence of non-relapse mortality (NRM) was at day +100 7% (RIC=3%, MAC=17%) and at 3 years 28% (RIC=27%, MAC=31%), respectively. Engraftment of neutrophils (ANC>500/µL) was significant different with a median of 16 (range 9-31) days after MAC compared to 23 (range 13-60) days after RIC (*P*= 0.0051). Platelet recovery (PLT>20.000/µL) occurred at a median of 24 (range 11-51) days after MAC and 21 (range 8-41) days after RIC (*P*= 0.2866). Graft rejection was rare, occurring only in 3 patients after RIC.

The cumulative incidence of acute GVHD was 29% (*n*=17/59, grade I *n*=13/17, ≥ grade II *n*=4/17). The risk for aGVHD for patients after MAC was significantly higher compared to patients after RIC (71% (*n*=12) versus 29% (*n*=5), *P*< 0.0001). The cumulative incidence of chronic GVHD was 54%. Extensive cGVHD was seen in 44% and limited cGVHD in 56%. Survival of patients with an extensive cGVHD was poor compared to patients with a mild or limited cGVHD (18% versus 39%, *P*= 0.2059).

Discussion: Allogeneic HCT following RIC is a feasible and safe treatment option for patients with primary and secondary MF with a 3-year overall survival of 64%. Even allogeneic HCT with MMUD may offer curation to an older and more comorbid patient population.

Disclosure of Interest: None Declared.

PH-P202

ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS; A NON-INTERVENTIONAL STUDY (NIS) BY THE PLASMA CELL DISORDER SUBCOMMITTEE OF THE CHRONIC MALIGNANCY WORKING PARTY OF THE EBMT

S. O. Schönland[1,], U. Hegenbart[1], S. Iacobelli[2], J. Hoek[3], J. Passweg[4], D. Bordessoule[5], T. de Witte[6], L. Garderet[7], N. Kröger[8]*

[1]Amyloidosis Center, University Hospital, Heidelberg, Germany, [2]Centro di Biostatistica, Università Tor Vergata Rome, Rome, Italy, [3]Data Office, EBMT, Leiden, Netherlands, [4]Hematology, University Hospital, Basel, Switzerland, [5]Hematology, CHRU, Limoges, France, [6]Hematology, Radboud University, Nijmeegen, Netherlands, [7]Hematology, Hospital Saint Antoine, Paris, France, [8]UKE, Hamburg, Germany

Introduction: Systemic Light-chain (AL-) amyloidosis is a rare protein folding and deposition disorder which is caused by a monoclonal plasma cell or B cell disorder with poor prognosis. Based on small series of patients and case reports allogeneic transplant (allo SCT) has emerged as potentially effective (EBMT retrospective data: Schönland *et al*., Blood 2005, DLI data: Haematologica, 2008). However, TRM was 40%: Therefore, a more formal proof of concept of using allogeneic hematopoietic transplantation for treatment of AL Amyloidosis is lacking.

Materials (or patients) and Methods: The primary endpoint of this NIS is efficacy (best hematological remission (HR) and organ response). Secondary endpoints are acute and chronic GvHD, TRM, non-hematological toxicity, event-free and overall survival. We selected those centres that in the past performed any allogeneic HSCT for AL amyloidosis. We approached 24 centres, of which 4 are participating in the study today. So far 10 patients have been included and were transplanted between 2006 and 2012. Age at allo SCT was 50 years in median (range, 42 – 60 years). Five patients had cardiac, 7 kidney, 5 liver and 2 patients nervous system involvement. As underlying disease one patient had symptomatic multiple myeloma, 9 patients a clonal plasma cell dyscrasia or another B cell disorder. All patients were in a good performance status measured by Karnofsky Index (3 patients 80%, 6 patients 90%, 1 patient 100%). Previous chemotherapy included high-dose melphalan with autologous stem cell transplantation in 8 patients as well as bortezomib, lenalidomide, melphalan and steroids. Disease stages at allo SCT were as follows: 1 CR, 1 VGPR, 3 PR, 3 stable disease, 1 progression. Two patients received myeloablative and 7 patients received RIC conditioning using TBI 2 Gy / fludarabine (2 patients with ATG). High-dose melphalan 200 mg/m^2 was applied prior to the one syngeneic SCT. Donors were: 5 sibling matched donors, 1 mismatched relative donor, 1 syngeneic donor, 2 matched unrelated donors and 1 mismatched unrelated donor (MMUD). Source of stem cells was peripheral blood in 9 and cord blood in 1 patient, respectively.

Results: All patients engrafted. Acute GvHD grade II-IV occurred in 6 patients and chronic GvHD in 6 patients (3 patients with limited and extensive disease, respectively). The patient who received a graft from a MMUD died of steroid-refractory acute GvHD (Grade IV) of the gut. Best HR after allo SCT was CR in 4, VGPR in 1 and PR in 2 patients. Nine patients are alive with a median follow-up of 23 months. Two patients relapsed or progressed.

Discussion: Preliminary analysis of the first 10 patients of this NIS showed that allo SCT is feasible and effective in patients with AL amyloidosis. In opposite to our retrospective analysis we have observed a low TRM using mostly reduced-intensity conditioning with TBI 2 Gy and fludarabine. Allo SCT might be a reasonable treatment option in young and medically fit and heavily pretreated patients.

Disclosure of Interest: None Declared.

PH-P203

IN PATIENTS ≥ 60 YEARS WITH ADVANCED MYELOID DISEASE - ALLOHCT IS A FEASIBLE CURATIVE THERAPY: - RESULTS IN 250 CONSECUTIVE PATIENTS WITH AML/MDS

H. Bertz[1,], M. Luebbert[1], K. Ohneberg[2], R. Zeiser[1], R. Wäsch[1], R. Marks[1], J. Finke[1]*

[1]Hematology/Oncology, University Clinic Internal Medicine, [2]Medical Biometry and Medical Informatics, University Medical Center, Freiburg, Germany

Introduction: Because of the changes of the demographic development and long-livity there will be more patients (pts) in their 7.th and 8.th decade, which will be diagnosed with a myeloid malignancy such as AML/MDS. Further, the personal fitness of these elderly has changed with more of them fit enough and asking for receiving a more intensive therapy for cure. After the introduction of reduced-toxicity conditioning we transplanted from 1999 to 2012, 250 consecutive pts with AML/MDS aged ≥ 60 years (yrs).

Materials (or patients) and Methods: The 144 male and 106 female pts with a median age of 66 yrs (range 60-77) were transplanted for de novo AML (*n*=95), s/tAML (*n*=104) and MDS (*n*=51) with mainly unfavorable cytogenetics. The donor was matched/mismatched unrelated in 74% and in 26% related in 26%. Only 16% were transplanted in CR1/2, 84 % with advanced or untreated disease. The conditioning regimen was the FBM protocol (**F**ludarabine 4x30mg/m^2, **B**CNU/carmustine 2x150mg/m^2, **M**elphalan 110mg/m^2; Bertz *et al*., JCO 2003) in 98%, and the graft in 97% PBSC. For GVHD prophylaxis in 91% a combination of cyclosporine A plus alemtuzumab or ATG-F™ was administered.

[PH-P203]

variable	value	Hazard Ratio	95% CI lower limit	95% CI upper limit	P value
Overall survival					
Remission at alloHCT	advanced	1.37	0.86	2.16	0.1825
HLA mismatch	yes	1.40	1.01	1.96	0.0463
HCT-CI (Sorror)	≥ 2	1.31	1.01	1.96	0.1007
Peripheral blood blasts	yes	1.21	0.84	1.76	0.3034
Disease-free survival					
Remission at alloHCT	advanced	1.29	0.72	2.30	0.3946
Donor	related	0.64	0.43	0.95	0.0258
HLA mismatch	yes	1.44	0.99	2.09	0.0561
CD34+ cells	> median	0.76	0.55	1.04	0.0867
Bone marrow blasts	> 5%	1.21	0.78	1.88	0.3915

Results: At a median follow up of 57 months (3-157) 37% of the pts are alive; main causes of death were relapse (*n*=62), infection (*n*=35) and age-related diseases (*n*=13). In the day +30 standard diagnostic measures, 94% of the pts achieved CR. Probability of OS/DFS was at 1yr 61%/49%, at 2 yrs 49%/41% and at 5 yrs 37%/34%, respectively. The probability for NRM/relapse at 1yr is 24%/28% at 2yrs 29%/32% and at 5 yrs 36%/35%. Clinically-relevant acute GvHD °II-°IV developed in 60/250 (24%) and °III-°IV in 30/250 (12%) evaluable patients after engraftment. Limited cGvHD was observed in 44 (18%) and extensive cGvHD in 57 patients (23%). Nineteen known prognostic factors for outcome were evaluated: e.g. patient and donor age, graft size, days between diagnosis and alloHCT, CMV, early/advanced disease, cytogenetics, Sorror and Gratwohl score, donor type, HLA-identity. In the multivariate analysis a better OS (factors with *P*<0.1; table 1) was seen with a matched donor; a better DFS with a related donor, and high CD34+ graft content; in contrast, a mismatched donor is a risk factor for reduced DFS.
Discussion: Our unique large cohort of older pts with AML/MDS with mainly advanced disease and unfavorable cytogenetics shows a high feasibility, safety and efficacy of alloHCT after the FBM protocol. AML/MDS pts in their 7th and 8th decade of life fit for transplant should be evaluated for alloHCT as a very important long-term curative option.
Disclosure of Interest: None Declared.

PH-P204
FLUDARABINE COMBINED WITH REDUCED OR MYELOABLATIVE DOSES OF INTRAVENOUS BUSULFAN RESULTS IN SIMILAR OUTCOME AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION (SCT) IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA IN REMISSION.

A. Shimoni[1,], N. Shem-Tov[1], Y. Volchek[1], I. Danylesko[1], R. Yerushalmi[1], A. Nagler[1]*
[1]Division of Hematology and Bone Marrow Transplantation, Chaim Sheba Medical Center, Tel-Hashomer, Israel

Introduction: Allogeneic SCT is potentially curative therapy in AML. Myeloablative conditioning (MAC) may be associated with high non-relapse mortality (NRM) in older patients (pts). Reduced intensity conditioning (RIC) allows SCT in these pts by reducing NRM but may be associated with increased relapse rate. Reduced toxicity myeloablative regimens (RTC) were designed to increase dose intensity and disease control while retaining low NRM.
Materials (or patients) and Methods: The study included 55 pts with AML, age 45-65, in CR1 or CR2, considered at high risk for MAC. Pts were sequentially assigned (based on the day of admission) to either RIC (FB2, fludarabine and busulfan 6.4 mg/kg, *n*=28) or RTC (FB4, fludarabine and busulfan 12.8 mg/kg, *n*=27).
Results: The median age was 59 years (46-65). 39 pts were in CR1 and 16 in CR2. 21 pts (38%) had poor risk cytogenetics. 10 pts (18%) had comorbidity score>2. The donor was a sibling (40%) or matched unrelated (60%). Pts given FB2 were older than pts given FB4 (60 Vs 55 years, *P*=0.001) but all other characteristics were similar. 54 pts achieved primary engraftment in a median of 16 days (11-20) and 12 days (9-22) after FB2 and FB4, respectively (*P*=0.01). CTCAE Grade III-V organ toxicity occurred in only 2 FB2 recipients (7%) compared to 5 FB4 recipients (19%, *P*=0.20). Mucositis grade ≥ III was rare after FB2 occurring in only 1 pt (4%) and significantly higher after FB4 (8 pts, 30%, *P*=0.01). Hepatic toxicity occurred in 7% and 19% respectively (*P*=0.20). The cumulative incidence of acute GVHD grade II-IV was 18% and 44% (*P*=0.03), respectively. The cumulative incidence of chronic GVHD was 42% after both regimens. In all, the 2-year NRM was 11% and 22% after FB2 and FB4, respectively (*P*=0.27). The rates became similar later during the course with 5 year NRM rate of 18% and 22%, respectively. The 5-year relapse rates were 39% and 33%, respectively. In all, with a median follow-up of 54 months (27-83) 26 pts are alive, 29 have died, 10 of NRM and 19 of relapse. There was no difference in survival rates between the 2 regimens. The 5-year OS rates were 49% (95%CI, 31-68) and 44% (95%CI, 26-63), after FB2 and FB4, respectively. The 5-year LFS rates were 43% (95%CI, 24-61) and 44% (95%CI, 26-63), respectively. Multivariate analysis that was limited due to the sample size, identified high comorbidity index and poor cytogenetics as adverse prognostic factors for OS with hazard ratios of 3.9 (*P*=0.006) and 1.8 (*P*=0.08), respectively. Age, gender, CR1 versus CR2, donor type and protocol used were not significant.
Discussion: FB2 (RIC regimen) and FB4 (RTC regimen) are both effective therapies in older pts with AML in CR1/CR2 who are not eligible for standard MAC. A 5-year OS rate of 46% is encouraging in this setting. FB2 is associated with less initial toxicity and a lower rate of acute GVHD, but later on, NRM becomes similar to FB4. Relapse rates seem similar, such that increasing dose intensity in these pts in CR, does not translate into significantly better disease control. In all, OS after the 2 regimens is similar. NRM is relatively low with these iv busulfan based regimens, however better strategies are required to reduce the moderately high relapse rate in order to further improve outcome. These observations merit further study in a larger randomized trial.
Disclosure of Interest: None Declared.

PH-P205
COMPARISON OF INTENSIVE CHEMOTHERAPY AND HYPOMETHYLATING AGENTS PRIOR TO ALLOGENEIC STEM CELL TRANSPLANTATION FOR ADVANCED MDS (RAEB, RAEB-T). A STUDY OF THE MDS SUBCOMMITTEE OF THE CMWP OF EBMT

V. Potter[1,], S. Iacobelli[2], A. van Biezen[3], J. Maertens[4], J.-H. Bourhis[5], J. Passweg[6], I. Yakhoub-Agha[7], R. Tabrizi[8], B. Jacques-Olivier[9], M. Robin[10], P. Chevallier[11], Y. Challandon[12], A. Hyunh[13], T. de Witte[14], N. Kroeger[15] and CLWP EBMT*
[1]Department of Haematological Medicine, Kings College Hospital, London, United Kingdom, [2]Interdipartimentale di Biostatistica e Bioinformatica, Universita tor Vergata, Roma, Italy, [3]EBMT Data Office, University Medical Centre, Leiden, Leiden, Netherlands, [4]Hematology, Uz Gasthuisberg, Leuven, Belgium, [5]Division of Hematology, Institut Gustave Roussy, Villejuif, France, [6]Hematology, University Hospital of Basel, Basel, Switzerland, [7]Hôpital HURIEZ, Hôpital HURIEZ, Lille, [8]Hematology, CHU, Bordeaux, [9]Cellulaire et d'hématologie clinique adulte, Clemont-Ferrand, [10]Hematology-Bone Marrow Trasplantation, Saint-Louis Hospital, Paris, [11]Hematology, CHU, Nantes, France, [12]HUG, Geneva, Switzerland, [13]Hopital de Purpan, Toulouse, France, [14]University Medical Centre St. Radboud, Nijmegen, Netherlands, [15]University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: Since the approval of hypomethylating agents for the treatment of myelodysplastic syndromes (MDS) increasing numbers of patients have received these agents prior to haematopoietic stem cell transplant (HSCT). A number of small studies have suggested that this approach results in similar outcomes to conventional chemotherapy. In light of this we utilised the EBMT dataset to retrospectively analyse the outcomes of hypomethylating agent (HMA) therapy compared with conventional chemotherapy (CC) pre-HSCT.
Materials (or patients) and Methods: Hypomethylating agents were approved in early 2000; consequently we selected MDS patients receiving allogeneic stem cell transplantation between 2004 and 2011 reported to the EBMT. In order to include a homogeneous group of patient with blasts at time of diagnosis we included only patients classified as RAEB or RAEB-T at time of diagnosis with sufficient data on anthracycline containing chemotherapy or hypomethylating agents. 209 patients (HMA =77(37%), CC =132(63%)) were identified with median follow-up of 22 months. The median age of the group was 58 with 37% of the population aged greater than 60yrs. 92(44%) patients received sibling HSCT and 119 (56%) unrelated donor HSCT. The majority of patients (124 (59%)) received reduced intensity conditioning. 104 (52%) patients had abnormal cytogenetics and 70 (34%) and 57 (27%) were classified as Int-2 and High IPSS scores respectively. At time of HSCT 66 (32%) did not achieve CR and 28 (13%) had primary refractory disease.

Results: On univariate analysis outcomes at three years were not significantly different between HMA and CC for Overall Survival (OS), Relapse Free Survival (RFS), cumulative incidence of relapse (CIR) and Non Relapse Mortality (NRM); OS (42 vs 35%), RFS (29 vs 31%) CIR (45 vs 40%) and NRM (26 vs 28%). The median OS was 31 vs 18 months for HMA vs CC respectively (*P*=NS). On comparing groups there were more patients in the chemotherapy group below 55 years. Additionally there were more patients in the CC group in CR (68 vs 32%) and less patients with primary refractory disease when compared to the HMA group (10 vs 19%, *P*<0.001). When compared to patients in CR those with primary refractory disease had significantly worse outcomes with regard to OS (HR 2.42(95% CI: 1.41-4.13) *P*=0.001), RFS (HR 2.27 (95%CI:1.37-3.76) *P*=0.001) and NRM (HR 2.49 (95%CI: 1.18-5.26) *P*=0.016). There was no significant difference in outcome with regard to increasing age, donor type (sib vs unrelated), conditioning protocol (MAC vs Sib). cytogenetics (normal vs abnormal) or IPSS stage. On multivariate analysis the presence of primary refractory disease retained significance with regard to outcomes. There was no effect of treatment group.
Discussion: Outcomes post HSCT are similar for patients receiving HMA compared with those receiving CC despite the higher proportion of patients with primary refractory disease in the HMA group. Whilst the inherent limitations of retrospective data analysis are acknowledged these findings support the growing body of evidence that HMA therapy is comparable to CC when used as pre-transplant induction treatment. Disease status is clearly important for best outcomes and the results of prospective studies which clarify the ability of hypomethylating agents vs other methods to achieve this are awaited.
Disclosure of Interest: None Declared.

PH-P206
RECONSTITUTION OF GPI-ANCHOR-NEGATIVE NK CELL POPULATIONS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOLLOWING ALEMTUZUMAB-BASED CONDITIONING

A. Naomi Lay[1,], R. Georg Meyer[1], D. Wolff[1], M. Theobald[1], E. Ullrich[2], E. Maria Wagner[1]*
[1]Hemytology, Oncology, Pneumology, University medical center Mainz, Mainz, [2]Childrens Hospital, hematology and oncology, cellular immunology, Childrens Hospital, Goethe university Frankfurt, Frankfurt, Germany

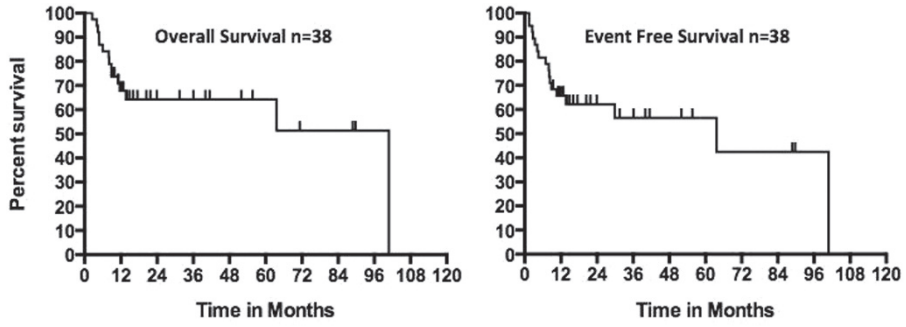
Introduction: The anti CD52-antibody Alemtuzumab is used for T cell depletion (TCD) in the context of allogeneic hematopoietic stem cell transplantation (HSCT). We recently showed long-term persistence of CD52-/Glykosylphosphatidyl-Inositol (GPI)-negative T cells with impaired antiviral function in patients after HSCT. The persistence of GPI^{neg} regulatory T cells with impaired immunosuppressive capacity correlates with severe acute GvHD. Since natural killer (NK) cells could influence antitumor reactivity, defense against infections, and GvHD, we further investigated on GPI^{neg} NK cells in our patients.
Materials (or patients) and Methods: We examined peripheral blood samples of 12 patients after HCST following conditioning including Alemtuzmab-mediated T-cell depletion. By using multicolor FACS-analysis, we phenotypically distinguished the NK cell subpopulations $CD56^{bright}CD16^{dim}$, $CD56^{dim}CD16^{bright}$, $CD56^{dim}CD16^{int}$ and $CD56^{dim}CD16^{neg}$ at d+60 and d+365 after HSCT. GPI^{neg} NK cell subsets were characterized regarding expression of specific surface antigens involved in cytotoxicity (NKp44, NKp46, NKG2A and NKG2D), maturation and activation (CD62L, CD69). In two patients, we investigated on reactivity against CMV-infected fibroblasts by IFN-γ ELISPOT-assay.
Results: Early after HSCT, we found $CD56^{dim}CD16^{int}$ to be the major NK cell population. At d+356, $CD56^{dim}CD16^{bright}$ becomes the most prominent population. $CD56^{dim}CD16^{bright}$ and $CD56^{bright}CD16^{dim}$ showed 70-90% GPI^{neg} cells (d+60), decreasing over time. $CD56^{dim}CD16^{neg}$ and $CD56^{dim}CD16^{int}$ NK cells were mostly GPI^{pos}. Independent from GPI expression, NKp46 was present in the $CD56^{dim}CD16^{bright}$/$CD56^{bright}CD16^{dim}$ populations. In contrast, $CD56^{dim}CD16^{neg}$/ $CD56^{dim}CD16^{int}$ NK cells remained NKp46 negative at d+60. Early after HSCT, all NK cell subsets lacked NKG2D, whereas NKG2A expression remained stable over time. Interestingly, NKp44 was only observed on GPI^{pos} NK cells, and its expression increased late after HSCT, especially in $CD56^{dim}CD16^{int}$ NK cell populations. The activation marker CD69 was expressed mainly on $CD56^{dim}CD16^{neg}$ and $CD56^{dim}CD16^{int}$. Early after HSCT, CD62L was prominent in $CD56^{dim}CD16^{neg}$ and $CD56^{dim}CD16^{int}$, but increased in the $CD56^{dim}CD16^{bright}$/$CD56^{bright}CD16^{dim}$ NK cell population at d+365. Remarkably, we observed the highest frequency of CMV-specific IFN-γ production by NK cells early after transplantation in the absence of T cells.
Discussion: We previously showed reconstitution of functionally impaired GPI^{neg} T cells that may be responsible for some of the viral complications after Alemtuzumab-based conditioning. In this setting, NK cells play a major role, especially early after HSCT. Here we provide evidence of a GPI^{neg} population among reconstituting NK cells. Interestingly, the distribution of these GPI^{neg} cells differed among NK subpopulations and changed over time. Expression of natural cytotoxicity receptors, maturation- and activation markers varied depending on the time after HSCT and the expression of GPI-anchors. GPI-anchor negative NK cells might be altered in their function towards allogeneic cells and pathogens. These alterations may affect NK cell mediated immune responses similar as shown for T cells. Further investigations on different NK subsets and their specific function might identify patients with need for specific cellular therapies including NK cell based DLI in the future.
Disclosure of Interest: None Declared.

PH-P207
ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS 60 YEARS AND OLDER: POTENTIALLY CURATIVE THERAPY FOR HAEMATOLOGICAL MALIGNANCY WITH LOW TREATMENT RELATED MORTALITY.

D. Susan Richardson[1,], A. Publicover[1], K. Hill[1], C. Hurlock[1], L. Jarvis[1], J. Newman[1], S. Main[1], N. McKeag[1], K. Harold Orchard[1]*
[1]Wessex Blood & Marrow Transplantation Unit, University Hospital Southampton, Southampton, United Kingdom

Introduction: Many haematological malignancies occur with higher incidence in older patients; the median age of diagnosis of the most common adult indication for allogeneic stem cell transplantation (alloSCT), Acute Myeloid Leukaemia (AML), is 65 years of age. Concerns regarding greater risk of transplant related morbidity and mortality in the older age group has meant that alloSCT as a potentially curative therapy has traditionally been denied to many of these patients. Despite increasing numbers of older patients undergoing alloSCT in the last decade, registry data confirms that a relatively low proportion of older patients diagnosed with AML and other haematological malignancies currently undergo alloSCT. Here we review our outcomes from 2004 to 2012 for patients with haematological malignancies aged 60 years and over.
Materials (or patients) and Methods: 38 patients aged 60 years and over have undergone alloSCT at our institution since 2004. Of these 30 were male and 8 female, with a median age of 62 years (range 60-66) and with a median follow-up (survivors) of 20.4 months (range 9.4 – 89.8). Matched sibling donors were used for 8 and unrelated donors for 30 patients. 27 patients were transplanted for high risk AML; other indications included advanced CML, myelofibrosis, and MDS. 3 of 38 patients were transplanted for lymphoid malignancies. All patients received reduced intensity or toxicity conditioning schedules based on Melphalan (*n*=7) or intravenous Busulphan for 2 to 3 days (*n*=30). 1 patient received LACE conditioning for multiply relapsed CLL. All patients underwent *in vivo* T-cell depletion using Alemtuzumab at a dose of 30 to 50mg, depending on donor type. All patients received GvHD prophylaxis with ciclosporin and short methotrexate.
Results: The non-relapse mortality at day 100 and 6 months was 0% and 0% respectively for all patients. The Kaplan Meier

Figure 1



probabilities of 3 year overall and event free survival are 64.2% and 56.6% respectively. The Kaplan Meier curves for overall and event free survival are shown in figure 1. 23 of 38 (61%) patients remain alive. Of the surviving patients, 2 have relapsed and one of these has responded to further therapy, thus 22 are currently in CR. 15 patients have died: 9 from relapsed disease, 1 another malignancy and 5 from transplant related complications. One of 38 patients experienced grade 3-4 acute GvHD and of the surviving patients none has extensive chronic GvHD.
Discussion: The results presented compare favourably with recently published retrospective analyses of outcome for older patients undergoing alloSCT and demonstrate excellent tolerability and outcome for reduced intensity or toxicity conditioning alloSCT in the treatment of high-risk haematological malignancy in older patients, comparable to that seen in younger patients. Our results confirm that chronological age alone should not exclude patients from being offered this potentially curative therapy. Older patients should be considered actively for allogeneic stem cell transplantation and potential candidates referred to a transplant centre.
Disclosure of Interest: None Declared.

PH-P208
ALPHA/BETA T CELL DEPLETION OF HLA-IDENTICAL SIBLING STEM CELL TRANSPLANTATION: TWO-CENTER EXPERIENCE

L. Kaynar[1,], A. Uzay[2], G. Akyol[1], Ç. Pala[3], S. Ratip[2], S. Şıvgın[3], B. Eser[3], A. Ünal[3], E. Ovalı[2], M. Çetin[3]*
[1]Hematology, Erciyes University, Kayseri, [2]Acıbadem Kozyatağı Hospital, İstanbul, [3]Erciyes University, Kayseri, Turkey

Introduction: Our approach which is alpha/beta T cell depletion in HLA-identical sibling stem cell transplantation aims to achieve maximal graft versus leukemia, minimal GvHD, lower graft rejection and transplant related mortality rate during allogeneic HLA identical sibling hematopoietic stem cell transplanatation. We evaluated our Alpha/Beta T Cell Depletion of HLA-identical sibling stem cell transplantations results. All data were obtained prospectively and observationally.
Materials (or patients) and Methods: Nineteen patients were done Alpha/Beta T Cell Depletion of HLA-identical sibling stem cell transplantation. The transplantations were performed at Erciyes University Transplant Center (Cappadocia Transplant Center) and Acibadem International Hospital between May 2012 and December 2013. The distribution of cases was as follows; 5 Acute lymphoblastic leukemia (ALL), 4 Acute myeloid leukemia (AML), 3 myelofibrosis (MF), 2 multiple myeloma (MM), 2 chronic myeloid leukemia (CML), 1 myelodysplastic syndrome (MDS), 1 aplastic anemia, 1 Hodgkin lymphoma (HL) patient. All donors were HLA identical (full matched) siblings. Alpha/Beta T Cell Depletion procedure was made by Clinimacs Depletion Device. Conditioning regimen was Fludarabine + ATG + Melphalan (2 MM cases), Melpahalan + Busulfan + ATG + Cyclophosphamide (1 case), Fludarabine + Thiotepa + Melphalan (14 cases), Fludarabine + Busulfan + ATG (2 MF cases). MMF was started if residual alpha/beta T-cells > 25000/kg, otherwise we have not given any immunosuppressive therapy.
Results: The median age was 42 years (19-64) and 11 of patients were men (11/19). Median CD34+ cell number $9.47*10^6$/kg (6.45-14.75), median Alpha/Beta selecting rate 99.85% (99.28-99.99), median Alpha/Beta T cell number $1.05*10^5$/kg (0.018-8.69). Seven patients did not complete remission before transplantation. The median time to neutrophil engraftment was 11 day (9-15 days), platelet engraftment time was 12 and median follow-up time 183 days (30-557 days). Only AML patient developed moderate acute graft versus host disease (GVHD) (5%). Chronic GVHD has not been seen in any patient. Chimerism occurred all patients except two patients. One of the patients did not in remission and he died from relapse disease, the other died because of intracranial hemorrhage (ICH) before chimerism study. CMV is reactivated in ~16% of patients (3/19). After transplantation four patients (20%) were died. The reasons of deaths were as follows; primary disease (11%; two ALL cases), transplant related mortality (5%; the patient had hypertension and died from ICH).
Discussion: Transplant approach using alpha/beta T cell-depleted stem cells looks like a very promising approach with lower graft rejection, mortality, GVHD and viral infection rates.
Disclosure of Interest: None Declared.

PH-P209
ASSESSMENT OF THE HEMATOPOIETIC CELL TRANSPLANTATION COMORBIDITY INDEX IN OLDER PATIENTS RECEIVING REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

J. Patrick Galvin[1,], J. Mehta[2], J. Monreal[2]*
[1]Medicine, [2]Northwestern University, Chicago, United States

Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) is an established therapy for malignant and nonmalignant hematologic disorders. Reduced-intensity conditioning (RIC) regimens have expanded the use of HSCT to elderly and higher risk patients. However, HSCT still remains associated with a significant mortality and morbidity and the careful assessment of risks and benefits before transplantation is essential. Major factors which influence non-relapse mortality (NRM) and overall survival (OS) after HSCT are diagnosis, type of transplant, remission status and the patient's risk profile, which includes age and presence of comorbidities. The use of the hematopoietic cell transplantation-specific comorbidity index (HCT-CI) has been proposed to predict the probability of non-relapse mortality (NRM) and overall survival (OS) following HSCT. However, the HCT-CI usefulness for older patients receiving a reduced intensity allogeneic HSCT remains unclear.

Materials (or patients) and Methods: A retrospective medical record review was performed to collect data from patients who underwent an allogeneic HSCT at a large, urban, NCI Comprehensive Cancer Center during a five year period (January 2005 to December 2010). Patients 55 years and older who were transplanted using RIC for a hematologic malignancy were selected to be analyzed. Comorbidities and test results were collected to determine the HCT-CI score and other clinical data collected included disease and remission status at time of transplant.
Results: 85 patients aged >55 years old who received a RIC HSCT for hematologic malignancies between January 2005 to December 2010 were analyzed. The median patient age at the time of transplantation was 63 years (range: 55-75 years). The patient diagnoses included AML (39%), NHL (29%), MM (9%), MDS (11%), CLL (8%), ALL (4%). The median pre-transplantation HSCT-CI score was 2 (range: 0-9). Among 85 patients, OS at 2 years was 44%. The 2 yr OS was 31%, 37% and 44% in the low-, intermediate-, and high-risk HSCT-CI groups ($P = 0.61$), respectively. The corresponding NRM at 2 years was 25%, 17% and 18% ($P = 0.91$). We found no predictive value of HSCT-CI for either OS or NRM in older patients having an allogeneic HSCT with reduced-intensity condition. Stratification of age (55-59, 60-64, and 65+) showed no significant impact on 2 yr OS or NRM. However, there was a trend towards a better 2 yr OS for patients who had achieved a CR versus no CR at time of transplant (48% vs 41%).
Discussion: For older patients with hematologic malignancies, the prospect of a RIC HSCT is often considered high-risk. A method to reliably stratify these older HSCT candidates is greatly needed. However, the HCT-CI score in this retrospective analysis did not help predict the NRM or 2 year OS for patients 55 years and older with hematologic malignancies receiving a RIC HSCT. Further research is warranted into what combination of factors may provide a reliable predictive score for older patients with hematologic malignancies.
Disclosure of Interest: None Declared.

Regenerative Medicine

PH-P210
INFLAMMATION CONVERTS HUMAN MESOANGIOBLASTS INTO TARGETS OF ALLOREACTIVE IMMUNE RESPONSES: IMPLICATIONS FOR ALLOGENEIC CELL THERAPY OF DMD

M. Noviello[1,], F. S. Tedesco[2], A. Bondanza[3], R. Tonlorenzi[3], M. R. Carbone[3], M. Gerli[3], S. Marktel[3], S. Napolitano[3], M. P. Cicalese[3], F. Ciceri[3], G. Peretti[4], G. Cossu[5], C. Bonini[3]*
[1]Experimental Hematology, San Raffaele Scientific Institute, Milan, Italy, [2]University College London, London, United Kingdom, [3]San Raffaele Scientific Institute, [4]IRCCS Galeazzi Orthopaedic Institute, Milan, Italy, [5]University of Manchester, Manchester, United Kingdom

Introduction: Duchenne Muscular Dystrophy (DMD) is a severe and incurable genetic disorder leading to disability and death early in life. Recently, different approaches have been proposed to ameliorate disease progression. Among these, cell therapy with donor Mesoangioblasts (MAB) produced significant functional improvements in animal models of muscular dystrophy. Following this preclinical evidence, a phase I-II clinical trial based on the systemic infusion of MAB isolated from HLA-identical familiar donors is started at our Institution.
Materials (or patients) and Methods: We characterized the immunological properties of MAB isolated from healthy donors, both in resting conditions and upon treatment with pro-inflammatory cytokines. Moreover, we longitudinally analyzed T-cell dynamics in DMD patients enrolled in a phase I-II clinical trial testing the safety of intra-arterial infusions of allogeneic mesoangioblasts under a regimen of immunosuppression. Patient samples were harvested before the beginning of treatment, prior to each infusion and bimonthly for one year.
Results: *In vitro* isolated and expanded human MAB proved poorly immunogenic in resting conditions and intrinsically resistant to T-cell killing. However, upon exposure to INF-g or differentiation in myotubes, MAB acquire the ability to promote expansion of alloreactive T cells and become sensitive to T-cell killing. Resistance of mesoangioblasts to T-cell killing is largely due to the expression of the intracellular serine protease inhibitor PI-9. Strikingly, this intrinsic mechanism of stem cell immune evasion does not interfere with the development of T-cell based immune responses against stem cell progeny. In patients receiving MAB infusions, despite standard corticosteroid plus tacrolimus continuous treatment, the numbers of circulating lymphocytes, the relative proportion of naïve/memory T-cell subset and of Tregs were comparable to that measured before treatment. Thus, the treatment does not perturb T-cell homeostasis, and accordingly, infectious adverse events were not recorded. We described alloreactive T-cell responses in three out of five patients, and an inverse correlation between alloreactivity and clinical benefit.
Discussion: Altogether these results suggest that hypoimmunogenic MAB might become immunostimulatory in the inflammatory milieu encountered in dystrophic muscles, justifying and recommending the use of immunosuppressive and/or anti-inflammatory drugs in allogeneic cell therapy of DMD.
Disclosure of Interest: None Declared.

PH-P211
BONE MARROW MONONUCLEAR CELLS LOCAL IMPLANTATION IN PATIENTS WITH AVASCULAR NECROSIS OF THE HEAD OF THE FEMUR

R. Zdziarski[1], J. Lange[2], M. Mordak-Domagala[2], D. Dlubek[2,3], E. Jaskula[3], W. Wodzislawski [1], A. Lange[2,3,]*
[1]Orthopedic and Traumatology Department, 4th Military Clinical Hospital, [2]Lower Silesian Center for Cellular Transplantation with National Bone Marrow Donor Registry, [3]L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Introduction: The incidence of avascular necrosis (AVN) of the head of the femur is increasing what is partly due to greater use of steroids. Up to 50% of patients with AVN experience severe joint destruction what ultimately may lead to total hip replacement. Our 10 years experience with use of marrow derived mononuclear cells for revascularization of critically ischemic limbs prompted use to use these cells to stimulate revascularization of affected bones.
Materials (or patients) and Methods: For this procedure 5 patients (4 males, 1 female, age 25–35 years) were enrolled. All suffered from nontraumatic osteonecrosis. In two of them circulating lupus anti-coagulant was detected what was previously associated with deep venous thrombosis. In one of these two the procedure was performed in both hips. The diagnosis was confirmed by radiology followed by magnetic resonance imaging evaluation. Clinical observation was based on Harris, Ficat and Mitchel scores.
The procedure was performed as follows: (i) bone marrow harvested from posterior iliac crest under a general anesthesia in a volume of 500 mL was enriched in mononuclear cells (BMMNC) with the use of a Cobe Spectra separator, (ii) on the same day orthopedic surgery was performed: 4 channels were drilled with the use of Kirschner wire starting from the greater trochanter area, the channels (4.5 mm in diameter) penetrated to the affected head of the femur and were filled with the marrow mononuclear cell population in a volume of 30 mL. (iii) patients were kept in bed for one day and then they could move with the use of crutches. In a few days the patients started passive and then active rehabilitation in the out-patient clinic.
Results: In the transplanted inoculum were: CD34-CD45-CD90+ cells: 0.026%, CD34+CD45-VEGFR+: 0.012%, CD16+CD14+ cells having physical parameters of monocytes 0.027%, which included 70% Tie2+ cells.
The observation following the surgery included magnetic resonance imaging and the clinical scoring. In the patient with two hips affected, one hip had to be replaced. Histopathology revealed an

increased number of small collagen IV positive vessels with erythrocytes. The second hip 18 months after the marrow mononuclear cells implantation is stabilized in function and MRI documented the presence of bright areas corresponding to the repairing zones. Also other cases being from one year to 8 months after the cellular treatment enjoy stabilization of the affected hips.
Discussion: The procedure seems to be feasible offering some improvement in the function of affected hips with rather low personal and financial costs as compared to total hip replacement. In the cellular population used there are both MSC and ESC which may play a role in healing the affected areas.
This study was supported by the National Centre for Research grant N N407 121940.
Disclosure of Interest: None Declared.

PH-P212
BONE MARROW DERIVED MONONUCLEAR CELLS IMPLANTATION DECREASES SYMPTOMS OF LOWER LIMB ISCHEMIA: LONG TERM POST TREATMENT OBSERVATION

A. Lange[1,2,], L. Maslowski[3], D. Dlubek[1,2], S. Mizia[2], J. Lange[2], W. Witkiewicz[3]*
[1]L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, [2]Lower Silesian Center for Cellular Transplantation with National Bone Marrow Donor Registry, [3]Regional Specialist Hospital in Wroclaw, Research and Development Center, Wroclaw, Poland

Introduction: In years 2003–2005 twelve patients at the end stage of lower limb ischemia (Fontaine IV) had marrow cells population (harvested from the iliac posterior crest) enriched in mononuclear (BMMNC) cells implanted to the affected limbs. The patients suffered from atherosclerosis and all appropriate surgical procedures were exaggerated prior to the cellular therapy. These patients constituted the first cohort (experimental) and were followed for 4 to 6 yrs. After that 10 patients enrolled the second cohort (validation).
Materials (or patients) and Methods: Procedure employed for the first and the second cohort was the same and included: (i) bone marrow harvest from posterior iliac crest in a volume of 500 mL, (ii) mononuclear cells enrichment with the use of a Cobe Spectra separator which ended with a volume of 100 mL, (iii) 70 mL of the cell suspension were injected in 0.5 mL portions to calf muscles of the affected limb.
Results: Analysis of the implanted cells revealed the presence of (mean±SEM): (i) hematopoietic progenitors: CD34+, CD45+: 1.29%±0.19, (ii) mesenchymal stem cells: CD45-CD34-CD90+: 0.1%±0.02, CD45-CD34-CD105+: 2.4%±0.33, CD45-CD34-CD73+: 0.08%±0.01, and 24.4±4.5 CFU-F/10^6 WBC, (iii) endothelial stem cells: CD45-CD34+: 0.28%±0.04, CD45-CD34+CD309+: 0.09%±0.02, CD45-CD34+CD31+: 0.23%±0.03, in monocyte gate: CD14+CD16+Tie-2+: 0.019%±0.008.
In seven patients the gastrocnemius muscle was biopsied for immunohistochemistry and genetic study. HIF-1, SDF-1 and CXCR4 gene expression was normalized to the average expression of GAPDH and HPRT1 genes. The transcripts of all these genes were noted and the highest values were for HIF-1 transcripts. Immunostaining revealed the presence of HIF-1+ cells especially in biopsies strongly showing HSP 70 positivity. CD34+ mononuclear cells were seen in all biopsies.
Discussion: The outcome of the procedure was as follows: pain reduction in 83%, 67% and 28%; wound healing in 25%, 42% and 50% cases at 1, 3 and 12 months post implantation, respectively. Distance to claudication increased from 50 to 200 meters (median) 12 months post the procedure. In 2 cases affected limbs were amputated.
Disclosure of Interest: None Declared.

PH-P213
GMP MESENCHYMAL STEM CELL PRODUCTION FROM PATIENTS AFFECTED BY PROGRESSIVE SUPRANUCLEAR PALSY: A PHASE I STUDY.

R. Giordano[1,], T. Montemurro[1], M. Viganò[1], V. Parazzi[1], B. Baluce[1], C. Lavazza[1], S. Budelli[1], L. Marino[2], L. Lazzari[1], G. Pezzoli[3]*
[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, [2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, [3]Parkinson Center, Istituti Clinici di Perfezionamento, Milano, Italy

Introduction: Mesenchymal stem cells (MSCs) are multipotent cells that can be isolated from many sources. Recently, several cellular therapy products have been developed for therapeutic applications in neurodegenerative disorders in compliance with Good Manufacturing Practices (GMP). Since BM MSC production from elderly and diseased patients in the autologous context is extremely challenging, some technical aspects of cell culture should be considered (in particular culture duration and the use of FBS or other substitutes) factors.
Materials (or patients) and Methods: Here we describe the results of GMP procedures to obtain BMMSCs suitable for autologous use in patients affected by a rare form of parkinsonism (Progressive Supranuclear Palsy) within a specific clinical protocol (NCT01824121). A potency assay, based on the secretion of the two major neurotrophic factors (BDNF and GDNF) putative mediators of MSC neuroprotection have been developed.
Results: The target cell dose of $1.5 + 0.5 \times 10^6$ cells was reached in 8/10 patients. The quality controls (MSC-specific immunophenotype, viability >80%, absence of bacterial, fungi and mycoplasma contamination, low endotoxin level, normal karyotype) were conform in all the preparations. MSC from the enrolled patients were able to rescue 6-OH-DA damage in a co-culture system via BDNF/GDNF secretion.
Discussion: This study supports the concept that each clinical trial should be designed in order to choose the production protocol that is able to obtain the best results in view of the specific clinical needs.
Disclosure of Interest: None Declared.

Solid Tumours

PH-P214
NATURAL KILLER CELL BASED THERAPIES FOR OSTEOSARCOMA

L. Fernandez[1,], J. Valentín[2], I. Martínez-Romera[3], E. López-Collazo[2], A. Patiño[4], A. Pérez-Martínez[5]*
[1]Investigación Clínica, Centro Nacional de Investigación del Cáncer, [2]Grupo de Inmmunidad Innata, IdiPaz, [3]Universidad Autónoma de Madrid, Madrid, [4]Pediatría. Genética Clínica., Clínica Universitaria de Navarra, Pamplona, [5]Servicio de Hemato-Oncología Infantil. Hospital La Paz., Universidad Autónoma de Madrid, Madrid, Spain

Introduction: Metastatic osteosarcoma occurs predominantly in adolescents and young adults and has an overall survival rate of about 20%, despite chemotherapy and surgery. Therefore, new therapeutic approaches are urgently needed to improve survival in these patients. Natural Killer (NK) cells are lymphocytes with cytotoxic activity toward virus-infected or malignant cells. Crosstalk between NK cells receptors and tumour cells ligands is necessary for a NK cell activation and anti-tumour activity. In the present study we explored the feasibility of NK cell–mediated therapies for osteosarcoma.
Materials (or patients) and Methods: The expression of ligands for activatory NKG2D (MICA, MICAB, ULBP1, ULBP2 and ULBP3), DNAM (CD155, CD112), inhibitory (HLA class I), apoptosis (Fas) receptor on NK cell receptors was analyzed by flow cytometry in

11 metastatic primary cell lines: 319M, 531MII, 588M, 595M, 654M, 685MII, 699B, 709M, 716M, 722MII, 728MII. All cell lines derived from patients were lung metastases except for the 531MII, which was one-rib metastases, and for the 699B, which was a primary tumour. Mean Fluorescence Intensity (MFI) was calculated by MFI of the specific staining relative to the MFI of the appropriate isotype control staining. Activated and Expanded NK (NKAE) cells were obtained by co culture of PBMCs from healthy donors with the K562mbIL15-41BBL cell line (previously irradiated with 100Gy) in a ratio 1:1.5 for three weeks in RPMI supplemented with 10% human AB serum (Sigma), 100 IU/mL IL-2 (Miltenyi), and 1% PS. The NK cell purity was assessed by flow cytometry. The cytotoxicity of NKAE/NKAE IL-2/IL-15 cells was monitored using a conventional 2-h europium-TDA release assay (Perkin-Elmer Wallac, Turku, Finland). The number of NK cells was calculated by multiplying the lymphocyte counts to the percentage of $CD3^{-}CD56^{+}$ NK cells. To explore the importance of the NK cell receptors and their ligands interactions for a NK cell elimination of osteosarcoma, cytotoxicity assays were performed in the presence of different blocking antibodies.
Results: At least 3/5 NKG2D ligands and HLA class I on primary osteosarcomas had a low expression (MFI/MFI isotype ≤ 10) in 10/11 and 8/11, respectively. However, Fas and CD112 were highly expressed (MFI/MFI isotype ≥15) in 8/11 of the primary osteosarcomas. Cytotoxicity assays revealed primary osteosarcoma were resistant to criopreserved NK cell–mediated cytolysis, although cytolytic activity was strongly enhanced by IL-15/IL-2 overnight activation, and when fresh NKAEs were used as effectors. The contribution of each ligand to NK cell–mediated cytolysis was studied by specific antibody blockade, depending on the ligands expression. We also explored different ways to enhance NK cell cytotoxicity for each osteosarcoma. We did not find a common pattern; each osteosarcoma seemed to be dependent on different pathways.
Discussion: NKG2D and HLA class I ligands have a low expression on primary osteosarcoma, probably underlying a NK cell evasion mechanism. However, Fas/FasL and DNAM-1/CD112 pathways could be important for NK cell recognition. Although primary osteosarcomas were resistant to NK cell cytolysis, cytotoxic activity was strongly enhanced by IL-15/IL-2 overnight stimulation, and with activated and expanded NK cells. However, each osteosarcoma depended on different pathways. This fact shows the heterogeneity of osteosarcomas and underlies the need for personalized strategies to treat these tumours.
Disclosure of Interest: None Declared.

PH-P215
ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION WITH REDUCED-INTENSITY CONDITIONING REGIMEN IN CHILDREN AND YOUNG ADULTS WITH NEUROBLASTOMA AND EWING SARCOMA

I. Kazantsev[1,2,], A. Gevorgyan[1,3], A. Kozlov[1,2], S. Shiryaev[1,2], T. Andreeva[1,2], T. Jukhta[1,4], E. Morozova[5], A. Klimov[6,7], A. Shvetsov[6,7], Y. Punanov[4], S. Safonova[4,8], L. Zubarovskaya[2,5], B. Afanasyev[2,5]*
[1]Pediatric BMT, R.M.Gorbacheva Memorial Institute for Pediatric Hematology, Oncology and Transplantation, [2]BMT, I.Pavlov First State Medical University, [3]BMT, I.Pavlov First State Medical Universty, [4]Pediatric Cancer, N.Petrov Federal Research Institute for Oncology, [5]BMT, [6]Surgery, R.M.Gorbacheva Memorial Institute for Pediatric Hematology, Oncology and Transplantation, [7]Oncology, I.Pavlov First State Medical University, [8]Pediatric Cancer, R.M.Gorbacheva Memorial Institute for Pediatric Hematology, Oncology and Transplantation, Saint-Petersburg, Russian Federation

Introduction: Disseminated high-risk pediatric solid tumors are a biologically heterogeneic condition with dismal prognosis. Although the state-of-art therapy combines aggressive local control with aggressive systemic therapy, the local and systemic relapse rate is still high. There is a body of evidence for immune effects in some pediatric solid tumors patients (pts), therefore allogeneic hemopoietic stem cell transplantation (allo-HSCT) with rediced-intensity conditioning (RIC) may be a feasible method creating a platform for further cell-based immune therapy.
Materials (or patients) and Methods: In the period from 2008 to 2013 thirteen pediatric and young adult pts (age 4-22, median age 6 years) with neuroblastoma (*n*=7) or Ewing sarcoma (*n*=6) underwent allo-HSCT with RIC. At the time of transplant 2 pts achieved the 1st PR, 3 pts 2nd or further CR, 2 patients had 2nd or further PR and 3 pts had stable disease. All the patients were heavily pretreated: 3-4 lines of chemotherapy (*n*=13), irradiation (*n*=3), surgical treatment (*n*=9), high-dose chemotherapy with auto-HSCT (*n*=9). In 10 of 13 pts allo-HSCT was performed as salvage therapy. The donors were haploidentical (*n*=10), matched unrelated (*n*=2) or matched related (*n*=1). The graft source was G-CSF - primed bone marrow (*n*=6), PBSC (*n*=3) or both (*n*=4). The median CD 34 count in the graft was 10.6×10^6, CD3 5.5×10^7. Conditioning regimen consisted of fludarabin and melphalan (*n*=8) or busulfan and thiotepa (*n*=5). The graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus or cyclosporine A with addition of ATG (*n*=7) or post-transplant cyclophosphamide (*n*=6) in haploidentical or matched unrelated donor graft recipient. Haploidentical allo-HSCT recipients also received MMF and sirolimus.
Results: All the patients engrafted at the median of D+15. Grade II-IV aGVHD was observed in 7 patients, 3 of them responded well to steroids therapy, 3 of the patients developed chronic GVHD. Five of 13 patients are currently alive with the follow-up of 3 to 44 months, the 2-year OS rate is 30%. Two of the patients died of aGVHD, 6 of tumor progression. There is some clinical evidence for graft-versus-tumor effect: long-term (5-32 months, *n*=5) or short-term (*n*=3) stabilization of aggressive disease in salvage group patients, partial response (*n*=2), PET-remission (*n*=1). Most of the patients received some form of post-transplant therapy: preemptive DLI (*n*=3), purified NK-cell infusion with *in vivo* expansion (*n*=2), surgery or irradiation (*n*=2), chemotherapy with targeted therapy (*n*=7). In 4 patients with post-transplant progression the lasting response was achieved.
Discussion: Allo-HSCT with RIC is a potentially effective method in patients with chemoresistant pediatric solid tumors, that may enhance the effects of further complex therapy and serve as a platform for immune-based interventions. RIC regimens are characterized by acceptable complications rate in heavily pretreated pediatric population. The indications for allo-HSCT and optimal post-transplant therapy tactics should be developed in a larger prospective study.
Disclosure of Interest: None Declared.

PH-P216
EVALUATION OF LIGANDS FOR NATURAL KILLER CELL ACTIVATING RECEPTORS IN METASTATIC COLORECTAL CARCINOMA CELLS AND THEIR ROLE IN NK CELL-MEDIATED LYSIS

I. Turin[1], S. Brugnatelli[1], T. Matteo[1], D. Lisini[2], M. Maestri[1], B. Rovati[1], O. Luinetti[1], M. Paulli[3], R. Maccario[1], P. Pedrazzoli[1], D. Montagna[3,]*
[1]Fondazione IRCCS Pol. San Matteo, Pavia, [2]Fondazione IRCCS Istituto Neurologico Besta, Milano, [3]Fondazione IRCCS Pol. San Matteo, Università di Pavia, Pavia, Italy

Introduction: Immunotherapy, including natural killer (NK) cells-based strategies, is an area of active investigation in metastatic colorectal cancer (mCRC). The function of NK cells is determined by a balance between activating and inhibitory signals. DNAM-1 and NKG2D, the most important NK cell activating receptors, transduce activating signals after binding their ligands, namely the poliovirus receptor (PVR) CD155 and its family member CD112 (nectin-2), and MICA/B on tumor cells.
Materials (or patients) and Methods: We evaluated the expression and function of these ligands in mCRC cells. After obtaining informed signed consent, 25 mCRC patients were included in the study. Tumor samples were obtained by biopsy or surgical resection, disaggregated by Gentle MACS Dissociator and cultured. After pathologic confirmation of neoplastic origin, early passage primary cultures were cryopreserved for further experiments.

The expression of the most important ligands was evaluated by cytofluorimetric analysis and gene expression on cultured tumor cells and in immunohistochemistry sections of the original sample embedded in paraffin.
Results: Gene expression of ligands was performed by real-time quantitative PCR (relative quantification) An endogenous control was used to account for differences in the amount and quality of total RNA added to each reaction. PVR, MICA/B, were highly and uniformly expressed in tumor cells (TC) of patients analyzed (mean value: 5.57, 6.17, 6.5 respectively), while, lower levels of ULBP1/3 were detected (mean value: 10.67 and 10.11, respectively). Membrane phenotype evaluation of cultured TC demonstrated the CD155 and CD112 were uniformly expressed by all TC, while variable expression of MICA/B was documented. In contrast with gene expression results, ULBP1/3 was virtually undetectable on the surface of all cultured TC. Immunohistochemistry analysis in original samples are ongoing. KIR receptors expression on patients' derived NK cells was in general down-regulate but could be enhanced after appropriate activation.
Discussion: Data on the roles of receptor-ligand interactions in NK cell-mediated lysis of mCRC cells could be useful in designing immunotherapeutic approaches based on the infusion of freshly or activated NK cells in patients affected by mCRC cells and for evaluating approaches to enhance susceptibility of lysis of tumor cells.
Disclosure of Interest: None Declared.

PH-P217
HAPLOIDENTICAL STEM CELL TRANSPLANTATION IN PEDIATRIC SOLID TUMORS: THE EXPERIENCE OF CHILDREN HOSPITAL OF BRESCIA, ITALY

B. Alessandra[1,], B. Federica[1], C. Marta[1], D. Carmelita[2], N. Lucia Dora[2], S. R Fabian [2], P. Fulvio[2], L. Arnalda[1]*
[1]Servizio di Medicina di Laboratorio,Sezione di Ematologia e Emoc oagulazione,Laboratorio Cellule Staminali, [2]UO Oncoematologia Pediatrica e Trapianto Midolllo Osseo, Ospedale dei Bambini, Brescia, Italy

Introduction: Ruggeri *et al* have shown allogeneic NK cells can mediate antileukemic effects against AML after allogeneic haploidentical and partially matched unrelated hematopoietic cell transplantation when KIR/KIR ligand incompatibility is present in the graft-versus-host (GVH) direction (defined as an MHC class I KIR ligand absent in the recipient, but present in the donor). Little is known about clinically beneficial allograft-vs-tumor effect associated with natural killer cells (NK) donor-recipient mismatch in pediatric solid tumors. In this study we focus on isolation and characterization of primary tumoral cultures from biopsy and evaluation of NK cell alloreactivity against tumoral cells *in vitro*, for an identification of a familiar donor with relevant citotoxic activity.
Materials (or patients) and Methods: We characterized 29 pediatric patients affected by neuroblastoma (NB *n*=11), Ewing sarcoma (ES *n*=3), Wilms'tumor (WT *n*=6) and rabdomiosarcoma (RB *n*=9). Expression of phenotypic markers of each lineage was evaluated by immunohistochemical markers (HIC). HLA typing, evaluating KIR mismatches and cytotoxicity assays were performed.
Results: Cells were isolated from tumoral tissue derived from biopsy, cultivated under the appropriate conditions until confluence.The cells had to be characterized by HIC. The HIC evaluation was performed on the basis of tumoral markers at diagnosis. 7 NB were non tumoral with a low expression of neoplastic markers and only 3 were neoplastic with an espression of cancer markers above of 40%, only one didn't grow. 3/9 RB were neoplastic with an expression of tumoral markers on 70% of the cells, 1/9 didn't grow *in vitro* and 5 weren't tumoral. As concern ES only one was neoplastic. 4/6 WT were neoplastic with an high expressions of tumoral markers on average 70% of the cells. These data confirm the technical difficulty to obtain tumoral primary cultures from surgical biopsy especially in NB. Q-PCR was performed to determine the mRNA expression of tumoral markers detected at onset:preliminary data show relevant levels of mRNA, in keeping with HIC revaluation. We are now in the process of developing a multiparametric flow cytometric screening for solid. With this approach heterogeneous cell populations can be analyzed and this can provide informations on the functional status of regulatory processes by the simultaneous measurement of multiple key elements. Moreover patients candidate were assessed for HLA compatibility by serology and by high-resolution molecular analysis. NK alloreactivity assay against patient's blast was performed. On the basis of our pre-clinical studies, a child affected by stage IV ES in partial remission, after completing the whole of the AIEOP protocol, underwent haploidentical transplantation of hematopoietic stem cells (HCT) from his KIR alloreactive uncle.The transplant consisted of $1.5x10^6$/kg of CD34+ and $8x10^4$/kg of CD3+. After 4 months from HCT, patient was in complete remission. He had a overall survival of 3 years after transplantation respect 1-2 months expected. An additional child affected by stage IV NB in partial remission after 3 lines of chemotherapy was grafted from her KIR alloreactive father and survived in partial remission for 5 months.
Discussion: Alloreactive NK-cell mediated antitumor effects might provide useful perspectives with the aim to design innovative new cell therapy approaches against solid tumors in children.
Disclosure of Interest: None Declared.

PH-P218
DESMOPLASTIC SMALL ROUND CELL TUMOR (DSRCT): A RETROSPECTIVE ANALYSIS OF 26 ADULTS PATIENTS (PTS) FROM A SINGLE INSTITUTION

R. De Sanctis[1], A. Bertuzzi[1], V. Quagliuolo[1], L. Castagna[1,], A. Marrari[1], S. Marchetti[1], A. Santoro[1]*
[1]Humanitas Cancer Center, Rozzano, Italy

Introduction: DSRCT is a rare and highly aggressive mesenchymal tumor. Despite aggressive multimodal treatment, the prognosis remains poor. We performed a mono-institutional retrospective study to analyze treatment and outcome of pts with DSRCT.
Materials (or patients) and Methods: We retrospectively reviewed the medical records of pts with DSRCT treated from May 1997 to July 2012 at Humanitas Cancer Center. Demographic data, tumor characteristics, treatment and response rate (RR) according to RECIST criteria were collected; median Progression-Free Survival (mPFS) was analyzed with Kaplan-Meier method.
Results: We treated 26 pts with DSRCT (M/F ratio, 24/2), median age at presentation 30 (range 15-65). Primary tumor site was abdomen/pelvis (88%; *n*= 24) and mediastinum (12%; *n*= 2). Sixteen pts were metastatic at diagnosis (62%). Six pts underwent debulking surgery elsewhere, of whom 4 with local disease (LD) and 2 with metastatic disease (MD). Pts received induction chemotherapy (CT) as follows: 10 VAI (vincristine, adriamycin, ifosfamide) with 6 PR, 2 SD and 2 PD; 6 miniCARBOPEC (etoposide, carboplatin and cyclofosfamide) with 5 PR and 1 PD; 4 modified P6-regimen with 2 PR and 2 SD. 6 pts received different adriamycin-based CT of whom one was lost to follow-up. Overall, the overall response rate (PR + SD) was 73%. 19 responding pts were treated with high-dose chemotherapy (HDC) of whom 11 with MD and 8 with LD. At day+100 after HDC, the disease status was CR 1, PR 8, SD 5 and PD 5. Seven pts underwent surgery after HDC and 3 had post-surgery radiotherapy (RT). The mPFS of pts who had ASCT was 11.61 months (range, 5.07-97.2).
Discussion: DSRCT management requires multimodality treatments. Although investigational, HDC seems to obtain long-term disease control, even in the metastatic setting. The role of HDC in DSRCT needs to be prospectively evaluated.
Disclosure of Interest: None Declared.

PH-P219

BUSULPHAN-MELPHALAN VERSUS CARBOPLATIN-ETOPOSIDE-MELPHALAN CONDITIONING REGIMEN IN CHILDREN WITH NEUROBLASTOMA UNDERWENT AUTOLOGOUS STEM CELL TRANSPLANTATION

E. Atas[1,], V. Kesik[1], O. Babacan[1], N. Korkmazer[2]*
[1]Department of Pediatric Oncology, [2]Department of Pediatrics, Gulhane Military Medical Academy, Ankara, Turkey

Introduction: Neuroblastoma (NBL) is the most common extracranial solid tumor of childhood. The 5 year survival rate for Stage IV patients with conventional standard treatment regimens were reported as 30% (1). Autologous stem cell transplantation (ASCT) has been revealed better event-free survival rate (EFS) than conventional chemotherapy (30% vs 17%) (3). Herein, we evaluated the effect of BuMel and CEM conditioning regimens on outcome.
Materials (or patients) and Methods: The treatment features of 19 children with neuroblastoma object to ASCT between 1998 and 2013 were analyzed. The median age was 5 years (range 2-17) and male/female ratio was 14/5=2.8. Kaplan-Meier methods and log rank tests were used in analysis. Age, gender, involvement of bone and cranial bone, N-myc status, LDH, VMA, ferritin, erythrocyte sedimentation rate, primary tumor localization and conditioning regimens were analyzed as prognostic factor on survival rate with Cox regression analysis.
Results: The primary tumor localizations were left adrenal gland in 11 (57.9%), right adrenal gland in 5 (26.3%), left mediastinal in 1 (5.3%), paraaortic in 1 (5.3%), and right cervical in 1 (5.3%) cases. Sixty-three percent (n: 12) of children had primary disease and the rest had relapsed disease. Fifty-seven percent of children (n:11) were in remission. There were 17 (89.5%) patients with bone and 9 (47.4%) patients with cranial bone involvement. The stage distribution was stage III in 2 (10.5%) cases, and stage IV in 17 (89.5%) cases. The conditioning regimen prior to transplantation was BuMel in 10 (52.6%) cases and CEM in 9 (47.4%) cases. Five-year event-free and overall survival rates were 18.4% and 67.7%, respectively. With a median follow-up of 13 months (range 2-154) after transplant, disease-free survival (DFS) and overall survival (OS) are estimated to be 49.0% and 65.3% at 17 months, respectively. Post-transplant DFS and OS were 39.5% & 88.9% in BuMel, 55.6% & 55.6% in CEM cases at 15 months. Age, involvement of bone, N-myc status, LDH, VMA, ferritin, erythrocyte sedimentation rate, primary tumor localization and conditioning regimen were not found as a significant prognostic factor for EFS and OS (P>0.05). Cranial bone involvement, gender, status at transplantation (primary or relapsed) were found as a significant prognostic factor for EFS in univariate and multivariate analyses but was not significant in BuMel and CEM for DFS and OS.
Discussion: Although the overall survival rates for children with high risk neuroblastoma were within acceptable limits, the event free survival rates were much lower than overall survival rates in children. BuMel regimen had better survival rate than CEM. Cranial bone involvement was the most effective factor on EFS. Although the DFS rate of CEM was a little better than BuMel, OS was nearly two fold high in BuMel group. Cranial bone involvement can also be associated with low DFS in BuMel group [BuMel group; 60% (n:6) vs CEM group; 33%(n:3)]. Randomized prospective large scaled studies can be done for further investigation to improve the survival rates and preventing the late relapses.
Disclosure of Interest: None Declared.

PH-P220

ROLE OF NK CELLS DURING ANTITUMORAL IMMUNE ACTIVATION INDUCED BY X-RAY AND HYPERTHERMIA

P. Finkel[1], B. Frey[2], F. Mayer[1], K. Boesl[1], N. Werthmoeller[2], A. Mackensen[1], U. Gaipl[2], E. Ullrich[1,3,]*
[1]Department of Internal Medicine 5, Hematology and Oncology, [2]Department of Radiation Oncology, Friedrich Alexander University, Erlangen, [3]Childrens Hospital, Pediatric Hematology & Oncology, Goethe University Frankfurt, Frankfurt, Germany

Introduction: Classical tumor therapy is generally based on surgery combined with radio- and chemotherapy. However, recently additive immunotherapy has gained in impact. Different strategies have been developed with the aim to strengthen the antitumor capacity of the immune system. It has further been demonstrated that treatment with chemotherapeutics or use of ionizing radiation (X-ray) contributes to a beneficial tumor microenvironment.
Materials (or patients) and Methods: In order to gain deeper insight into how X-ray in combination with adjuvant hyperthermia treatment results in immunological responses, we examined the role of T-, B- and NK-cells by use of RAG1$^{-/-}$ mice as well as NK1.1-depleting antibodies in the following two scenarios.
(1) Immunization with whole tumor cells that have been exposed to X-ray and hyperthermia
(2) Local *in situ* treatment of solid tumors with X-ray and hyperthermia
Results: (1) *In vitro* treatment of tumor cells by X-ray in combination with hyperthermia induces a mixture of apoptotic and necrotic cells. After subcutaneous injection of these cells as vaccines we could generate very effective immune activation and tumor protection. Furthermore, our *in vivo* results suggest that the impact of NK cells on antitumor immunity significantly differs dependent on the phase of tumor progress and treatment. Importantly, during the antitumor immunization period, NK cells mediate a negative influence on the generation of the favored antitumor response in our model.
(2) The transfer in a therapeutic immunization protocol of established tumors in situ showed similar effects. Depletion of NK cells during the period of local treatment with X-ray and hyperthermia provided a significant better outcome than in non-depleted mice.
Discussion: Summarized, we demonstrated that the treatment with X-ray in combination with hyperthermia induced the best immunogenic effects. Moreover, our preclinical studies indicate importance of immune editing effects of NK cells. These experiments are of high relevance for the further development of multimodal immunotherapeutic protocols combining X-ray and hyperthermia with cell therapeutic approaches for the treatment of solid tumors.
Disclosure of Interest: None Declared.

PH-P221

SUCCESSFUL GENERATION OF HPV-SPECIFIC T CELL LINES FOR T CELL THERAPY OF PATIENTS WITH HPV-POSITIVE HEAD AND NECK CANCER

S. Basso[1,], I. Guido[1], S. Secondino[2], G. Quartuccio[1], A. Gurrado[1], C. Paglino[2], A. Pellerano[1], G. Acquafredda[1], L. Rubert[1], R. Maccario[3], M. Zecca[1], P. Comoli[1], P. Pedrazzoli[2]*
[1]Pediatric Hematology/Oncology, [2]Medical Oncology, [3]Cell Factory, Fondazione iRCCS Policlinico San Matteo, Pavia, Italy

Introduction: Virus-related tumors seem to be distinct clinical entities, with a better prognosis than virus-negative head and neck cancer. Viral positivity in biological fluids and tissues is emerging as a valid biomarker not only for diagnostic purposes, but also for identifying patients amenable to lower toxicity treatment strategies, and to evaluate response to treatment. Moreover, viral antigens on tumor cells may represent suitable therapeutic targets. Phase I-II studies demonstrated that clinical and immunological responses can be obtained in patients with radiotherapy- and chemotherapy-resistant, stage IV EBV-related undifferentiated nasopharyngeal carcinoma by administration

of EBV-specific autologous polyclonal CTL therapy. Human papillomavirus (HPV) 16 is a prognostic marker for enhanced overall and disease-free survival in oropharyngeal cancer, but its uses as a predictive marker or a therapeutic target have not yet been proven in the specific setting.
Materials (or patients) and Methods: The aim of this study was to evaluate the feasibility of expanding HPV-specific CTL from 10 healthy donors and 5 patients with HPV+ oropharyngeal squamous cell carcinoma (OSCC), to be employed as targeted therapy for OSCC. We conducted experiments to validate an *in vitro* culture method to expand HPV-specific CTL, by peripheral blood mononuclear cell (PBMC) stimulation with 15mer peptide pools derived from the HPV16 E6 and E7 proteins.
Results: T-cell lines (TCL), that included a median 75% CD4+ and 22% CD8+ T lymphocytes, were successfully generated from 7/10 healthy individuals and 4/5 OSCC patients. While only 70% of TCL expanded from healthy donors presented specific cytotoxic activity against PHA blasts pulsed with HPV E6/E7 peptide mix, all TCL obtained from OSCC patients exerted specific cytotoxicity, also at very low effector to target ratio. Some of the T-cell lines showed HPV16-specific INFg production in Elispot assays, with those from OSCC patients yielding consistently higher frequencies than healthy subjects (median 90 SFU/10^6 cells vs. 24 SFU/10^6 cells). Noncultured PBMC from the same individuals did not show any specific cytotoxicity, nor a measurable HPV16-specific IFNg-producing cell frequency.
Discussion: Our data indicate that HPV16-specific cytotoxic T cell lines may be expanded from healthy donors and, more importantly, from OSCC patients, after stimulation with HPV E6/E7-derived peptides. Their efficacy in tumor containment remains to be evaluated in clinical trials.
Disclosure of Interest: None Declared.

PH-P222
STEM CELL RESCUE FROM IRRADIATION OF MULTIPLE TUMOR SITES COMBINED WITH HIGH-DOSE CHEMOTHERAPY, FOLLOWED BY REDUCED INTENSITY CONDITIONING AND HAPLODISPARATE STEM CELL TRANSPLANTATION IN PATIENTS WITH ADVANCED PEDIATRIC SARCOMAS: PRELIMINARY RESULTS

U. Thiel[1], A. Wawer[1], I. Teichert von Luettichau[1], H.-U. Bender[1], F. Blaeschke[1], T. G. P. Grunewald[1], F. Bauer[1], M. Steinborn[2], B. Röper[3], N. Andratschke[3], M. Molls[4], C. Salat[5], R. D Issels[5], T. Klingebiel[6], P. Bader[6], E. Koscielniak[7], U. Dirksen[8], H. Jürgens[8], H.-J. Kolb[1,9], S. Burdach[1,]*
[1]Department of Pediatrics, Wilhelm Sander Sarcoma Unit and Roman Herzog Comprehensive Cancer Center, Klinikum rechts der Isar, Technische Universität München, [2]Department of Pediatric Radiology, Schwabing Hospital Medical Center, [3]Department of Radiation Oncology, Klinikum rechts der Isar, Technische Universität München, [4]Klinikum rechts der Isar, Technische Universität München, Department of Radiation Oncology, [5]Department of Internal Medicine III, Klinikum Großhadern Medical Centre Ludwig-Maximilians-University, Munich, [6]Pediatric Hematology and Oncology, Children's Hospital III, Johann Wolfgang Goethe University, Frankfurt, [7]Pediatric 5 (Oncology, Hematology, Immunology), Olga Hospital, Klinikum Stuttgart, Stuttgart, [8]Department of Pediatric Hematology and Oncology, University Children's Hospital Münster, Westfalian Wilhelms-University Münster, Münster, [9]Department of Medicine III, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

Introduction: Advanced pediatric sarcomas are associated with poor prognosis. They include Ewing's sarcomas (ES), metastatic to more than one bone or early relapse as well as stage IV rhabdomyosarcomas (RMS). We assessed toxicity, relapse free survival (RFS) and overall survival (OS) of ES and RMS patients treated with the MetaEICESS 2007 protocol.
Materials (or patients) and Methods: From 2007 to 2013 six ES and two RMS patients were enrolled. Four ES patients were eligible due to multifocal disease (≥ three bones/organs or marrow involved at diagnosis) and two due to early relapse (<24 months after diagnosis). The two RMS patients were in stage IV. The protocol comprised induction-chemotherapy, whole-body MRI/PET directed radiotherapy to the primary tumor and to all metastases, optional surgery, tandem high-dose chemotherapy with autologous stem cell transplantation and reduced intensity conditioning for allogeneic stem cell transplantation (allo-SCT). Data was censured on November 1st 2013. Definitive radiotherapy was delivered to the primary tumor (*n*=7, total dose 50-60 Gy), to the lungs (*n*=4, 15-18 Gy), and to lymph node and multiple osseous metastases (*n*=3 and *n*=7, 45-50 Gy) in individual combinations. Sums of planning target volumes ranged from 453–9407 cm^3, with a median of 2762 cm^3. One patient received local proton irradiation.
Results: 5/8 patients are surviving at a median of 28 months after diagnosis: 4/8 patients in CR and 1/8 in stable disease. Seven patients reached complete remission (CR) before allo-SCT, three of whom relapsed thereafter, predominantly outside the radiotherapy treatment fields. Four patients with relapsed or progressive disease after allo-SCT received donor lymphocyte infusions (DLI), of whom one showed ongoing remission after one year, one has stable disease for >44 months after transplant and 28 months after relapse; the remaining two patients progressed. 1/8 patients had to be re-transplanted after initial graft rejection, 3/8 patients suffered ADV reactivation, 3/8 patients developed acute- and 1/8 developed chronic GvHD. Median RFS after allo-SCT was 16.5 months (range 0-48+ months) and median OS was 21 months (range 5-48+).
Discussion: The MetaEICESS 2007 protocol constitutes a feasible option for patients with advanced pediatric sarcomas. A larger cohort is mandatory to verify OS or RFS improvements over current protocols.
Disclosure of Interest: None Declared.

Stem cell donor

PH-P223
USE OF UNRELATED DONORS IN ELDERLY PATIENTS (AGE >60 YEARS) UNDERGOING REDUCED-INTENSITY CONDITIONING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES

J. Elcheikh[1,], R. Devilier[1], R. Devilier[1], S. Furst[1], S. Furst[1], R. Crocchiolo[1], R. Crocchiolo[1], A. Granata[1], A. Granata[1], C. Faucher[2], C. Faucher[2], B. Mohty[1], B. Mohty[1], S. Harbi[1], S. Harbi[1], R. Bouabdallah[1], R. Bouabdallah[1], N. Vey[1], N. Vey[1], L. Castagna[1], L. Castagna[1], C. Chababnnon[1], C. Chababnnon[1], D. Blaise[1], D. Blaise[1]*
[1]institut Paoli Calmettes, Marseille, France, [2]Transplantation, Institut Paoli Calmettes, Marseille, France

Introduction: The lower morbidity and mortality of reduced-intensity conditioning (RIC) regimens have allowed allogeneic hematopoietic cell transplantation (HCT) in older patients. However, there are only limited data on the feasibility and outcomes of URD HCT in elderly patients. The aim of the study was to compare the outcome in OS and PFS for patients transplanted using unrelated donor (URD) in patients age 60 or older.
Materials (or patients) and Methods: We retrospectively analyzed outcomes in 62 consecutive hematologic malignancy patients aged > or =60 years (median, 62 years; range: 60-70 years) undergoing reduced intensity conditioning regimens (RIC) from URD. In this study, URD was used only when a MRD was not available.Then we compared the outcome of 17 elderly patients (age >65 years) with 44 younger patients aged between 60 and 65 years.
Results: No patients experienced graft rejection. The median HCT comorbidity index score was 2 (range, 0 to 6). With a median follow up of 36 months (range, 5-74), the cumulative incidence of grades II to IV acute GVHD was 28% and of grades III to IV acute GVHD, 13%. At 2 years, the cumulative incidence of chronic GVHD was 27%, progression-free survival (PFS) was 62%, overall survival (OS) was 63%, and relapse was 14%. Non relapse mortality (NRM) was 24% at 2 years. The cumulative incidence of grade II–IV Acute

GVHD was 43% for the younger group and 17% for the older group (P = 0.056). The cumulative incidence of chronic GVHD was not different between the two groups (23% vs. 45% (P=0.3), respectively). Two-year OS and PFS was 57% versus 86% (P = 0.059) and 55% versus 86% (P = 0.03), in the younger and the older group respectively. The 2-year NRM and relapse was 26% versus 14% (P = 0.4) and 19% versus 0% (P = 0.04), in the younger and older group respectively.
Discussion: This retrospective study suggest that RIC HCT from URD is a safe and effective option for patients aged > or =60 years or older, and in the absence of suitable related donors, well-matched URD may offer a very reasonable alternative, and that does not appear to be associated with a detrimental outcome. However these results are encouraging showing once again that with an adequate selection, age is not a definitive limitation.
Disclosure of Interest: None Declared.

PH-P224
COMPARISON BETWEEN HLA ALLELE AND ANTIGEN MISMATCHED UNRELATED BONE MARROW TRANSPLANTATION IN 6183 JMDP RECIPIENTS

S. Kato[1,], Y. Atsuta[2], Y. Morishima[3], T. Kawase[4], K. Ohashi[5], T. Fukuda[6], K. Miyamura[7], T. Eto[8], C. Kato[9], K. Iwato[10], A. Sawada[11], Y. Kanda[12]*
[1]Cell transplantation, Tokai University, Isehara, [2]HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine, [3]Epidemiology and Prevention, [4]Aichi Cancer Center Research Institute, Nagoya, [5]Division of Hematology, Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital, [6]Division of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, [7]Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, [8]Hematology, Hamanomachi Hospital, Fukuoka, [9]Hematology, Meitetsu Hospital, Nagoya, [10]Hematology, Hiroshima Red Cross Hospital, Hiroshima, [11]Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, [12]Hematology, Saitama Medical Center Jichi University, Saitama, Japan

Introduction: HLA compatibility between the donor and the recipient in unrelated bone marrow transplantation is the key parameter for the clinical outcomes. Although both serological and allelic disparities have been reported to be associated with the post-transplant complications and survival, reports on direct comparison between antigen and allele mismatched UBMT are limited. We analyzed the JMDP registry data to compare the outcomes between antigen and allele mismatched UBMT.
Materials (or patients) and Methods: 6183 UBMT recipients transplanted as first HSCT for AML/ALL/CML/MDS between 1933 and 2011 in Japan were included in this analysis. UBMT before 1993 (n=62), or those reported with no GVHD prophylaxis (n=14) were excluded. 338 HLA-A one mismatched (317 allele-level, 21 antigen-level), 56 B 1 mm (50 allele, 6 antigen), 950 C 1 mm (91 allele, 859 antigen) and 1090 DR 1 mm (590 allele, 500 antigen) were compared with 3749 HLA 8/8 matched recipients. Furthermore, comparison between allele-level and antigen-level mismatched UBMT was made at each locus.
Primary endpoint is overall survival (OS), and other endpoints are relapse, non-relapse mortality (NRM), and aGVHD. Cox model for overall mortality, and Fine and Gray's model for other endpoints were employed. P values, less than 0.05 considered significant Variables considered for multivariate analyses: patient age at transplant, patient sex, donor-recipient sex mismatch, donor-recipient ABO major mismatch, diagnosis (AML/ALL/CML/MDS), disease status at transplant (standard vs. advanced), RIC/MAC, tacrolimus-based GVHD prophylaxis vs. cyclosporine-based GVHD prophylaxis, transplant year (-2000 vs. 2001-)
Results: 1. Overall mortality; Predictable variables for overall mortality were HLA-A allele 1 mm (P<0.001), B allele 1 mm (P=0.001), C antigen 1 mm (P<0.001), DRB1 allele 1 mm (P=0.048) and DR antigen 1 mm (P<0.001). No statistically significant differences were found between allele and antigen-level mismatches at any HLA-locus.
2. Relapse; Relapse rate was statistically decreased in HLA-C antigen 1 mm recipients. No statistically significant differences were found between allele and antigen-level mismatches at any HLA-locus.
3. TRM; Significantly worse variables for TRM were HLA-A allele 1 mm (P<0.001), B allele 1 mm (P=0.032), B antigen 1 mm (P=0.023), C antigen 1 mm (P<0.001) and DR antigen 1 mm (P<0.001). Marginally significant difference was found between allele and antigen 1 mm at DR locus (P=0.049).
4. aGVHD; Predictable variables for increased incidence in grade 2 to 4 aGVHD were A allele 1 mm (P<0.001), B allele 1 mm (P=0.001), C antigen 1 mm (P<0.001), DRB1 allele 1 mm (P-0.048) and DR antigen 1 mm (P<0.001). HLA-C antigen 1 mm was the only predictable variable for grade 3 to 4 aGVHD (P=0.019).
Discussion: Although HLA 1 mm UBMT at A, B, C, DR loci showed higher overall mortality, transplant related mortality and aGVHD than in HLA 8/8 matched UBMT, no significant differences were found in overall mortality between allele-level and antigen-level mismatches. Therefore, the effect of one HLA antigen disparity on transplant outcome can be considered not to be different from that of one HLA allele disparity as far as HLA-C and DR are concerned. Numbers of patients who received HLA-A or B antigen 1 mm UBMT are too small to draw any conclusion.
Disclosure of Interest: None Declared.

PH-P225
WHO IS THE BEST HAEMATOPOIETIC STEM CELL DONOR FOR A MALE PATIENT WITH ACUTE LEUKAEMIA?

O. Ringdén[1,], M. Labopin[2], M. Solders[1], D. Beelen[3], R. Arnold[4], G. Ehninger[5], N. Milpied[6], D. Niederwieser[7], R.-M. Hamladji[8], S. Kyrcz Krzemien[9], A. Ganser[10], G. Socié[11], M. Stelljes[12], L. Volin[13], C. Craddock[14], M. Mohty[2] and Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation*
[1]Laboratory Medicine, Therapeutic Immunology, KAROLINSKA INSTITUTET, Stockholm, Sweden, [2]Department of Hematology, Hopital Saint Antoine, Paris, France, [3]University Hospital Essen, Essen, [4]Charité University Medicine, Berlin, [5]Universitätsklinikum Dresden, Dresden, Germany, [6]CHU Bordeaux, Pessac, France, [7]University Hospital Leipzig, Leipzig, Germany, [8]Centre Pierre et Marie Curie, Alger, Algeria, [9]Silesian Medical Academy, Katowice, Poland, [10]Hannover Medical University, Hannover, Germany, [11]Hopital St. Louis, Paris, France, [12]University of Münster, Münster, Germany, [13]Helsinki University Central Hospital, Helsinki, Finland, [14]Centre for Clinical Hematology, Birmingham, United Kingdom

Introduction: Female donors for male recipients worsens outcome of allogeneic haematopoietic stem cell transplantation (HSCT). Unrelated donors are being used increasingly in HSCT. Using genomic typing, outcome following unrelated donor transplant is improved compared to serological typing and similar to that using HLA-identical sibling donors. We wanted to find out whether a male HLA-matched unrelated donor (MUD, 8/8, n=2,014) might be an alternative to a female HLA-identical sibling donor (n=2,656) for males with acute leukaemia.
Materials (or patients) and Methods: We compared male patients with acute leukaemia who received grafts from MUD (n=2,014) with male patients receiving HLA-identical sibling grafts from a female donor (n=2,656). Univariate and multivariate analysis were performed.
Results: The relative risk (RR) of acute GVHD of grades II–IV was increased in the MUD group with acute myeloid leukaemia (AML) (RR 1.47, P<0.001) and acute lymphoblastic leukaemia (ALL) (RR 1.76, P<0.001). There was no difference in incidence of chronic GVHD and non-relapse mortality between the two groups. Probability of relapse was lower in the MUD group than in the sibling group in ALL patients (HR 0.75, P=0.04) but not in AML patients (HR 0.89, P=0.17). Survival was not different between the groups. Leukaemia-free survival (LFS) was also similar in the sibling and MUD groups in patients with AML (HR 1.01, P=0.81) or ALL (HR 0.93, P=0.45). Factors significantly associated with reduced LFS included active disease, poor cytogenetics, age, year of HSCT,

reduced-intensity conditioning, and the use of anti-thymocyte globulin.
Discussion: The main problem in using a female donor for a male recipient in HSCT has been the increased risk of acute GVHD. This has contributed to worse survival. In the present study, we found that unrelated male donor transplants to male recipients resulted in significantly more acute GVHD of grades II-IV than when using a female sibling donor. This was seen despite the fact that MUD patients were more often treated with ATG, which has been shown to reduce the risk of acute GVHD in MUD patients to a similar extent to that seen in HLA-identical sibling transplants without ATG.
To conclude, male patients with acute leukaemia who received grafts from MUD donors had a higher risk of acute GVHD and the same survival and LFS as using grafts from female HLA-identical sibling donors. Therefore, a female sibling donor should be preferred.
Disclosure of Interest: None Declared.

PH-P226
COMPARABLE OUTCOME OF HAPLOIDENTICAL AND MATCHED SIBLING DONOR PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOLLOWING MYELOABLATIVE CONDITIONING FOR POOR RISK ACUTE MYELOID LEUKEMIA
S. RANI Jaiswal[1,2,], N. Khattry[3], S. Punatar[3], A. Gupta[3], B. Bagal[3], S. Chakrabarti[2,4]*
[1]Hemato-Oncology and BMT, Apollo Gleneagles Hospital, Kolkata, [2]hemato-Oncology and BMT, Dharamshila Hospital and Research Centre, New Delhi, [3]BMT, Actrec, Tata Memorial Center, Mumbai, [4]hemato-oncology and BMT, Manashi Chakrabarti Foundation, Kolkata, India

Introduction: Acute Myeloid Leukemia (AML) with poor risk cytogenetics or relapsed and refractory disease are unequivocal indications for allogeneic transplantation. Only 20% of such patients find a matched family donor and matched unrelated donors are not available to the majority of noncaucasian origin.
Materials (or patients) and Methods: In a multicentre study, we compared the outcome of Myeloablative PBSC transplantation from Haploidentical (HAPLO) (*n*=15) and Matched Sibling Donors (MSD) (*n*=30) for patients with poor risk AML. The MSD group received conditioning with Cyclo-TBI or Fludarabine based myeloablation with Cyclosporine and Methotrexate as GVHD prophylaxis. The HAPLO group received Fludarabine, based myeloablation with posttransplant Cyclophosphamide, Cyclosporine and MMF as GVHD prophylaxis.
Results: The groups were matched for age and gender; however, more patients in the HAPLO group were transplanted with active disease (11/14 vs 8/30, *P*=0.004). Both groups were matched in graft composition and had neutrophil engraftment at a median of 14 days, although platelet engraftment was faster in the MSD group. There was no difference in the incidence of aGVHD, but cGVHD was less in the HAPLO group (*P*=0.04). NRM was remarkably low in both groups (2/15 vs 2/30), with 0% mortality at day100. Relapse was the major cause of treatment failure in both groups (46% vs 40%). Of patients transplanted in remission, 0/4 relapsed in the HAPLO group compared to 8/22 in the MSD group. 4/11 and 2/6 patients with active disease in the HAPLO and MSD group achieved sustained CR. Two year disease free survival was 53% in HAPLO vs 55% in the MSD group. cGVHD was associated with lower relapse in the MSD group (*P*=0.07). In the HAPLO group, transplantation from a NK alloreactive donor was associated with significantly lower probability of relapse (15% vs 85%; *P*=0.05).
Discussion: In conclusion, Haploidentical HCT with myeloablative conditioning and posttransplant cyclophosphamide is associated with very low NRM and GVHD, with outcome as good as matched sibling donor transplants. In addition, Haplodentical HCT from a NK alloreactive donor might reduce the risk of relapse further.
Disclosure of Interest: None Declared.

PH-P227
HLA-DPB1 DISPARITY AS A RICK FACTOR FOR 10/10 HLA MATCHED-UNRELATED DONOR HSCT
E. Kuzmich[1,], O. Makarenko[1], A. Alyanskiy[1], I. Alekseikova[1], I. Fediukova[1], S. Bondarenko[1], N. Ivanova[1]*
[1]First Pavlov State Medical University of St. Petersburg, Raisa Gorbacheva Memorial Institute for Pediatric Oncology, Hematology and Transplantation, St-Petersburg, Russian Federation

Introduction: Hematopoietic stem cell transplantation (HSCT) with 10 of 10 allele matched unrelated donors is still associated with a significant risk of acute graft-versus-host disease (aGVHD). In order to estimate the HLA-DPB1 disparity as a risk factor of aGVHD for our cohort patients, we used algorithm for the classification of HLA-DPB1 mismatches based on T-Cell Epitope (TCE) groups (www.ebi.ac.uk/ipd/imgt/hla/dpb.html).
Materials (or patients) and Methods: 60 patients with hematologic malignancies underwent a first allogeneic transplantation from a 10/10 allele (HLA-A, -B, -C, -DRB1, -DQB1) matched unrelated donors from 2009 to 2012 were included in the study. HLA-DPB1 typing of the donor/recipient pairs was retrospectively performed on the four-digit resolution level by SBT, PCR-SSOP methods.
Statistical analysis was done by SAS Enterprise Guide v. 5.1. The effect of a TCE-permissive or TCE-non-permissive mismatches in host-versus-graft (HvG) or graft-versus-host (GvH) direction on the risk of aGVHD was evaluated in univariate and multivariate analyses (including other clinical variables) using a logistic regression model. The impact on overall survival was estimated in Cox regression analyses. Clinical variables included age of patient (continuous variable), type of disease, risk of disease, donor/recipient gender-matching, donor/recipient CMV status, stem cell source, conditioning regimen, GVHD prevention regimen.
Results: 85% pairs were mismatched for the HLA-DPB1 locus. We could determine immunogenicity based on the TCE algorithm for 45 HLA-DPB1 mismatched pairs. TCE- non-permissive mismatches in the HvG or GvH directions were determined for the 25 pairs. It was shown that TCE-non-permissive mismatches in the HvG direction had a trend for the increased risk of grades III-IV aGVHD compared TCE-permissive mismatches – OR 7.54 (CI 0.83-68.47, *P*=0.071) in multivariate model.
We selected the group of the patients underwent HSCT with using non-myeloablative conditioning regimens. All patients of this group received peripheral blood stem cells (PBSC) as a stem cell source. The influence by TCE- non-permissive mismatches in the HvG direction on the risk of severe aGVHD was more statistically significant in this group (OR 32.7, *P*=0.044).
TCE-non-permissive mismatches in the GvH direction were not associated with the increased risk of grades III-IV aGVHD. Patients with TCE-non-permissive mismatches in both directions (GvH and HvG) had a trend for decreasing overall survival compared the group with TCE-permissive mismatches (HR 2.09, *P*=0.18).
Discussion: TCE-non-permissive HLA-DPB1 mismatches in the HvG direction are potentially negative prognostic factor of the risk of severe aGVHD for our cohort patients undergoing unrelated allogeneic HSCT with using non-myeloablative conditional regimes and PBSC as a stem cell source. HLA-DPB1 disparity is an essential factor risk for 10/10 unrelated matched donor HSCT outcome.
Disclosure of Interest: None Declared.

PH-P228

IS THE BONE MARROW ASSESSMENT AN EVALUATION THAT OFFERS MORE SAFETY IN ALLOGENEIC STEM CELLS TRANSPLANTATION? RESULTS IN 725 HEALTHY RELATED DONORS

S. Gattillo[1,], S. Malato[1], P. Ronchi[1], E. Guggiari[2], M. Coppola[1], E. Sala[2], F. Lorentino[2], C. Corti[2], J. Peccatori[2], M. Bernardi[2], M. Marcatti[2], M. Ponzoni[3], R. Milani[4], L. Bellio[1], M. Lupo Stanghellini[2], F. Ciceri[2]*

[1]Immunohematology and Transfusion Service, [2]Hematology and Bone Marrow Transplant Unit, [3]Pathological Anatomy Service, [4]Flow Cytometry Service, IRCCS Ospedale San Raffaele, MILAN, Italy

Introduction: Use of Peripheral Blood Stem Cells (PBSC) in allogeneic transplantation is a practice become increasingly consolidated. This technique involves the need to employ a mobilization therapy with growth factor granulocyte (GSCF) in order to allow the achievement of adequate doses of CD34 + cells to ensure engraftment. PBSC collection is considered as a safe practice but are also known risks related with the use of GCSF. In our hospital there is a chance, for related donors, to undergo to morphological and cytogenetic assessment by performing a bone marrow aspirate prior the administration of GCSF and prior the issue of the judgment of suitability for donation.

Materials (or patients) and Methods: Between 1997 and June 2012 we evaluated 725 related donors for donation to relatives (395 males, 330 females, median age 43; 263 donors aged> 50 years). 556 donors underwent to diagnostic bone marrow aspirate.

Results: 514 of 556 donors who underwent bone marrow aspirate were judged suitable. Abnormalities were found in 85 of 556 donors. The anomalies are divided as follow: 77 morphological abnormalities, 4 isolated cytogenetic anomalies and 4 both cytogenetic and morphological abnormalities. In 43 of the 85 donors such anomalies were judged to be incompatible with the donation.

Discussion: Morphologic and cytogenetic evaluation is an assessment that completes the clinical information obtained from routine investigations and offers greater safety to candidates for donation. Marrow evaluation is integrated in the regular workup of candidates subjects; this not cause daley in the process of clearance declaration for putative donor. In addition, flow cytometric assessment performed on the bone marrow and recently introduced in our Institution may aid in identifying related donors who are not eligible to donate.

Disclosure of Interest: None Declared.

PH-P229

THE PYROSEQUENCING: A NEW PROMISING APPROACH TO PERFORM HLA TYPING AND SELECT THE STEM CELL DONOR IN A QUICKLY, SIMPLE AND ACCURATE WAY

S. Bernardi[1,], F. Cattina[1], S. Soverini[2], E. Ottaviani[3], G. Carella[4], A. Malagoli[4], M. Malagola[1], C. Skert[1], C. Filì[1], E. Alghisi[1], S. Perucca[1], A. Di Palma[1], C. De Benedittis[2], M. T. Bochicchio[3], C. Bergonzi[1], G. Martinelli[2], D. Russo[1]*

[1]Chair of Haematology, University of Brescia, Brescia, [2]University of Bologna, [3]Policlinico S. Orsola Malpighi, Bologna, [4]Spedali Civili di Brescia, Brescia, Italy

Introduction: The HLA genes are the most polymorphic in the human genome and are characterized by a large number of alleles and haplotypes. Nowadays a variety of methodologies are available for HLA typing both at the protein and at nucleic acid level, but ambiguity can affect the possibility of the right call for each HLA-locus. In particular, phase ambiguities arise from the incomplete genomic coverage or the contemporary Sanger sequencing of two heterozygous alleles that determines different haplotypes. The new generation sequencing (NGS) technologies have the potential to perform HLA-typing in a simply, rapid and accurate way without phase ambiguities. In this study we performed high-resolution HLA-typing using an NGS platform based on pyrosequencing and subsequent bioinformatic analysis and we evaluated his feasibility, reliability and robustness in 40 samples.

Materials (or patients) and Methods: Fourteen amplicons for sample were synthesized using two custom assay. The output file was then uploaded into JSI SeqPilot software to align all sequences with the reference database (ref 3.9 2012).

Results: The PCR reactions generate 560 amplicons who correspond to HLA-A/B/C exons 2, 3 and 4, DQB1 exons 2 and 3 and DPB1, DQA1, DRB1/3/4/5 exon 2. Using Multiplex Identifier (MID) tag method, we pooled amplicons from different samples to analyze them contemporary. We pooled our 40 samples into 8 pools (5 different samples in each one) and performed 8 sequencing runs. We have obtained over of 150 reads for most amplicons. The assignment of unambiguous genotype was possible on 45.5% of alleles. The ambiguities were related to the assay design (above all for class-II). Notably, some ambiguities on the locus B and C have a little biological importance because the genomic differences between the two alleles were located on the transmembrane domain coding region, so both alleles code for the same peptide binding domain, instead. Ten cases analyzed in this study were also genotyped using conventional strategies resulting in a concordance of 100%.

Discussion: A rapid and accurate HLA-typing method without phase ambiguities is necessary to obtain a quickly and deep HLA compatibility analysis between donor a recipient. Using a NGS platform we obtained a high-resolution HLA-typing for the most important loci of the samples without phase ambiguities. We discriminated very well the alleles that are responsible of differences on the peptide binding domain in three days, so clonal amplification and pyrosequencing seems a feasible and promising approach.

This work was supported by Lions Club Bassa Bresciana, BCC Pompiano e Franciacorta, Roche Diagnostics, 60% Prof. Domenico Russo, 60% Dr. Michele Malagola.

Disclosure of Interest: None Declared.

PH-P230

STRATEGY, POLICY AND ALGORITHM OF ADULT PATIENTS WITH HIGH RISK HAEMATOLOGICAL MALIGNANCIES CANDIDATED TO AN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT: AN ANALYSIS FROM THE ROME TRANSPLANT NETWORK

A. Picardi[1,], L. Cudillo[1], R. Cerretti[1], G. De Angelis[1], P. De Fabritiis[2], T. Dentamaro[2], G. Avvisati[3], M. C. Tirindelli[3], A. Mengarelli[4], F. Marchesi[4], E. Montefusco[5], A. Ferrari[5], A. Chierichini[6], B. Anaclerico[6], M. Andreani[7], M. Testi[7], I. Mangione[1], F. Di Piazza[1], W. Arcese[1] on behalf of Rome Transplant Network (RTN)*

[1]Stem Cell Transplant Unit, Tor Vergata University of Rome, [2]Hematology, S.Eugenio Hospital, [3]Hematology, Campus Biomedicine University, [4]Hematology, Regina Elena Institute, [5]Hematology, S.Andrea Hospital, [6]Hematology, S.Giovanni Hospital, [7]HLA Laboratory, Tor Vergata Policlinic, Rome, Italy

Introduction: Pts with high-risk hematological malignancy eligible for HSCT should proceed timely to transplant. Several retrospective studies comparing transplants from volunteer unrelated (VUD), cord blood (CB) and haploidentical related donor (HRD) have shown no substantial differences in terms of outcome. On these basis and changing the concept of "donor versus no donor" with that of "transplant versus no transplant", the aim of RTN was the identification of the most suitable donor in order to perform HSCT in adequate timing.

Materials (or patients) and Methods: The HLA typing was extended to all available first familiar components; to drive the subsequent search strategy, a preliminary search was always performed. Selection criteria for VUD consisted of ≥8/10 HLA matching and single CBU was selected according to HLA and cell dose. HRD was contemporary considered. RTN policy considered a unique conditioning regimen regardless of the underlying disease and graft. GVHD prophylaxis depended on the type of HSCT.

Results: Over 6 years, 637 pts have been candidated to an HSCT. Id-Sib donor was identified in 194 (30%) but only 157/194 (81%) and 336/443 (76%) pts lacking an Id-Sib were considered eligible. By excluding ongoing searches (*n*=28) and early deaths (*n*=12),

296/336 (88%) pts were evaluable for donor identification. Of these 296 pts, an alternative donor was identified for 271 (92%). By adding the 157 pts with an Id-Sib, donor was identified for 428/493 (93%). After donor identification, 51 (19%) pts did not reach HSCT and 17 (6%) are in standby. Overall 360/428 (84%) pts underwent HSCT: Id-Sib=157, VUD=83, CB=31, Haplo=89. Id-Sib recipients underwent HSCT significantly earlier than the others, but there was no significant difference in the time between VUD, CBU and Haplo. The median age was 46 yrs (16-70) and the probability of OS was 40±3% at 7-yrs (median follow-up: 2.5 yrs). Most of pts had acute myeloid leukemia (AML, *n*=145) with a 7-yrs OS of 44±5% without differences between Id-Sib (*n*=62; 45±7%) and alternative (*n*=83; 44±7%) HSCT. In particular, for AML pts transplanted in early (1st/ 2nd CR) phase, 7-yrs OS was 55±8% and 57±7% (*P*=ns) for recipients of Id-Sib or alternative donor. By excluding the low number of CB-HSCT (*n*=10) in early phase, the probability of 7-yrs OS was 67±11%, 61and 55±8% (*P*=ns) for VUD, Haplo and Id-Sib.
Discussion: Our prospective study shows that the strategy and transplant policy of RTN based on a widespread and prompt donor search enable to transplant 84% of unselected, eligible pts. The long-term OS of AML pts transplanted in any disease phase from VUD or Haplo donor is not significantly different from pts transplanted from an Id-Sib.
Disclosure of Interest: None Declared.

PH-P231
MARROW HARVESTING FROM INFANT DONORS IN COMPARISON OF YOUNG DONORS IN TERM OF THE GRAFT PRODUCT

K. Paciaroni[1,], C. Alfieri[1], G. DeAngelis[1], C. Gallucci[1], M. Ribersani[1], M. Marziali[1], A. Isgrò[1], D. Armiento[1], L. Cardarelli[1], J. gaziev[1], P. Sodani[1], G. Lucarelli[1]*
[1]International Center for Transplantation in Thalassemia and Sickle Cell Anemia, Mediterranean, Rome, Italy

Introduction: Data regarding the marrow harvesting from infant donor are lacking. To compare the graft products obtained by infants with which obtained by young matched HLA-identical sibling donors for thalassemic and sickle cell recipients and to evaluate the feasibility to collect an adequate number of progenitor cell when a large weight discrepancy is present in the pair donor/recipient.
Materials (or patients) and Methods: From March 2004 to November 2012, 189 sibling donor donated bone marrow (BM): 27 healthy infant donors (<36 months of age: 7-36 months, median 20 months) and 162 young donors (3-51 years, median 15,37 years). Graft composing was assessed by the determination of NC, CD34 and CD3 cell by local standard immunophenotyping and compared between the two study groups. Other main variables were analyzed: donor weight, patient weight and the pair ratio (donor weight/patient weight) in order to quantified large discrepancy between donors and recipients weights (ratio <1) and consequently to evaluate the risk to no collect the minimum nucleated cell (NC) yield 2x108/kg required.
Results: The weight of infant donors was significantly less then the young donors and consequently the bone marrow volumes harvest were significantly smaller (see the table). In the infant group the median ratio was significantly lower then the value observed in the young donor group (0,63 vs 1,72, *P*<=0.001), it means that the infant donor weight was significantly less than the recipient; indeed the median weight recipient is rather two-fold greater than the respective infant donors unlike what observed in the young group (see the table). Despite of thus, the median counts per kg recipient weight of NC, CD34 cells and CD3 cells were not different in the infant bone marrow product compared to that of young donors (see the table). It is interesting to note that the median of the CD34 count calculated per kg of the donor weight is significant increased in comparison with the young donor's one (CD34 17.168x10^6/Kg vs 4.75x10^6/Kg (*P*=0.000)). No major adverse events or life-threatening complications occurred in the infant donors during the donation process . Nevertheless allogeneic red cells blood transfusions were more commonly used in the infants donors (48.1% vs 9.5%, *P*<0.001) in the presence of similar post donation haemoglobin levels (Hb 9.8 gr/dl vs 9.9 gr/dl, *P*=0.92).
Discussion: The present study supports the feasibility to collect an adequate number of progenitors cell from infant donors despite their low weights and the elevated CD34 count in the infant graft encourage the eligibility of the infant as donors. Higher rate of transfusion has been observed in the absence of more severe anaemia suggesting that a more conservative approach may be implemented.
Disclosure of Interest: None Declared.

PH-P232
SAFETY OF HEMATOPOIETIC STEM CELL DONATION FOR VOLUNTEER DONORS: THE EXPERIENCE OF THE SECOND OPINION SIMTI COMMITEE OF ITALIAN BONE MARROW DONOR REGISTRY (IBMDR)

A. Vassanelli[1,], N. Sacchi[2]*
[1]Azienda Ospedaliera Universitaria Integrata Verona, Transfusion Medicine Department, Verona, [2]Italian Bone Marrow Donor Registry, Ospedale Galliera, Genova, Italy

Introduction: The Italian Bone Marrow Donor Registry was established in Italy in 1989, with the role of providing an unrelated volunteer with immunogenetic characteristics to haematological patients waiting for a transplant and who do not have an identical sibling. The minimum eligibility criteria for a prospective donor must fit the Italian law for transfusion activity. The Donor Center (DC) is responsible for protecting donor safety: at every meeting with the donor, a medical evaluation of the volunteer donor is performed, to verify donor eligibility and to evaluate his suitability. In case of donors partially compliant with eligibility criteria, a "second opinion" SIMTI Committee, designated by the pertinent scientific society, helps DC to establish the suitability of the donor, with the aims to protect donor welfare and safety. The SIMTI Committee evaluates not only the suitability of donor partially compliant with eligibility criteria, but also the donors follow-up and management of HSC collection and minimum

[PH-P231]

Table: Donor characteristic and graft product

	INFANT DONORS: **27**	YOUNG DONORS: **162**	P
Age: median (range)	1,86 (0.7-2.9)	15.37 (3-51)	<0.001
Weight (Kg) Median(range)	11.93 (7-19)	42.13 (11.6-106)	<0.001
Weight Recipient(Kg) median (range)	20.81 (10-43)	27.6 (9.5-73)	0.05
Pair Ratio: median (range)	0.63 (0.23-1.1)	1.76 (0.5-5)	<0.001
Bone Marrow Volume (mL)	276.54 (180-450)	627,35 (230-1500)	<0.001
GRAFT PRODUCT			
NC (x 10^8/ Kg recipient)	3.31 (1.4-6.82)	4.23 (1.3-10.8)	<0.001
CD34 (x 10^6/ Kg recipient)	9.38 (1.76-34.7)	6.89 (0.78-20.8)	0.04
CD3 (x 10^6/ Kg recipient)	53.41 (3.82-102.2)	68.65 (1.1-208.2)	0.056

quality requirements. All second HSC donations and further lymphocyte donation requests are submitted to the SIMTI Committee.
Materials (or patients) and Methods: We evaluated all the requests of "second opinion" to SIMTI Committee, analyzing the experience of Italian Registry and the impact of the number of donations and occurrence of donor adverse events.
Results: From 1989 to 2012, Italian donors donated 2844 HSC products (both bone marrow and peripheral blood stem cells). The SIMTI Committee received 147 requests of "second opinion": 54/147 for a second/further HCS donation, 29/147 for a unstimulated lymphocyte donation, 36/147 for donor suitability, 14/147 for HSC product management, 14/147 for donor blood samples management.
On 85% of the requests, the SIMTI Committee answered with a positive evaluation.
Discussion: The incidence of unsuitability of Italian donors decreased during the last 5 years (not eligible and not suitability donors: 958 on 2006 vs 509 on 2012), despite of the increase of second opinion requests, with an incidence of adverse event after HSC donation very low 1,7/1000, suggesting that a good selection of donors, protecting the safety of the donor, increases the safety of the transplant process. On 2013 the second opinion committee started the evaluation on related donors too, with the aim to protect safety on HSC donation both for related and unrelated donors.
Disclosure of Interest: None Declared.

Stem Cell Mobilisation and Graft Engineering

PH-P233
BIOSIMILAR G-CSF (FILGRASTIM) IS (COST)EFFECTIVE FOR PERIPHERAL BLOOD STEM-CELL MOBILIZATION BEFORE AUTOLOGOUS TRANSPLANTATION-A SINGLE CENTRE EXPERIENCE

M. D Michael[1,], C. Stylianou[2], I. Konstantelos[1], T. Tsitskari[3], L. Elena[1], M. Antoniades[1], F. Melanthiou[1], M. Braimi[1]*
[1]Haematology, [2]Stem Cell Laboratory, [3]Nicosia General Hospital, Nicosia, Cyprus

Introduction: Biosimilars are similar but non-identical, versions of existing biological drugs for which patents have expired. Biosimilars of filgrastim, based on the originator product Neupogen®, have been available in Europe since 2008, but concerns have been expressed about the efficacy and safety of such products. In our Centre, we have been using biosimilar figrastim (Zarzio®, Sandoz Biopharmaceuticals), since 2010 and we set out our 3-year 'real-world' clinical practice experience in patients with haematological malignancies, mobilized for autologous stem cell collection and compared the safety, efficacy and- to our knowledge for the first time- cost effectiveness with a historical control mobilized with originator.
Materials (or patients) and Methods: A total of 141 patients were included in this study, 80 of those were treated with Zarzio®, during the period October 2010 to October 2013 and 61 were mobilized with originator. The former group was composed of 42 male patients and 38 females, with a median age of 55 years (15-68 years). Patients suffered from multiple myeloma (51), Hodgkin's Lymphoma (26), Non-Hodgkin's Lymphoma (54) or other diagnosis (8) and median lines of prior treatment received were 2 (1-4) for both groups. After administration of mobilizing regimens (primarily ESHAP and high dose Cyclophosphamide) patients were administered standard daily 10 μg/kg dose of Zarzio® (*n*=80) or Granocyte® (*n*=61).
Results: Median duration of G-CSF administration was 6 days for both Zarzio® (4-13) and Granocyte® (4-14). Both groups had a median of one apheresis and the group mobilized with Zarzio® achieved the goal of more than $2x10^6$/kg CD34+ cells per body weight in 57 patients with one session and in 17 with two sessions (one of them co-administered plerixafor) of apheresis, while 6 patients failed to mobilize (1 co-administered plerixafor). In the originator group 44 achieved the goal with one session of apheresis, 9 with two, while 8 failed (1 co-administered plerixafor). Median patient body weight was 73kg (45-143kg) in the biosimilar group and 72kg (48-137 kg) in the originator group. Median WBC count at collection day was 14610/μl (2100-95490/μl) and 9880/μl (1290-75290) and CD34+ cells/Kg body weight collected per apheresis day were 3.6 [0-47] and 3.4 [0.1-45] for the biosimilar and originator groups respectively. Adverse events included bone pain, headache and/or neutropenic fever and was observed in 17 and 15 patients in both groups respectively. According to local pharmaceutical services purchase values, financial benefit was estimated at 16400 euros, during the recent, three years in favor of the biosimilar agent, i.e 5467 euros saving per year.
Discussion: Zarzio® demonstrated similar efficacy and safety profile compared to the originator in this 'real-world' non-selected patient cohort confirming previous reported trials. What is more, for the first time we depicted the remarkably better profile in terms of cost effectiveness. Further studies are required to assess the latter finding.
Disclosure of Interest: None Declared.

PH-P234
SPECTRA OPTIA VERSUS COBE SPECTRA APHERESIS DEVICES: HOW DOES THE NEW TECHNOLOGY SUIT BETTER PBSC COLLECTION?

R. Eshel[1,], A. Pinchasov[1], M. Shpringer[1], G. Senyor[1], B. Tartakovsky[1], E. Naparstek[1]*
[1]Bone Marrow Transplantation, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel

Introduction: Peripheral blood stem cell collection (PBSC) by apheresis has become the preferred source of stem cell grafts in hematopoietic stem cell (HSC) transplantation setting. The apheresis process involves establishment of an extracorporeal circulation, separation of whole blood into its components, and selective collection of stem cell-rich fraction for use as a stem cell graft.
A novel apheresis system, the Spectra Optia- a modern electronic automated device- has been recently introduced into clinical practice in most transplant centers to overcome some shortcoming such as the high amount of irrelevant white blood cells (WBC) in the final product and the high variability in CD34 collection efficiency (CE2 -calculated by $CD34^+$/ml product x Poduct Volume)/ $CD34^+$/ml pre x Total Processed Blood Volume).
Materials (or patients) and Methods: We have recently introduced a new Optia device into our collection facility and consequently the aim of this study was to assess and compare the quality of the apheresis process and the stem cell product in HSC donors subjected to the procedure by Optia and Cobe Spectra devices. We have tested 31 Optia and 49 COBE Spectra procedures or PBSC collection.
Results: The following results were obtained:
- Significantly higher volume of product with the Optia, median 298 ml (108-440) vs. 180 ml (135-280) with the Spectra.
- Significantly lower absolute number of WBC with the Optia, median 551 x 10^8 (167-1032) vs. 841 x10^8 (216-2141) with the Spectra.
- CD34 collection efficiency (CE2): median CE2 is similar in both instruments (45.3% by the Optia vs. 47.6% by Spectra), and average CE2 is lower with the Optia (45.9% +/– 13.3 by Optia vs. 59.4% +/– 30.5 by the Spectra).
- In both instruments a slight decrease in CE2 was noted as a function of increasing CD34 cells pre collection, although this decrease was less pronounced with the Optia instrument.
- The process collection with the Optia requires about one hour more.

Discussion:
1. Both Spectra and Optia devices satisfied CD34 yield efficiently although a greater variability in collection (CE2) was observed with the COBE Spectra (median vs. average CE2).

2. High product volume using the Optia is significant and is of concern in respect to the number of frozen bags to be stored and later on transplanted to patients particularly due to DMSO exposure.

3. The lower total WBC in the product is clearly an advantage of the Optia.

4. The longer time needed with the Optia instrument is inconvenient for both the donors and the operating staff.

The results presented here should be taken with some precautions since the operator experience and performance with the Optia are considerably less than with the COBE Spectra in our institution.

At this point in time, both devices were found suitable for PBSC collections Yet, the high product volume obtained using the Optia, should be resolved possibly by reducing plasma flush volume.

Disclosure of Interest: None Declared.

PH-P235

CHEMOMOBILIZATION WITH HIGH-DOSE ETOPOSIDE AND G-CSF RESULTS IN EFFECTIVE AND SAFE STEM CELL COLLECTION IN HEAVILY PRETREATED LYMPHOMA PATIENTS

H. A. Ozkan[1,], Z. Gulbas[1]*

[1]Anadolu Medical Center Hospital, Istanbul, Turkey

Introduction: The optimal mobilization strategy prior to autologous stem cell transplantation (auto-SCT) for patients with lymphoma is yet to be determined.

Materials (or patients) and Methods: We reviewed our institutional experience using chemomobilization with high-dose (HD) etoposide (1.6 gr/m^2) and G-CSF (300 µg/day) in 79 patients with lymphoma. The majority (76%) had received at least two prior regimens of chemotherapy and 12 (15.2%) patients had previously failed to mobilize following HD cyclophosphamide or DHAP or ICE with G-CSF.

Results: HD etoposide and G-CSF chemomobilization resulted in successful collection (>2 x 10^6 CD34+ cells/kg) in 82.3% of patients within a median 2 (1-6) apheresis days. Patients had stem cells collected between days +8 and +15, with a median +12 day. Median total CD34+ cells/kg collected was 5.95 x 10^6 (0.1-36.8). Seventy - one percent of patients yielded > 2 x 10^6 CD34+ cells/kg in ≤ 2 days of apheresis, and were defined as good mobilizers. While median CD34+ cells/kg collected for good mobilizers was 7.6 x 10^6, it was 3.9 x 10^6 for poor mobilizers (P<0.001). This regimen was safe with a low rate of febrile neutropenia (7.6%) and acceptable rates of RBC (40.5%) and platelet transfusions (22.8%). Hematopoietic recovery after auto-SCT was achieved on expected time. Therapy - related myelodysplastic syndrome/acute myeloid leukemia occurred in only one patient (1.3%) with in a median follow-up of 16 months after chemomobilization. Patient's characteristics, efficacy and safety of the chemomobilization are summarized in Table 1-4.

Discussion: In conclusion, HD etoposide (1.6 gr/m^2) and G-CSF (300 µg) chemomobilization in the majority of heavily pretreated lymphoma patients appears to result in effective, tolerable and safe stem cell collection. This regimen seems to overcome the effects of most of the prior high-risk features frequently associated with poor mobilization. Large, prospective, randomized studies are needed to analyze whether 'on-demand' use of plerixafor in chemomobilized lymphoma patients with etoposide decreases mobilization failure rate without increase in cost.

Disclosure of Interest: None Declared.

PH-P236

CRYOPRESERVATIVE VERSUS NON CRYOPRESERVATIVE IN AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (EGYPTIAN EXPERIENCE)

M. Moussa[1,], M. Tawfik Elzimaity[1], M. Hamdy Attia[1], H. Mohamed Elsayed[1], N. Mohamed Shalaby[2], A. Ahmed Mohamed[2]*

[1]Internal Medicine and Hematology, [2]Clinical Pathology, Ain Shams University, Cairo, Egypt

Introduction: Autologous peripheral blood stem cell transplantation is a 'rescue' of patient's self hematopoietic stem cells (HSC) from myeloablative effects of chemotherapy or irradiation. These HPSC are either cryopreserved or liquid preserved and reinfused to repopulate the patient's marrow after myeloablation.

Cryopreservation of HSC using 10% dimethyl sulphoxide (DMSO) as a cryoprotectant under liquid nitrogen storage conditions (-196°C) for long-term usage is a well-established procedure while liquid preservation of stem cells is usually done for storage at 4°C for 36 to 96 hours**.**

Aim of the study was to compare the outcome of autologous stem cell transplantation using cryopreserved or non-cryopreserved stem cells.

Materials (or patients) and Methods: Twenty adult patients younger than 60 years were enrolled in this study. They had undergone autologous peripheral stem cell transplantation. They had been stratified into 2 groups: Ten patients received cryopreserved autologous PBSCT and the remaining ten patients received non cryopreserved autologous PBSCT. With a follow up period of 6 months to 55 months.

Results: No significant difference was detected in engraftment of neutrophils and platelets between both treatments groups. When we compared cryopreserved and non cryopreserved groups for clinical outcome, there had been a longer overall survival (OS) and disease free survival (DFS) in favor of the cryopreserved group but this had not been translated into statistically significant values. In addition, there was no significant difference as regard recurrence of disease. However, non cryopreserved group had shorter hospital stay and was less costly than cryopreserved, 8,149 $ versus 8,900 $ respectively. This could be attributed to the fact that liquid nitrogen was not used, shorter hospital stay (P value 0.000) thus lower cost for medications, laboratory tests and procedures; however this was not translated into a statistically significant value. However, cryopreserved was found to be cost-effective as regards keeping the patients alive for those who cannot tolerate to proceed to autologous stem cell transplant following mobilization immediately.

Discussion: Non cryopreserved therapy required less number of aphaeresis sessions, is associated with shorter hospital stay and is less costy than cryopreserved therapy and give us the possibility of transplantation of patients with hepatitis without the need for buying a special liquid nitrogen tank.

Disclosure of Interest: None Declared.

PH-P237

NON INTERVENTIONAL PROSPECTIVE CLINICAL STUDY ON PERIPHERAL BLOOD STEM CELL MOBILIZATION IN PATIENTS WITH RELAPSED LYMPHOMAS

G. van Gorkom[1,], H. Finel[2], J. Luan[2], S. Giebel[3], D. Pohlreich[4], A. Shimoni[5], M. Ringhoffer[6], G. Sucak[7], M. Schaap[8], P. Dreger[9], A. Sureda[10], H. Schouten[1] and on behalf of the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation (EBMT)*

[1]Maastricht University Medical Center, Maastricht, Netherlands, [2]EBMT Lymphoma Working Party, Paris, France, [3]Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Gliwice, Poland, [4]Charles University Hospital, Prague, Czech Republic, [5]Chaim Sheba Medical Center, Tel HaShomer, Israel, [6]Municipal Hospital Karlsruhe, Karlsruhe, Germany, [7]Gazi University, Ankara, Turkey, [8]Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [9]Heidelberg University Hospital, Heidelberg, Germany, [10]Addenbrooke's Hospital, Cambridge, United Kingdom

Introduction: Autologous stem cell transplantation (ASCT) is the standard of care for many patients with relapsed chemosensitive

lymphoma. Peripheral blood stem cells have become the main source for the ASCT worldwide, because of its advantages over bone marrow. Several risk factors have been identified for poor stem cell mobilization, and diagnosis of lymphoma is one of the most important ones, with an inadequate stem cell harvest in 4 to 25% of the cases. Even though stem cell mobilization in relapsed lymphoma patients can be relatively difficult, mobilization strategies have not been standardized and there is a significant variation amongst centers. The aim of this study was to review the mobilization strategies used by EBMT centers in relapsed lymphoma and to evaluate the failure rates.
Materials (or patients) and Methods: EBMT centers were invited to participate in this non-interventional prospective clinical study that was started in 2010. Centers were requested to collect data on all consecutive patients with relapsed lymphomas considered to be candidates for an ASCT. Data collected included age, sex, diagnosis, number of prior chemotherapy regimens, mobilization regimen, collected CD34+ cells, marrow harvests.
Results: In total, 217 patients with relapsed lymphomas from 30 EBMT centers were included in this study; 135 patients (62%) with non-Hodgkin's lymphoma (NHL) and 82 patients (38%) with Hodgkin's lymphoma (HL). There were 124 males and 93 females with a median age of 54 (range 19 – 77) years. Median number of chemotherapy lines received before this relapse was one (range 1–8). Twohundred-and-seven patients (95%) were mobilized with chemotherapy and cytokines, being DHAP (36%) and ESHAP (13%) the most frequent protocols at first mobilization, and 11 patients (5%) were mobilized with cytokines alone. All patients used G-CSF at the first mobilization. Ten patients (5%) were at first mobilized with G-CSF, but switched to plerixafor (PLX) during the first mobilization. These were all patients that were mobilized with chemotherapy as well. Twenty-seven patients (12.4%) failed to mobilize adequate stem cells (<2 x 10^6 CD34+ cells/kg) during first mobilization. Four of those patients received PLX. The median number of stem cells collected at first mobilization was 5 x 10^6 CD34+ cells/kg (range: 0 – 82). In 198 patients (91%) only one mobilization course was given, 17 patients (8%) had two mobilization courses, 2 patients (1%) underwent three courses. Three patients had a mobilization failure after only G-CSF; they all were successful in a second attempt after chemotherapy. Five of the patients failing the first mobilization with chemotherapy received PLX at second mobilization, but only three of them were successful. One patient failed both first and second mobilization and received PLX at third mobilization without success. Nineteen patients (8.7%) still had an inadequate amount of stem cells after those mobilizations. Of those, only 4 patients (2%) underwent bone marrow harvest.
Discussion: Mobilization strategies for patients with relapsed lymphoma are very diverse but more than 90% of them are mobilized using the combination of salvage chemotherapy plus G-CSF. PLX was used in less than 10% of the mobilization procedures during the time period analyzed. In our experience, the failure rate was 12.4% after the first mobilization attempt and 8.7% after several attempts.
Disclosure of Interest: None Declared.

PH-P238

LENOGRASTIM (MYELOSTIM®) VS BIOSIMILAR FILGRASTIM (ZARZIO®) FOR AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS MOBILIZATION IN ADULT PATIENTS WITH HEMATOLOGIC MALIGNANCIES: A SINGLE INSTITUTION EXPERIENCE

F. Marchesi[1,], S. Gumenyuk[1], M. Vacca[2], A. Pandolfi[2], M. L. Dessanti[1], F. Palombi[1], F. Pisani[1], A. Romano[1], A. Spadea[1], A. Mengarelli[1]*
[1]Hematology and Stem Cell Transplantation, Regina Elena National Cancer Institute, [2]Leukapheresis and Cellular Therapy, S. Camillo-Forlanini Hospital, Rome, Italy

Introduction: Biosimilar Granulocytre Colony-Stimulating Factor (G-CSF) has been approved on the basis of comparable quality, safety and efficacy as the originator product. So far, biosimilar G-CSF Zarzio® has been approved also for autologous peripheral blood stem cell (PBSC) mobilization and for prophylaxis of sever neutropenia duration following conditioning chemotherapy and stem cell infusion. However, there is still general skepticism about safety and efficacy of Zarzio® in these setting of patients.
Materials (or patients) and Methods: From March to November 2013, 22 consecutive adult patients with hematologic malignancies (acute leukemia *n*=5, lymphoma *n*=13 and multiple myeloma *n*=4) underwent autologous PBSC mobilization after administration of chemotherapy associated to biosimilar Filgrastim (Zarzio®) in our Institution. Zarzio® was administered according to the study protocol in which patients were enrolled for acute leukemia (5 mcg/Kg/day in 2 patients and 10 mcg/Kg/day in 3 patients), at dosage of 10 mcg/Kg/day for multiple myeloma and 5 mcg/Kg/day for lymphoma. The target of CD34+ cell dose was 4 x 10^6/Kg recipient body weight. This cohort of 22 patients was retrospectively compared with 53 consecutive patients (acute leukemia *n*=2, lymphoma *n*=28 and multiple myeloma *n*=23) who underwent autologous PBSC mobilization after administration of chemotherapy associated to Lenograstim (Myelostim®) at the same dosage from March 2011 to February 2013.
Results: The two groups of patients were similar as baseline clinical features, including sex (*P*=1), age and body weight at leukapheresis (*P*=0.124 and 0.357, respectively), bone marrow involvement and disease status at leukapheresis (*P*=0.451 and 0.501 respectively), previous radiotherapy (*P*=0.551), previous chemotherapy lines (*P*=0.977) and mobilization regimes of chemotherapy received (*P*=0.198), with the only exception for diagnosis distribution (high rate of acute leukemia in the Zarzio® group; *P*=0.014). As for PBSC collection data, median days of G-CSF administration, median CD34+/mcL number at leukapheresis and median number of CD34+ x 10^6/Kg collected at first leukapheresis were similar between two groups of patients. However, in group of patients who received Zarzio®, we observed an higher rate of mobilization failures (22.7% compared to 3.8% of Myelostim® group; *P*=0.02), an higher rate of patients unable to reach the target of CD34+ cell planned dose (40.9% vs 7.5%; *P*=0.001) and an higher (but not statistically significant) rate of patients needing Plerixafor administration (13.6% vs 1.9%; *P*=0.076). We not observed any adverse effect directly related to Zarzio® administration.
Discussion: Despite the limitation due to the low number of patients, our data suggest that Zarzio® could be less effective when compared with Myelostim® for PBSC mobilization after chemotherapy in adult patients with hematologic malignancies and more expensive considering the high rate of patients needing Plerixafor administration. Further studies on a larger number of patients are warrant to better evaluate the role of Zarzio® in this setting.
Disclosure of Interest: None Declared.

PH-P239

EFFECTS OF A NEW DOSING SCHEME OF ATG-F OF DONOR NK CELLS AND IMMUNE RECOVERY IN HAPLOIDENTICAL T AND B CELL DEPLETED STEM CELL TRANSPLANTATION

P. Lang[1,], T. Feuchtinger[1], R. Meisel[2], F. Schuster[2], C. Kyzirakos[1], P. Schlegel[1], C. Klose[1], H.-M. Teltschik[1], B. Gruhn[3], W. Schwinger[4], C. Urban[4], H. Martinius[5], U. Pflueckhahn[1], R. Teltschik[1], M. Schumm[1], R. Handgretinger[1]*
[1]Hematology/Oncology, University Children's Hospital, Tuebingen, [2]Hematology/Oncology, University Children's Hospital, Duesseldorf, [3]Hematology/Oncology, University Chidren's Hospital, Jena, Germany, [4]Hematology/Oncology, University Children's Hospital, Graz, Austria, [5]Scientific Development, Fresenius Biotech GmbH, Planegg Martinsried, Germany

Introduction: T and B cell depletion of haploidentical peripheral stem cells with CD3/CD19 coated magnetic microbeads effectively prevents from GvHD and allows to coinfuse large numbers of donor NK cells and other accessory cells Additional *in vivo* depletion of the graft with serotherapy is not mandatory. Thus, a dosing scheme of ATG is needed, which provides a rejection prophylaxis by depleting lymphocyte subsets of the patients but which does not harm the stem cell graft. We present data with reduced ATG

doses given at the beginning of the conditioning regimen in order not to impair cotransfused NK cells and immune recovery.
Materials (or patients) and Methods: A total of 33 pediatric patients received 3x10 mg/kg ATG-F (Fresenius) starting at day -12, followed by fludarabine (160 mg/m^2, d -8 to -5), thiotepa (10 mg/m^2 d -4) and melphalan (140 mg/m^2 d -3 to -2). Most patients received MMF untill day 30-60. ATG serum levels were measured in 19 patients by flow cytometry (amount of ATG binding to the Jurkat cell line, defined as T cell specific rabbit IgG). Apoptosis/necrosis of donor NK cells was assessed with Annexin V/PI staining and flow cytometry.
Results: Median time to ANC>500 and to independence from platelet transfusion was 9.5 and 13.5 days. Graft rejection occurred in 7/33 patients (21%). All rejectors could be rescued with reconditioning and 2nd stem cell donation or with infusion of autologous back ups. Acute GvHD grade I and II-IV was observed in 12/26 (46%) and 2/26 (8%) patients without rejection, respectively. Limited (extensive) chronic GvHD occurred in 5/24 (1/24) evaluable patients. Median peak levels of 22.1 µg/ml specific rabbit IgG were reached between day -8 and -6 and dropped to 2.9 µg/ml at day 0.
In vitro incubation of NK cells from healthy donors with a comparable dosage of ATG-F (1 µg/ml) resulted in 26 % apoptosis and 0.2 % necrosis (70% vital cells) after 24 hours. Moreover, NK cells were incubated with patient´s serum, taken after ATG treatment and adjusted to a concentration of 1 or 2 µg/ml. Cell death occurred in 20% of NK cells each. Functional activity was measured against K562 targets. Specific lysis of decreased from 83% to 71% (corresponding to a loss of 15% activity) after incubation with 2µg/ml and to 11% (87% loss of activity) with 1000µg/ml.
Immune recovery was monitored and compared with a historical group receiving OKT3 and the same chemotherapy (*n*=34). Recovery of CD56+ NK cells was fast with a mean number of 414 vs. 232 cells/µl (ATG group vs. OKT3 group, *P*<0.01) at day +14, 252 vs. 342 cells/µl at day +30 (*P*=0.1) and 189 vs. 217 cells/µl at day +90 (*P*=0.3). CD3+ T cells reached 36 vs. 12/µl at day +30 and 189 vs. 225/µl at day +90 (no significant differences for all data pairs).
Discussion: Conclusions: ATG-F was started at an early time point, resulting in low serum levels of specific ATG at day 0 and in a fast NK cell recovery. In vitro results suggested, that the majority of NK cells will not be damaged herewith. Immune recovery of T and NK cells was comparable to that of a historical control group who received OKT3. However. The rejection rate was higher than expected and an increase of the ATG dose has to be considered. This approach will be also of interest for other transplantation strategies in which various components of the grafts and additionally given T cells have to be preserved.
Disclosure of Interest: P. Lang: None Declared, T. Feuchtinger: None Declared, R. Meisel: None Declared, F. Schuster: None Declared, C. Kyzirakos: None Declared, P. Schlegel: None Declared, C. Klose: None Declared, H.-M. Teltschik: None Declared, B. Gruhn: None Declared, W. Schwinger: None Declared, C. Urban: None Declared, H. Martinius Conflict with: Head of Laboratory Fresenius Biotech, U. Pflueckhahn: None Declared, R. Teltschik: None Declared, M. Schumm: None Declared, R. Handgretinger: None Declared.

PH-P240

BIOSIMILAR COMPARED WITH ORIGINATOR FILGRASTIM FOR RELATED-DONOR ALLOGENEIC STEM CELL MOBILISATION: A PROSPECTIVE-HISTORICAL CONTROL STUDY

F. Lefrère[1,], J.-A. Ribeil[1], M. Turner[2], O. Hermine[1], S. Blanche[3], M. Cavazzana-Calvo[4]*
[1]Service de Biothérapie, Service d'Hématologie Adulte, Service d'Immuno-Hématologie Pédiatrique, Hôpital Necker, APHP, Paris, France, [2]Sandoz Biopharmaceuticals, Holzkirchen, Germany, [3]Unité d'Immunologie Pédiatrique, Hôpital Necker, APHP, [4]Biotherapy Department, Hôpital Necker Enfants Malades, APHP, Paris, France

Introduction: Biosimilars of filgrastim have been available since 2008 and are now in widespread use for the prevention of chemotherapy-induced neutropenia and stem cell mobilisation. However, some professional bodies have raised concerns over their use in healthy donors for allogeneic mobilisation given the lack of clinical data in this setting. Here we report the use of biosimilar filgrastim compared with a matched historical control group in allogeneic transplant.
Materials (or patients) and Methods: A total of 26 healthy related-donors (parent or sibling) received biosimilar filgrastim (Zarzio®) for allogeneic stem cell mobilisation at a single centre between 2011 and 2013. Donors and recipients were compared with a matched historical control group (*n*=48) who had been treated with original filgrastim (Neupogen®) at the same centre between 2005 and 2011. Donors and patients in both groups were treated according to the same clinical protocol, with G-CSF 10 µg/kg/day administered to donors on days 1-5 with leukapheresis performed on day 5.
Results: Donor and recipient characteristics (age, gender, body weight) were similar in both groups. Both the biosimilar and originator groups had similar donor mean white blood cell counts at baseline (6.13 vs 6.24 x 10^9/l) and on day 5 (46.9 vs 45.3 x 10^9/l). Mean donor CD34+ cell count on day 5 was 92/µl in the biosimilar group and 88/µl in the originator group (*P*=0.713). Median number of CD34+ cells per recipient body weight was 9.7 x 10^6 in the biosimilar group and 8.00 x 10^6 in the originator group (*P*=0.437). Occurrence and intensity of bone pain was similar in donors in both groups. The majority of recipients in both groups had acute leukemia, myeloma, lymphoma or congenital immunodeficiency syndrome and around half underwent a non-myeloablative transplant. Median time to neutrophil engraftment (>500/µl) was similar in both the biosimilar and originator groups (16.5 [range 11-44] vs 15.0 [range 9-23] days; *P*=0.078), as was platelet recovery (>20 g/l) (12.5 [range 8-28] vs 12.5 [range 3-38] days; *P*=0.990). Acute graft-versus-host disease (GVHD) occurred in 27% of patients in the biosimilar group and 38% patients in the original group, while chronic GVHD occurred in 15% and 19% of recipients, respectively.
Discussion: Biosimilar G-CSF is as effective and well tolerated as originator G-CSF for related-donor allogeneic stem cell mobilisation. Long-term follow-up of donors is required to confirm the safety of biosimilar and originator G-CSF.
Disclosure of Interest: F. Lefrère Conflict with: Sandoz Biopharmaceuticals, J.-A. Ribeil: None Declared, M. Turner: None Declared, O. Hermine: None Declared, S. Blanche: None Declared, M. Cavazzana-Calvo: None Declared.

PH-P241

BLOOD GRAFT STEM CELL SUBCLASSES AND LYMPHOCYTE SUBSETS IN NHL PATIENTS MOBILIZED WITH CHEMOTHERAPY-G-CSF PLUS PLERIXAFOR INJECTION DUE TO POOR MOBILIZATION – A PROSPECTIVE GOA STUDY

V. Varmavuo[1,], J. Valtola[1], A. Ropponen[2], T. Siitonen[3], H. Kuitunen[4], E.-R. Savolainen[5], T. Kuittinen[1], M. Pyörälä[1], P. Mäntymaa[6], J. Pelkonen[2], T. Nousiainen[1], E. Jantunen[1,7]*
[1]Department of Medicine, Kuopio University Hospital, [2]Department of Clinical Microbiology, University of Eastern Finland, Kuopio, [3]Department of Medicine, [4]Department of Oncology, Oulu University Hospital, [5]Nordlab of Oulu, Oulu, [6]Laboratory of Eastern Finland, [7]Clinical Medicine, University of Eastern Finland, Kuopio, Finland

Introduction: Plerixafor, a CXCR4 antagonist, can be used with G-CSF or chemotherapy plus G-CSF mobilization (chemomobilization) to enhance the mobilization of hematopoietic stem cells. Previous studies have shown that plerixafor have impact on cellular composition of the collected graft. Some characteristics of the blood stem cell grafts (e.g. stem cell primitivity and lymphocyte content) have been linked with engraftment and patient outcome after ASCT.
Materials (or patients) and Methods: Thirty-seven patients with NHL were included in this prospective GOA study (Graft and Outcome in Autologous Stem Cell Transplantation). All patients received chemomobilization. Fourteen patients (38 %) mobilizing poorly received plerixafor to enhance mobilization (*plerixafor group*). Twenty-three patients served as controls (*control group*). The samples of each cryopreserved leukapheresis products

were studied by flow cytometry. CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19 antibodies with BD Trucount tubes were used to determine the absolute counts of T, B, and NK cells as well as the CD4 and CD8 subpopulations of T cells. Stem cell primitivity was investigated by using the following antibodies: CD34, CD38, CD133, CD19, CD117 and CD45.
Results: The median number of aphereses was two in the plerixafor group and one in the control group ($P = 0.006$). The median of total stem cell yield was 2.8 x 10^6/kg $CD34^+$ cells (range 1.9 – 5.1 x 10^6/kg) in the plerixafor group and 3.9 x 10^6/kg $CD34^+$ cells (range 2.0 – 16.8 x 10^6/kg) in the control group ($P = 0.017$). The median proportion of the primitive stem cells ($CD34^+CD133^+CD38^-$) cells from all $CD34^+$ cells was significantly higher in the plerixafor group when compared to the control group (3.0 % vs. 2.1%, $P = 0.049$) but there was no significant difference in the total amount of these cells (medians 0.07 x 10^6/kg vs. 0.05 x 10^6/kg, $P = 0.914$).
The median amounts of total $CD3^+$ T cells (152 x 10^6/kg in the plerixafor group vs. 64 x 10^6/kg in the control group, $P = 0.001$), helper ($CD3^+CD4^+$) T subsets (76 x 10^6/kg vs. 38 x 10^6/kg, $P = 0.001$), suppressor ($CD3^+CD8^+$) T subsets (73 x 10^6/kg vs. 22 x 10^6/kg, $P = 0.002$) and NK ($CD3^-CD16/56^+$) cells (19 x 10^6/kg vs. 5 x 10^6/kg, $P < 0.001$) in the graft were all significantly higher in the plerixafor group when compared to the control group. $CD19^+$ cells were apparent only at in a few patients and there was no difference between the groups ($P = 0.998$).
Discussion: Plerixafor added to chemomobilization in poor mobilizers results in collection of higher amount of lymphocytes. In addition, the proportion of the most primitive stem cells in the graft is higher. The clinical relevance of these observations will be studied in regard to hematopoietic and immune reconstitution as well as progression free survival in this prospective GOA study.
Disclosure of Interest: None Declared.

PH-P242
SUCCESSFUL TRANSITION FROM COBE SPECTRA TO SPECTRA OPTIA APHERESIS DEVICE FOR ALLOGENEIC AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

C. Tietze-Bürger[1,], B. Berg[1], B. Reufi[1], A. Wieczorek[1], A. Lorusso[1], K. Movassaghi[1], L. Uharek[1]*
[1]Charite Stem Cell Facility, Charite-Universitätsmedizin Berlin, Berlin, Germany

Introduction: Since 1994 we have been collecting over ten thousand peripheral blood stem cells (PBSC) in an allogeneic and autologous setting using the COBE Spectra apheresis device, mainly using the MCN program. As our large center embraces innovation, we have recently transitioned to Spectra Optia in March 2012 and compared both machines in a similar setting.
Materials (or patients) and Methods: 68 Patients were mobilized with chemotherapy + G-CSF 7 days before apheresis. 38 of them underwent procedures using Spectra Optia; 30 patients were put on COBE Spectra. In addition, PBSC from 255 normal donors, receiving only G-CSF, were collected on the Spectra Optia and compared with 30 donors on COBE Spectra. For all subjects, pre-counts and post-counts of total blood cells and CD34+ cells were obtained. From apheresis products, the same quality control parameters were obtained. Both apheresis devices were run in a consistent fashion, depending on the setting (auto or allo). With Spectra Optia, a collection preference of 20 (in allo) and 20 to 40 (in auto) was used with a chamber flush of 16 ml and chamber chase of 2 ml. Collection phases were carried out every 500 to 700 ml in an allogeneic setting and in the autologous setting were determined by the device. For COBE Spectra, a collect Hct of 2-3 % was maintained and collections were done at a collect flow of 0.8 ml/min. All results were analyzed using a non-parametric Mann-Whitney U test.
Results: Pre-procedure and post-procedure blood counts for all patients and donors were comparable between the two device groups, except for the recipients body weight which was higher in the COBE Spectra group (76 (7-130) kg vs 85 (58-105) kg; P=0.0068). In the allo setting we could process less blood on Spectra Optia (11.3 (3.5-15.4) L vs 13.2 (7.3-17.0) L) to get to the same CD34+ target dose (7.5 (0.13-224) x 10^6 vs 8.1 (1.5-21.9) x 10^6 c/kg). However, the collection efficiency (CE1) was similarly high (82 (5-204) % on Optia; 83 (49-151) % on Spectra). In the auto setting, we obtained similar results: less blood was processed to get to a higher transplant dose. However, the CE1 was similar, also in this setting: 85 (48-239) % on Optia vs 85 (46-184) % on COBE Spectra). Interestingly, we observed a much higher CD34+ dose/kg obtained per liter that we processed on Spectra Optia, than on COBE Spectra (0.67 (0.10-4.6) vs 0.46 (0.06-2.5) x 10^6/kg/L; P=012) in the autologous setting. The RBC contamination of Spectra Optia products was much lower, both in the allo (7 (1-22) ml vs 15 (3-52) ml) and auto setting (5 (2-16) vs 6 (3-13)). The large difference in the allogenic setting would be a benefit for ABO-incompatible transplants.
Discussion: The high collection efficiency values on both devices enabled us to get high transplant doses in a short time and by processing relatively low amounts of blood. Spectra Optia appeared superior in collecting higher doses per liter of blood processed, especially in the autologous setting. Product cross cellular contamination was low, especially in the amount of RBC collected on Spectra Optia. Taking all these data together, we intend to move forward only with Spectra Optia in our large transplant setting.
Disclosure of Interest: None Declared.

PH-P243
QUANTIFICATION OF THE TIME AND EFFORT REQUIRED FOR AUTOLOGOUS PERIPHERAL BLOOD STEM CELL COLLECTION: A EUROPEAN PERSPECTIVE

M. Mohty[1,], N. Azar[2], J. F. Reitan [3], R. P Kadota[4], S. U. Iqbal[5], S. Naoshy[5], K. Hübel [6]*
[1]Hopital Saint-Antoine, [2]Hematologie Clinique, Hôpital de la Pitié-Sâlpétrière, Paris, France, [3]RJM Group LLC, Crown Point, [4]Global Oncology Medical Affairs, [5]Global Evidence & Value Development, Sanofi, Cambridge, United States, [6]University Hospital of Cologne, Cologne, Germany

Introduction: Plerixafor is approved for autologous peripheral blood stem cell mobilization in patients with Non-Hodgkin Lymphoma (NHL) or Multiple Myeloma. This agent may reduce the failure rate and/or the number of apheresis procedures required without increasing the toxicity, and this may reduce total transplant costs. With this background, the aim of this prospective non-interventional analysis is to assess resource utilization to document provider costs associated with peripheral stem cell mobilization and apheresis. Of note, the study aims to evaluate the impact on the time and effort associated and costs to the hospital when using plerixafor (P) with a primary analysis to compare measures of time/effort from patients drawn from the Pre-P versus P eras.
Materials (or patients) and Methods: The study population includes NHL patients undergoing peripheral blood stem cell mobilization. Part I of the study is a retrospective medical record review study of 200 NHL patients from 7 centers in France and Germany. Selected patients will be evenly divided between two eras: 1) prior to approval of plerixafor = Pre-P era (until June 1, 2009) 2) after approval of plerixafor = P era (July 1, 2010 and onwards).
Part II of the analysis is an ongoing prospective study consisting of time/motion evaluation of actual apheresis – 20 events at each center. The actual apheresis events are being measured in consecutive patients scheduled to be candidates for peripheral blood stem mobilization. Outcome measures include number of visits for administration of mobilizing agents; duration (days) of administration of mobilizing agents; agents used as mobilizing agents; adverse events detected during mobilization; number of apheresis sessions; hours of apheresis sessions; attainment of CD34+ target (yes, no); days until CD 34+ target level was met. In addition, time-motion assessments will be obtained retrospectively (Part I) and concurrently (Part II) and included the total time to prepare the patient, perform apheresis and manage adverse events. Costs will be evaluated and quantified in terms

of micro-costing group interviews with local hospital administration. The primary study end point is difference in mean time to perform apheresis (including apheresis related adverse events, if any) and costs to the hospital in terms of micro-costing per patient, between patients in the Pre-P versus P eras.
Results: At the time of abstract submission, data collection is ongoing and results will be presented during the meeting. The key findings of this study will demonstrate the favorable impact of novel interventions on the number of apheresis procedures required to reach a target peripheral blood stem cell, and failure rate of mobilization, thus translating into reduced total transplant costs without increasing the toxicity.
Discussion: The financial implications for transplant centers would be significant and would pave the way for further studies aiming to optimize staff time and resource utilization related to apheresis in real-world practice.
Funding provided by Sanofi.
Disclosure of Interest: M. Mohty Conflict with: Sanofi, N. Azar Conflict with: Sanofi, J. Reitan Conflict with: Sanofi, R. Kadota Conflict with: Sanofi, S. U. Iqbal Conflict with: Sanofi, Conflict with: Stock Options, S. Naoshy Conflict with: Sanofi, Conflict with: Stock Options, K. Hübel Conflict with: Sanofi.

PH-P244
STEM CELL GRAFT VIABILITY AFFECT CLINICAL OUTCOME AFTER ALLOGENEIC HSCT

E. Watz[1], M. Remberger[1], O. ringden[1], J. Mattsson[1], M. Uhlin[1,]*
[1]Karolinska University Hospital, Centre for Allogeneic Stem Cell Transplantation, Stockholm, Sweden

Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) is a successful treatment strategy for hematopoietic malignancies and inborn errors of metabolism or the immune system. In about two thirds of the patients no suitable related donor can be identified. A suitable unrelated HLA-matched donor can be found through large international donor registries. Cell grafts from unrelated donors are almost always collected at distant collection sites and storage and transportation of cell grafts becomes a crucial link in the transplantation process. Several studies have investigated the impact of factors influencing the graft quality, such as liquid storage and transportation
Materials (or patients) and Methods: We studied the influence of graft quality on clinical outcome in 144 patients treated with allogeneic SCT. As a measurement of the graft quality we used the viability measured by 7AAD on a frozen/thawed sample from the PBSC graft in %.
Results: There was great variation in the viability of the frozen/thawed samples (median viability 64%; range 24-96%). When comparing the viability using 7AAD on freshly arrived PBSC grafts compared to frozen/thawed vials no clear correlation could be observed.
More patients who received PBSC with inferior quality (viability <64% on the frozen/thawed sample) developed acute graft versus host disease (aGVHD) of any grade than patients receiving grafts with better quality, 71% and 57% respectively (*P*=0.025). This also is reflected by the fact that Transplant related mortality (TRM) were 22% in the group receiving grafts with a viability of <64% on the frozen/thawed vial compared to only 8 % in the patient group with better graft quality (*P*=0.03).
To measure the impact of graft quality on viral complications with a relatively early onset after HSCT we decided to analyze EBV-PTLD and CMV reactivation.
Of the patients receiving grafts with inferior quality, 61% of the patients suffered from cytomegalovirus (CMV) reactivation as compared to 44% in the group receiving grafts of a better quality (*P*=0.05).
Discussion: We have found that poor graft quality, measured as viability on frozen/thawed samples from PBSC grafts, is associated with increased occurrence of acute GVHD. Also, patients receiving grafts with inferior graft quality have more CMV infections. There is a need for better analyses for assessing graft quality in routine transplantation care.
Our study also suggests that clinical follow-up of cell collection and processing as measurements of quality needs to be more elaborate, Engraftment- and survival data is not sufficient as quality markers.
Disclosure of Interest: None Declared.

PH-P245
AUTOLOGOUS HEMATOPOIETIC STEM CELL MOBILIZATION WITH PLERIXAFOR (P) PLUS G-CSF (G) IN PATIENTS <65 AND ≥65 YEARS OF AGE IN FRONTLINE AND MOBILIZATION FAILURE SETTINGS FOR MULTIPLE MYELOMA (MM) AND NON-HODGKIN'S LYMPHOMA (NHL)

J. Schriber[1,], R. P. Kadota[2], S. R. Llanos[2], I. Micallef[3]*
[1]Cancer Transplant Institute Virginia G Piper Cancer Center, Scottsdale, [2]Sanofi Oncology, Cambridge, [3]Mayo Clinic, Rochester, United States

Introduction: MM and NHL are the most common indications for autologous stem cell transplantation (SCT). Increasing age has been correlated with poor mobilization and in some areas is a primary factor for transplant eligibility. However, recent studies

[PH-P245]

Table 1.

	<65 Years of Age			≥65 Years of Age		
	RCT	CUP	Total	RCT	CUP	Total
MM						
Total (N)	115	199	314	33	104	137
>2 x 10^6 CD34/kg, n (%)	108 (93.9)	161 (80.9)	269 (85.6)	33 (100)	82 (78.8)	115 (83.9)
Mean apheresis days to achieve* (SD)	1.86 (0.93)	3.25 (1.56)	2.86 (1.40)	1.88 (0.89)	3.35 (1.41)	3.05 (1.35)
Proceeded to Transplant, n (%)	109 (94.8)	152 (76.4)	261 (83.1)	33 (100)	84 (80.8)	117 (85.4)
Engraftment Plt, n (%)	101 (92.7)	111 (73)	212 (81)	32 (97)	69 (82)	101 (86.3)
Engraftment WBC, n (%)	108 (99)	144 (95)	252 (97)	33 (100)	82 (98)	115 (98)
NHL						
Total (N)	117	374	491	33	161	194
>2 x 10^6 CD34/kg, n (%)	103 (88)	244 (65.2)	347 (70.1)	27 (81.8)	98 (60.9)	125 (64.4)
Mean apheresis days to achieve* (SD)	2.51 (1.12)	3.01 (1.56)	2.93 (1.41)	2.94 (1.11)	3.15 (1.57)	3.10 (1.42)
Proceeded to Transplant, n (%)	107 (91.5)	279 (74.6)	386 (78.6)	28 (84.8)	120 (74.5)	148 (76.2)
Engraftment Plt, n (%)	101 (92.7)	210 (75.3)	311 (80.5)	24 (85.7)	95 (79.2)	119 (80.4)
Engraftment WBC, n (%)	107 (100)	276 (98.9)	383 (99.2)	28 (84.5)	118 (98.3)	146 (98.6)

*Apheresis days of patients from the RCTs was recorded for only the first 4 days, and was reported up to 10 days for CUP patients.

suggest SCT can be performed safely on patients (pts) well into their 70s, which is critical since the median age of pts with MM is 70 and in NHL it is 66.
Materials (or patients) and Methods: We review results of 2 Ph 3 trials (RCTs) utilizing P upfront + G vs. G alone to collect stem cells and results of the compassionate use protocol (CUP) of P+G to mobilize stem cells in those who failed prior collections. We compare collection results and safety in pts ≥65 or <65 years of age (yrs).
Results: 137 MM pts ≥65 yrs (median 67 range 65-77) received P+G (38 RCT, 104 CUP). 314 MM pts <65 received P+G (115 RCT, 199 CUP). Median age was 57 (range 28-64). 194 pts with NHL ≥65 yrs (median 68 range 65-78) received P+G (33 RCT, 161 CUP) while 491 were <65 (median 56 range 10-64) (117 RCT, 374 CUP). Overall, there were more women in the pts ≥65; other baseline characteristics were similar. As seen in Table 1, 84% of MM pts ≥65 yrs collected sufficient cells to proceed with single SCT and was similar to pts <65 yrs (86%). Median days of apheresis to reach this target was 3 (range 1-8) and 3 (range 1-7). The number of MM patients who proceeded to SCT was similar between the 65< and ≥65 yrs pts (83 vs 85%) and the number of NHL pts who proceeded to SCT was similar for pts 65< or ≥65 yrs (79 and 76%, respectively). In NHL pts, 70% of pts <65 and 64% of pts ≥65 yrs collected sufficient cells to proceed with single SCT. Median days of apheresis to reach this target was 3 (range 1-10) and 3 (range 1-8). Overall, there was a higher percentage of pts who mobilized sufficient cells to proceed to SCT when treated with P+G upfront rather than after failing mobilization using standard techniques regardless of age. There was no difference in engraftment to WBC or Plts between those ≥65 or <65 yrs. In the RCTs, ≥Gr 3 febrile neutropenia was slightly higher in pts ≥65 yrs that received P+G (7.6 vs. 4.0% G alone), but similar to those <65 yrs who received P+G (6.9%). Other adverse events (AEs) were similar between the <65 and ≥65 pts in the RCTs. AEs were similar in the CUP pts 65< and ≥65 yrs.
Discussion: Although age has traditionally been a risk factor for poor mobilization, these data suggest that with the use of P+G there is no difference in the ability to mobilize or transplant MM and NHL pts ≥65 yrs. Engraftment seems no different for pts who proceed to SCT. Generally, AEs appear similar when comparing age and P+G vs G alone. Age alone should not be a consideration for SCT in pts with MM or NHL.
Disclosure of Interest: J. Schriber Conflict with: Consultant and Speaker for Sanofi, R. Kadota Conflict with: Sanofi Employee, S. Llanos Conflict with: Sanofi Employee, I. Micallef Conflict with: Research Funding, Consultant, and Speaker for Sanofi.

PH-P246
EFFECTIVE DEPLETION OF CD45RA+ NAÏVE T CELLS USING THE CLINIMACS® SYSTEM TO PRODUCE DONOR LYMPHOCYTE INFUSIONS FOR ANTIVIRAL BOOST FOLLOWING HAPLOIDENTICAL TRANSPLANTATION

J. Dykes[1,2,], A. Hult[1,2], Å. Persson[1], S. Scheding[3,4], S. Lenhoff[3], J. Toporski[5], D. Turkiewicz[5]*
[1]Clinical Immunology and Transfusion Medicine, Laboratory Medicine, [2]Division of Hematology and Transfusion Medicine, Lund University, [3]Department of Hematology, Lund University Hospital, [4]Stem Cell Center, Lund University, [5]Department of Pediatric Oncology, Lund University Hospital, Lund, Sweden

Introduction: Depletion of CD45RA+ naïve T cells from donor lymphocyte infusions (DLI) aims at the reduction of alloreactivity while preserving immunocompetent memory T-cells to provide pathogen-specific reactivity. Here, we investigated the performance of the CliniMACS system for depletion of CD45RA+ T cells from DLI intended for therapeutic or pre-emptive antiviral boost, following TCRαβ+ depleted haploidentical hematopoietic stem cell transplantation (HSCT).
Materials (or patients) and Methods: Whole-blood-derived buffy coats were obtained from two HLA-haploidentical donors. The cells were labelled with CD45RA reagent and processed with the CliniMACS device using the D3.1 program with the DTS (Depletion Tubing Set). In flow cytometric analysis viable CD45RA+ T cells were defined by their cell scatter properties of lymphocytes, positivity for CD3 and CD45RA, and negativity for propidium iodine staining, as well as lack of CD13-, CD19-, CD14- and CD45RO-expression. CD3+CD45RA- and CD3+CD45RO+ T cells were further defined as CD4+ or CD8+, respectively. Aliquots of CD45RA-depelted products containing 25×10^3 CD3+ cells per kilogram of recipient weight were prepared for fresh DLI and cryopreservation.
Results: The $\log_{10}$ CD3+CD45RA+ cell depletion rate was 3.84 and 3.85, corresponding to 0.0049 % and 0.0071 % residual CD3+CD45RA+ cells, respectively, in the target fractions. The recovery of CD3+CD45RA-CD45RO+ cells was 51% and 54% and the ratio of CD4+ to CD8+ cells was increased from 2.5 to 9.7 and 1.8 to 6.8. Two patients following TCRαβ+ depleted haploidentical HSCT with symptomatic (patient#1; CMV, VZV) and asymptomatic (patient#2; CMV, AdV) viral infection, received a fresh CD45RA+ depleted DLI of 25×10^3 CD3+ cells /kg from their original HSCT donor, at day +84 (patient#1) and +70 (patient#2), respectively. Patient#2 received a second dose of 50 x 10^3 CD3+ cells /kg on day +91. At the time of DLI, peripheral blood CD3+CD4+ and CD3+CD8+ levels were < 10/ul. Following the first DLI, the time to clearance of VZV (patient#1) and AdV (patient#2) viremia was 2 and 8 weeks, respectively, whereas decreasing levels of CMV in peripheral blood were detected at 3 (patient#1) and 5 weeks (patient#2). Peripheral blood CD3+CD4+ and CD3+CD8+ levels reached > 100/ul at 3 (patient#1) and 9 (patient#2) weeks post DLI. There was no GvHD before or after DLI.
Discussion: The ClinMACS system (D3.1 program, DTS) enables extensive depletion of naïve CD45RA+ T cells from DLI products, which can be safely used for therapeutic or pre-emptive antiviral boost following TCRαβ+ depleted haploidentical HSCT.
Disclosure of Interest: None Declared.

PH-P247
OSMOZ: AN OBSERVATIONAL STUDY OF THE USE OF PLERIXAFOR FOR STEM CELL MOBILIZATION AND COLLECTION IN 262 PATIENTS TREATED AT 33 FRENCH TRANSPLANT PROGRAMMES OVER A 1-YEAR PERIOD

C. Chabannon[1,], F. Bijou[2], M. Jean-Michel[3], N. Milpied[4], J.-M. Grouin[5], M. Mohty[6]*
[1]Institut Paoli-Calmettes, Marseille, [2]EFS Acquitaine, Bordeaux, [3]Hopital Saint Louis, Paris, [4]CHU de Bordeaux, Bordeaux, [5]CHU de Reims, Reims, [6]Hematology Department, Hopital Saint-Antoine, Paris, France

Introduction: In 2009, Plerixafor was granted Marketing Authorization by EMA, to be used in patients with lymphoid malignancies and who "…mobilize poorly …" in response to conventional mobilization treatment. Since there is no consensus across collection and transplant facilities on the definition of "poor-mobilization", and since the price tag of plerixafor is high and significantly adds on the cost of the collection and transplant procedure, French competent authorities as represented by the "Comité Economique des Produits de Santé" (CEPS), commissioned a nationwide survey on the use of plerixafor in France. Genzyme–Sanofi promoted constitution of the registry where data were collected on mobilization and collection; data analysis was conducted by a committee of experts, who here report on the results of this observational study.
Materials (or patients) and Methods: Contacts were established with haematologists/transplant physicians and their corresponding apheresis practitioners at all transplant programmes that annually report their activity to the "Agence de la Biomédecine" (ABM), the public health authority that monitors and supervises cell, organ and tissue transplantation in France.
Results: Thirty-three programmes/collection facilities (out of 37 registered with the ABM) reported on 262 patients (Male: 148, Female: 114; median age: 57, range: 2–72) who were prescribed plerixafor between November 2011 and November 2012. Patients were informed and provided written consent to access their health records. As expected, the vast majority of enrolled patients were treated for lymphoid malignancies; however, a small

proportion of patients (*n*= 21 or 8%) received plerixafor for other types of diseases where high-dose chemotherapy was deemed an indication. One hundred and sixteen patients had previously failed at least one attempt for mobilization/collection, while 146 patients received plerixafor in the course of their first attempt for mobilization/collection, using a "pre-emptive" strategy to immediately overcome poor or insufficient mobilization. Enumeration of circulating $CD34^+$ cells was widely used as a surrogate marker for stem cell mobilization: 67% and 78% of patients treated with plerixafor in the "failed collection" and "pre-emptive" subgroups had < 15 $CD34^+$ cells/mL of peripheral blood immediately before introduction of plerixafor. Patients experienced a median 3.89 (0.3; 52.0) fold increase in the number of circulating $CD34^+$ cells. Two hundred and fifty five treated patients reached the targeted number of collected cells; a median number of $3.66x10^6$ $CD34^+$ cells/kg (0.1; 16.4) was collected after introduction of plerixafor (and was eventually combined and transplanted with previously collected cell products).
Discussion: This "real-life" survey - that includes a significant proportion of candidate patients for plerixafor at a national level – suggests that prescriptions have not exceeded the expected figures, despite variations in criteria used to define poor-mobilization; plerixafor improved upon the mobilization status, and allowed for efficient collection of a blood graft for most treated patients.
Disclosure of Interest: C. Chabannon Conflict with: SANOFI, F. BIJOU Conflict with: SANOFI, M. Jean-Michel Conflict with: SANOFI, N. Milpied Conflict with: SANOFI, J.-M. Grouin Conflict with: SANOFI, M. Mohty Conflict with: SANOFI.

PH-P248
SEPARATION OF CD8+ LYMPHOCYTES FROM PERIPHERAL BLOOD PROGENITOR CELL PRODUCTS USING AFFINITY BEAD ACOUSTOPHORESIS

A. Urbansky[1], A. Lenshof[2], J. Dykes[3], T. Laurell[4], S. Scheding[5,]*
[1]Stem Cell Center, [2]Measurement Technology and Industrial Electrical Engineering, University of Lund, [3]Clinical Immunology and Transfusion Medicine, University and Regional Laboratories, [4]University of Lund, [5]Hematology, University Hospital Lund, Lund, Sweden

Introduction: Processing of peripheral blood progenitor cells (PBPC) for clinical transplantation or research applications aims to effectively isolate or deplete specific cell populations. We have previously reported the use of a novel ultrasound-based sorting technology (acoustophoresis) for sorting of platelets and CD4+ cells from PBPC products. Utilizing a further improved sorting device, this study aimed to optimize affinity bead acoustophoresis for smaller cell populations, in this case CD8+ cells.
Materials (or patients) and Methods: PBPC samples (*n*=16) were obtained from patients and healthy donors. Following density gradient centrifugation, mononuclear cells were labelled with anti-CD8 microbeads (Dynal) and sorted either on an acoustophoresis-microchip or magnetically. PBPC samples, target and waste fractions were analysed for purity, recovery, T-cell function and progenitor cell content.
Results: PBPC products contained a mean 11.6 ± 7.1% CD8+ cells before sorting. Purities obtained with acoustic sorting of CD8+ lymphocytes were 93.3 ± 6.8% compared to 94.4 ± 8.6% for magnetic sorting (*n*=16). Viabilities of sorted cells were 97.0 ± 3.9% (acoustic) and 97.5 ± 3.5% (magnetic). Recovery of acoustic sorted CD8+ cells was 57 ± 19% of the total CD8+ cells compared to a median recovery of magnetic sorted CD8+ cells of 43 ± 17%. Leukocyte subpopulation analysis performed after CD8 selection showed a relative increase of CD4 cells in the non-target fractions due to the removal of CD8 cells. Functional testing of sorted CD8+ cytotoxic T cells showed unimpaired mitogen-induced proliferation capacity with proliferation rates of 87.5 ± 7.8% and 64.3 ± 7.7% (acousto) compared with 92.2 ± 12.2 and 69.5 ± 15.2 (magnetic) after 6-day stimulation with CD3/CD28 and ConA, respectively. Furthermore, hematopoietic progenitor cell assays showed a preserved colony-forming ability of post-sorted non-target cells (CFU-C/5,000 seeded cells: 14.4 ± 6.5 pre-sort versus 11.3 ± 5.9 post-sort).
Discussion: Acoustophoresis is a promising technology to efficiently sort bead-labelled lymphocyte populations from PBPC samples with high purity and recovery without impairing lymphocyte function. Affinity-bead acoustophoresis is, thus, an interesting technology for PBPC processing.
Disclosure of Interest: A. Urbansky: None Declared, A. Lenshof: None Declared, J. Dykes: None Declared, T. Laurell Conflict with: co-founder and share holder of Acousort AB, S. Scheding Conflict with: co-founder and share holder of Acousort AB.

PH-P249
INVOLVEMENT OF SOLUBLE FORMS OF RECEPTOR OF THE UROKINASE-TYPE PLASMINOGEN ACTIVATOR (UPAR) IN HEMATOPOIETIC STEM CELL HOMING

N. Montuori[1,], C. Selleri[2], B. Serio[2], V. Giudice[2], M. Sessa[2], A. Gorrasi[3], A. Li Santi[3], M. Rocco[2], P. Ricci[2], P. Ragno[3]*
[1]Department of Translational Medical Sciences, "Federico II" University, Naples, [2]Hematology and Hematopoietic Stem Cell Transplant Center, Department of Medicine and Surgery, [3]Department of Chemistry and Biology, University of Salerno, Salerno, Italy

Introduction: The receptor (uPAR) of the urokinase-type plasminogen activator (uPA) plays a key role in cell migration processes. uPAR is expressed on the surface of various cell types, both in full-length and cleaved forms, lacking the N-terminal DI domain (DIIDIII-uPAR). uPAR binds uPA and vitronectin (VN) and regulates integrin activity. The receptor can be shed from the cell surface, generating full-length and cleaved soluble forms, suPAR and DIIDIII-suPAR, respectively. Both forms of soluble uPAR have been detected in human plasma and urine. suPAR is still able to bind uPA and VN, whereas DIIDIII-suPAR, when exposing the chemotactic SRSRY sequence (aa 88-92) at its N-terminus, is able to bind receptors for the formyl-methionyl-leucyl phenylalanine peptide (fMLF). We previously demonstrated the involvement of uPAR soluble forms in G-CSF-induced human CD34+ hematopoietic stem cell (HSC) mobilization. Further, we demonstrated that DIIDIII-suPAR can induce mobilization of hematopoietic stem/progenitor cells in mice. Since HSC mobilization and homing are specular processes which utilize same mediators and similar signaling pathways, we investigated whether the soluble forms of uPAR could be also involved in HSC homing.
Materials (or patients) and Methods: We examined suPAR and DIIDIII-suPAR expression in cultures of human marrow stromal cells. We then evaluated the levels of both suPAR forms in sera from five healthy donors and from five patients before and after chemotherapy-based conditioning regimens. We also examined the potential effects of the different soluble forms of uPAR in long term cultures (LTC) of G-CSF-mobilized CD34+ HSCs, in the presence of suPAR or of the uPAR84-95 peptide, containing the SRSRY chemotactic sequence of DIIDIII-suPAR.
Results: Interestingly, stromal cells produced suPAR and high amounts of the chemotactic DIIDIII-suPAR. We found a significant increase in DIIDIII-suPAR levels in sera from patients before conditioning, as compared to healthy donors; however, the chemotherapy-based conditioning regimen significantly decreased circulating DIIDIII suPAR levels. Both suPAR and the uPAR84-95 peptide increased the number of adherent clonogenic progenitors in LTC of G-CSF mobilized HSCs.
Discussion: Our results suggest that marrow stroma produces soluble forms of uPAR which could contribute to the engraftment of marrow CD34+ HSC. According with our previous observation on the mobilizing effects of DIIDIII-suPAR, the circulating cleaved suPAR seems to be lowered by chemotherapy-based conditioning regimen, likely allowing CD34+ HSC homing.
Disclosure of Interest: None Declared.

PH-P250

FILGRASTIM (BIOSIMILAR) IN ALTERNATE DAYS FOR THE MANAGEMENT OF NEUTROPENIA AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION – EXPERIENCE OF A SINGLE CENTER

C. Constanço[1], C. Vaz[1], F. Campilho[1,], R. Branca[1], S. Roncon[2], A. Campos[1]*
[1]Bone Marrow Transplantation Unit, [2]Celular Therapy Service, Instituto Portugues de Oncologia do Porto, Porto, Portugal

Introduction: Neutropenia after stem cell transplantation may contribute to the severity of infectious complications and may have a significant impact on overall morbidity and mortality in this setting. Granulocyte colony-stimulating factors (G-CSFs), filgrastim, is recognised to be useful in accelerating engraftment after autologous stem cell transplantation. Several forms of biosimilar non-glycosylated G-CSF have been approved by the European Medicines Agency, with limited published data supporting the clinical equivalence in peripheral blood stem cell mobilisation and recovery after autologous stem cell transplantation.
Materials (or patients) and Methods: With the aim of evaluate the use of G-CSF after autologous stem cell transplantation, we studied retrospectively 124 patients (pts) consecutively treated with a biosimilar of filgrastim, at a dosage of 5 mcg/kg/day, given intravenously from day 7, in alternate days, to absolute neutrophil count of 500/mmc for three days. Hospitalization period, number of days with fever, and time to neutrophils and platelets recovery (neutrophils> 500/1000 cells/mm^3 and platelets > 20000 cell/mm^3) were evaluated as primary endpoints.
Results: In order to assess the effect of filgrastim (biosimilar) on alternate days, beginning in D+7, on neutropenia duration and clinical outcome after transplant, 124 patients were included. The median age was 54 (range 18-69) years with 52.4% male and 47.6% female. The baseline malignancies were: Multiple Myeloma (57.3%), Non-Hodgkin's Lymphoma (35.5%), Hodgkin's Disease (6.5%) and others (0.8%). The most frequently received chemotherapy regimens were: high dose Melphalan200mg/m^2 (54%), FEAM (28.2%), BEAM (13.7%), Melphalan140mg/m^2 (2.4%), Melphalan80mg/m^2 (0.8%) and Carbopec (0.8%). The median of CD34+ cells infused were 2.93x10^6 (1-12) cells/kg. Median time to neutrophils> 500 cells/mm^3 12d (9-32); neutrophils> 1000 cells/mm^3 12d (9-195) and platelets > 20000 cell/mm^3 12d (6-42); days of fever: median 4d (0-18). Length of hospital stay: median 16d (11-184). No deaths related to transplant. The comparison with our previous practice (filgrastim (Neupogen 5 mcg/kg/day intravenous from day 7 each day to absolute neutrophil count of 500/mmc for three days.)
Discussion: Although the study population was not matched with previously analyzed patients (discrepancy regarding the predominance of each sex, age, prevalent diseases, and conditioning regimens used) the results of hematopoietic recovery were overlapping. The median of CD34+ cells infused was higher, but hospitalization was similar. The difference found in time for recovery of neutrophils and platelets appear to have no significant clinical impact. Filgrastim (biosimilar) on alternate days appears to be as effective as daily filgrastrim (Neupogen) in the context of autologous hematopoietic stem cell transplantation.
Disclosure of Interest: None Declared.

PH-P251

USE OF BIOSIMILAR FILGRASTIM FOR PERIPHERAL BLOOD STEM CELL MOBILIZATION IN AUTOLOGOUS TRANSPLANTATION: AN ANALYSIS OF EFFICACY AND SAFETY

R. Gerivaz[1], J. Schuh[1], F. Costa[1], G. Ferreira[1], A. Botelho de Sousa[1,]*
[1]Hospital dos Capuchos, Lisboa, Portugal

Introduction: Recombinant G-CSF filgrastim (Neupogenâ) has been widely used for mobilization of peripheral blood progenitor cells (PBPC), alone or in combination with chemotherapy. Although developing new cost effective mobilization regimens is important, the experience with biosimilar G-CSF agents for this indication has been limited by concerns about biological equivalence and unidentified functional differences, and scarce data have been published regarding the use of biosimilars in this context. In 2012, after a 2-month experimental period, we incorporated biosimilars in our mobilization protocols with the aim of prospectively assessing their efficacy and safety.
Materials (or patients) and Methods: From April 2012 to June 2013 filgrastim biosimilars (Zarzio®, Sandoz and Nivestim®, Hospira) were used in 70 consecutive PBPC collections in 64 patients (pts), with no other change in mobilization regimens nor in equipment. Collection and engraftment parameters were prospectively registered. A series of 72 consecutive collections in 64 pts mobilized with Neupogen® from April 2011 to June 2012 were used as the comparator group. A collection is defined as the PBPC harvested from a single mobilization procedure, regardless of the days of apheresis required to collect them.
Results: The study and control cohorts were well matched for median age (56/55), underlying disease (multiple myeloma 47/45%, lymphoma 48/45%), retransplant candidates (3 pts *vs* 5) and number of previous lines of therapy (2 ou more 41/53%, n.s.). Most pts were mobilized with cyclophosphamide + filgrastim (81/71%, n.s.); poor mobilizers received etoposide (4/8%) or plerixafor (10/14%) + filgrastim. Side effects attributable to filgrastim were minimal and transient in both groups, consisting of transient myalgias/arthralgias (which were grade 3 in 6/4%) with no reports of unexpected events. The number of days of apheresis required to reach the target CD34+ count did not differ (with a single apheresis in 58/46%, n.s.). The collection was considered inadequate in 7/15%. CD34+ counts in pre-apheresis PB were similar (median 32/24/ul) as were total CD34+ collections (median 3.79 *vs* 2.51 x10^6/kg, both n.s.). In the study group 59 pts were transplanted (92%) as compared to 54 pts in the control group (84%). Time to engraftment was identical in the 2 groups, with median time to reach 0.5x10^9/l neutrophils and 20x10^9/l platelets of 12 and 17 days respectively. The difference in costs was calculated at approximately 80,000€/year.
Discussion: Our results indicate that mobilization of PBPC with original and biosimilar filgrastim has equivalent results in the key parameters of collection and engraftment. Although we must acknowledge the fact that long-term toxicity data are not yet available, changing to a biosimilar appears safe and efficient, while allowing significant cost advantages for a transplant unit.
Disclosure of Interest: None Declared.

[PH-P250]

	Filgrastim n=32 (Neupogen)	Filgrastim n=124 (biosimilar)	p
CD34+ cells infused	2.59x10^6 (1-9)	2.93x10^6 (1-12)	.04
neutrophils> 500 cells/mm^3	11d (9-23)	12d (6-42)	.20
neutrophils>1000 cells/mm^3	12d (9-23)	12d (9-195)	.37
platelets > 20000 cell/mm^3	12d (9-23)	11d (6-42)	.001
days of fever	5d (0-16)	4d (0-18)	.016
Discharde day	16d (11-33)	16d (11-184)	.5

PH-P252

SELECTIVE PHOTODEPLETION OF RECIPIENT-ALLOREACTIVE T-CELLS ENABLES SAFE AND EFFICACIOUS HAPLOIDENTICAL HSCT: INTERIM RESULTS FROM A PHASE II TRIAL IN PATIENTS WITH AML, ALL, AND MDS

D.-C. Roy[1,], J. Maertens[2], I. Walker[3], R. Foley[4], P. Lewalle[5], D. Selleslag[6], J. Velthuis[7], L. Gerez[7], K. Reitsma[7], E. Wagena[7], J. Roy[1], S. Lachance[1], S. Mielke[8]*

[1]Hematology-HSCT unit, Hopital Maisonneuve-Rosemont, Montreal, Canada, [2]Hematology-Stem cell transplantation unit, University Hospitals Leuven, Leuven, Belgium, [3]Oncology-Bone Marrow Transplantation, [4]Pathology and Molecular Medicine, Juravinski Hospital and Cancer Centre, Hamilton, Canada, [5]Hematology, Jules Bordet Institute, Brussels, [6]Hematology, AZ Sint-Jan Brugge-Oostende, Brugge, Belgium, [7]Kiadis Pharma, Amsterdam, Netherlands, [8]Hematology-Oncology, Universitätsklinikum Würzburg, Wurzburg, Germany

Introduction: For patients in need of a hematopoietic stem cell transplant (HSCT) but lacking an HLA matched donor, a haploidentical family donor is a most appealing alternative. However, to prevent graft-versus-host disease (GVHD), haploidentical HSCT necessitates intensive T-cell depletion that results in frequent and often lethal infectious complications and/or in high relapse rates, thus decreasing overall survival.

Materials (or patients) and Methods: In an open-label, multicenter phase 2 study, 23 patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) or myelodysplastic syndrome (MDS) will undergo an immunotherapeutic approach consisting of donor lymphocytes selectively depleted of host alloreactive T-cells through the use of photodynamic therapy (ATIR). The myeloablative regimen consists of TBI (1200 cGy), thiotepa (5 mg/kg) and fludarabine (200 mg/m^2). Patients first receive a stem cell graft that undergoes *in vitro* immunomagnetic T cell depletion using CD34+ positive cell selection. ATIR is infused between 28-32 days after a haploidentical CD34-selected HSCT. No post-transplant GVHD prophylaxis is used. Enrolment is expected to be completed by early 2014.

Selectivity of the photodepletion process and reactivity toward recipient and third party cells is assessed using a CFSE-based proliferation assay. Data from this assay is analyzed by Modfit LT™ software leading to identification of dividing cell populations. From this analysis, a proliferation index, reflecting cell divisions, is calculated.

Results: As of December 12, 2013, 7 patients have been treated with ATIR (mean age 44, range 21-61), AML=4, ALL=3, CR1=2, CR2=5, with a mean follow-up of 4 months post HSCT (range 2-7 months). Two additional grafts have been treated *ex vivo* and 3 patients are being prepared for HSCT.Stable engraftment was achieved at a median of 11 days 11 (8-18) for neutrophils and 12 days (9-23) for platelets in all patients. No patient experienced graft rejection. None of the patients treated to date developed acute GVHD (any grade). In addition, following ATIR infusion no severe infections (grade 3 or 4) were reported, no patient has relapsed nor died. Based on the 9 batches manufactured to date, ATIR mainly consists of T-cells (>90%). Remaining cells (≤10%) are B lymphocytes and NK cells. Donor cell proliferation toward donor, recipient, and third party cells, as well as CD3/CD28 is measured and used as release criteria. Results of donor cell proliferation before the photodepletion process and in the ATIR cell product show elimination of anti-host reactivity and preservation of immune reactivity toward third-party cells. In addition, the pan-T-cell activator anti-CD3/CD28 causes robust proliferation in both populations. Moreover, donor cytotoxic T-lymphocyte precursors (CTLp) toward the host are decreased by more than 95%. These results confirm the selectivity of the photodepletion process.

Discussion: The interim data confirm that novel immunotherapy consisting of donor lymphocytes selectively photodepleted of alloreactive cells (ATIR), can be manufactured consistently and reproducibly. The data so far show that ATIR can preserve anti-infectious activity without promoting GVHD in patients with hematological malignancies.

Disclosure of Interest: D.-C. Roy Conflict with: Kiadis Pharma, J. Maertens: None Declared, I. Walker: None Declared, R. Foley: None Declared, P. Lewalle: None Declared, D. Selleslag: None Declared, J. Velthuis Conflict with: Kiadis Pharma, L. Gerez Conflict with: Kiadis Pharma, K. Reitsma Conflict with: Kiadis Pharma, E. Wagena Conflict with: Kiadis Pharma, J. Roy: None Declared, S. Lachance: None Declared, S. Mielke: None Declared.

PH-P253

PERIPHERAL BLOOD PROGENITOR CELL COLLECTION–A COMPARISON OF 2 APHAERESIS EQUIPMENTS

S. M. Lopes[1,], F. Bordalo[1], S. Ferreira[1], F. Amado[1], A. P. Bernardo[1], M. França[1], M. Rosales[1], S. Roncon[1]*

[1]Cellular Therapy, IPO Porto, Porto, Portugal

Introduction: Allogeneic haematopoietic progenitor cell (HPC) transplant is a widespread treatment in many oncological diseases. The HPC used can be collected directly from the bone marrow or mobilized to the peripheral blood (with the use of aproprieted growth factors) and then collected by apheresis. During the last 2 years, our service updated the aphaeresis equipment from the Cobe Spectra to the Spectra Optia (both from Terumo), which features a new algoritm of collection, in theory obtaining a graft of higher cellular purity.

Materials (or patients) and Methods: In order to evaluate actual differences in the grafts collected, we review data from 10^4 collections performed in healthy adult donors in our service in 2012 and 2013 in either one of the two equipments. The graft composition was determined by flow cytometry in a FACSCanto II (BD). We compared the volume of collection, WBC, CD34 and T lymphocytes collected.

Results: For each parameter evaluated, the mean and standard deviation was calculated, and differences accessed with the T test. The results are presented in table 1.

Discussion: There are significant differences between grafts collected by aphaeresis in the 2 equipments. The Optia Spectra collects cellular products with larger volumes, although much more diluted, resulting in a lower cellular concentration. Regarding the total CD34 cells collected, which are the target cells of the procedure, no significant differences were found. We can conclude that although both cellular separators are suitable for HPC collection,

[PH-P253]

Table 1: Comparison of the grafts collected in the Optia Spectra and Cobe Spectra equipments

	Volume (ml)	WBC (x10^6/ml)	CD34 (%)	CD3 (%)	CD4 (%)	CD8 (%)	WBC (x10^5)	CD34 (x10^6)	CD3 (x10^5)	CD4 (x10^5)	CD8 (x10^5)
Optia* (n=46)	349.8 (90.1)	239.8 (51.4)	0.7 (0.3)	21.9 (7.1)	13.6 (5.0)	6.8 (2.5)	840.8 (308.2)	553.1 (322.9)	176.1 (66.9)	109.7 (44.8)	54.9 (22.2)
Cobe* (n=58)	182.6 (38.3)	644.9 (146.7)	0.5 (0.3)	21.8 (5.7)	13.3 (4.2)	7.3 (2.4)	1167.7 (340.9)	600.8 (288.7)	248.1 (80.6)	148.4 (44.0)	84.7 (34.9)
p value+	<0.01	<0.01€	<0.05	0.96	0.78	0.3	<0.01	0.43	<0.01	<0.01	<0.01

*Mean (Standard deviation)
+T test

the Optia Spectra produces grafts with less WBC but higher percentage of CD34 cells, confirming its higher purity.
Disclosure of Interest: None Declared.

PH-P254

PLERIXAFOR ON-DEMAND COMBINED WITH CHEMOTHERAPY AND GRANULOCYTE COLONY-STIMULATING FACTOR TO MOBILIZE PERIPHERAL STEM CELLS FOR AUTOLOGOUS TRANPLANTATION. ANALYSIS OF COLLECTION AND ENGRAFTMENT CHARACTERISTICS OF 29 PATIENTS WITH LYMPHOMA AND MYELOMA

P. Vincenzo[1,], M. Morciano[1], A. Ostuni[1], A. Mele[1], R. De Francesco[1], M. Fina[1], G. Greco[1], S. Sibilla[1], D. Carlino[1], B. Rossini[1], C. De Risi[1], S. Citiso[1], G. Rizzo[1], L. Abbruzzese[1]*
[1]Haematology, Az Osp Card Panico, Tricase (LE), Tricase, Italy

Introduction: High-dose chemotherapy (CHT) and autologous stem cell transplantation (ASCT) is a standard procedure for many patients (pts) with malignant Lymphoma and Multiple Myeloma (MM). For a safe ASCT is important an adequate mobilization and collection of from the peripheral hematopoietic progenitor cells (PBSCs). CHT plus granulocyte colony-stimulating factor (G-CSF) is a common strategy to obtain PBSCs. Failure to mobilize CD34+ cells or to harvest the minimum amount of CD34+ cells required for ASCT affects a significant number of MM and lymphoma pts. Plerixafor (AMD) used in combination with G-CSF results in increased mobilization of CD34+ cells compared to G-CSF alone, also in poor mobilizer pts.
Materials (or patients) and Methods: Between October 2009 and November 2013, 29 pts (19 NHL, 2 HD, 8 MM) underwent mobilization with AMD (0,24 mg/kg/d) in association with CHT plus G-CSF (10µg/kg/d). Patients' characteristics are shown on table 1. 4 pts (14%) were identified as predicted poor mobilizer and 25 pts (86%) as proven poor mobilizer (if PB CD34+ cells count ≤10/µL on 2 consecutive days or CD34+ yield < 2.0x10^6/kg by ≤ 3 apheresis, according to GITMO consensus).
Results: In this report we compared our results with a control group of 29 consecutive pts with Lymphoma and MM undergoing mobilization with disease specific CHT plus G-CSF, referred to the same period. After AMD administration and before the first apheresis, the median value of the circulating CD34+ cells/µL, was 28.35 (range 7.6-58.2) with a median of 4.35-fold increase (1.18-11.8) and a significant difference (*P*=0.002) compared to the control group (median 50.4, 10.98-302.63). Time to collection was 16 days (9-22) versus 14 days (10-21) for control group (*P*=0.008). After AMD, all pts successfully reached the target dose of CD34+ cells for ASCT (median 4.98, 2.3-7.8), with a median number of 2 apheresis, in comparison with the control group (1; *P*=0.0001). 20 pts (69%) mobilized with AMD received ASCT with a CD34+ median dose of 2.76 x10^6/kg (1.82-6.19) and a significant difference (*P*=0.003) with the control group (4.0; 1.52-6.77). There was no significant difference in terms of neutrophils and platelets engraftment (>500 and >20000/ µL, respectively).
Discussion: In this study we confirm the efficacy of AMD to mobilize and collect an adequate dose of CD34+ cells in pts with malignant lymphoma and MM without significant difference in term of engraftment in respect of pts mobilized with CHT and G-CSF. Based on our data, we developed and so apply in clinical practice an algorithm for optimal utilization of plerixafor (Figure 1).
Disclosure of Interest: None Declared.

PH-P255

AUTOLOGOUS STEM CELL COLLECTION IN LYMPHOMA AND MYELOMA PATIENTS: SINGLE CENTER ANALYSIS WITH INTENT TO SUCCESSFULLY MOBILIZE AND TRANSPLANT

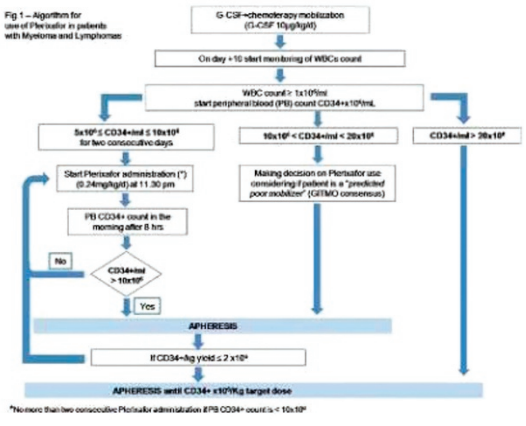
N. Hasbal[1,], H. Bilgen[2], F. Hindilerden[1], S. Serefhanoglu[3], S. Güvenç[1], R. Kucukkaya[4], E. Ozcelık[3], M. Arat[1]*
[1]Hematology, Istanbul Bilim University, [2]Apheresis Unit, Sisli Florence Nigtingale Hospital, [3]Hematology, Sisli Florence Nightingale Hospital, [4]Hematology, Istanbul Bılım University, Istanbul, Turkey

Introduction: High dose chemotherapy (HDCT) with autologous hematopoietic stem cell transplantation (HSCT) rescue is a potentially curative&consolidative therapy for advanced hematological malignancies, and it also permits the administration of higher doses of chemotherapy to overcome tumor cell resistance.In this study, our aim is to evaluate 167 consecutive myeloma (MM) and lymphoma (ML) patients referred to our center between August 2010 and May 2013.
Materials (or patients) and Methods: Patients data are analyzed in intent to successful hematopoietic stem cell (HSC) mobilization and collection. In our country we have limited access to plerixafor, as salvage HSC mobilizing agent, permitted only after a failed mobilization&collection trial of chemotherapy and G-CSF. Our center's policy is to collect HSC with G-CSF in all MM (exception of prolonged revlimide use and prior autologous HSCT), and non-heavily pretreated ML patients. Candidates for poor mobilization underwent first CT and G-CSF, and second line receive plerixafor.
Results: Under these circumstances 86 lymphoma patients (31 Hodgkin and 55 NHL) and 81 MM patients (F/M: 57/110, med. age 52, range 18-72) were included in this study. Approximately >15% of the patients received more than 2 cycles of chemother-

[PH-P254]

Table 1 Patients' demographic characteristics

	N° (%)	Median (min max)
N°	29	
NHL	19 (66)	
HD	2 (7)	
MM	8 (27)	
Hb		9.4 (7.2 D 13 D)
Sesso M/F	24/5	
Proven poor mobilizer	25 (86)	
Predicted poor mobilizer	4 (14)	
Disease status at mobilization		
Complete Remission	12 (41)	
Partial Remissione	11 (38)	
Advanced phase disease	6 (21)	
Previous chemotherapy courses		2 (1 – 5)
Mobilization regimen		
ARA-C containing regimen	16 (55)	
MDX HD	10 (35)	
Others	3 (10)	
Plerixafor (AMD) mobilization results		
WBC (x 10e3/µL) after AMD		20.9 (7.74-84.4)
Hgb (g/dL)		10.1 (7.80-13.3)
PLT (x 10e3/µL)		35.0 (15.0-368.0)
CD34/ µL before-AMD		6.6 (2.8-34.0)
CD34/ µL before apheresis		25.4 (6.3 160.98)
CD34/ µL after-apheresis		13.8 (2.10-191.36)
N. of apheresis		2 (1 3)
Time to collection (days)		16 (9-22)
Aphaeresis data		
Blood volume processed (L)		13.49 (5.65-18.89)
Procedure time (min)		320 (236 380)
CD34 collection efficiency (%)		67.73 (24.82-97.40)
MNC collection efficiency (%)		53.52 (17.74 91.42)
Platelet depletion efficiency (%)		4.11 (2.10-15.47)

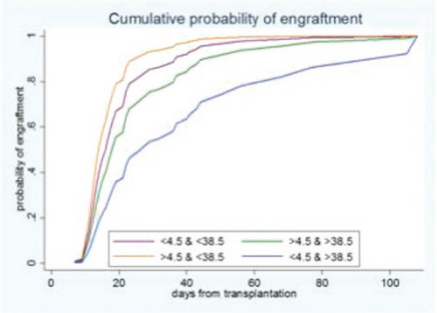


apy before HSC collection. Mobilization with G-CSF as a single agent resulted in optimal CD34+ cell yield for 121 (72%) patients. In myeloma G-CSF as first line resulted with 92.7% successful HSC mobilization and collection. Overall 17 patients received plerixafor as 2nd or 3rd line, and resulted with sufficient HSC collection in 57.3%. In three cases (MM:1, ML:2) additional support with autologous bone marrow collection necessary. Only in 9 (5.3%) patients all attempts for mobilization failed including plerixafor. After any type of mobilization regimen median count for pCD34+ cells obtained was 18/mcl. Median yield of 3.3 x 10^6/kg CD34+ cells/kg was collected with range of 0.2-33.9x10^6/kg in total apheresis sessions. MM patients have significant high levels of preapheresis circulating CD34+ count in comparison to ML patients (29 vs 15, *P*=0.001). pCD34+ cell did not correlate with body mass index, age, underlying disease and previous treatment cycles. There is a close correlation between pCD34+ cell count and collected CD34+ cells in all types of mobilization regimens as G-CSF, chemotherapy and plerixafor (relatively; *P*<0.001, *P*=0.002 and *P*=0.001). Successful ASCT is achieved in 144 patients transplanted so far. Mobilization is achieved in almost all multiple myeloma patients and most of lymphoma patients with only G-CSF based regimen in our cohort. Half of patients not mobilized with G-CSF were successfully mobilized with chemotherapy and plerixafor as second or third line regimen.
Discussion: The restricted use of plerixafor resulted in time and expense for additional chemotherapy and collection attempt, whereas this inconvenience did not impact the success of stem mobilization and collection in our current policy.
Disclosure of Interest: None Declared.

Stem cell Source

PH-P256
THE INFLUENCE OF AUTOLOGOUS GRAFT COMPOSITION ON ENGRAFTMENT
L. Pierelli[1,], M. Vacca[2], A. Pandolfi[2], P. Iudicone[2], R. Scocchera[2], D. Fioravanti[2], F. Ipsevich[2], P. Pezzotti[3], A. Severino[4], I. Majolino[4]*
[1]Experimental Medicine, Sapienza University, Rome, [2]Transfusion Medicine, San Camillo Forlanini Hospital, [3]Infectious, Parasitologica l,Immunological and Medical Diseases, National Institute of Health, [4]Hematology and Stem Cell Transplantation, San Camillo Forlanini Hospital, Rome, Italy

Introduction: Autologous peripheral blood stem cell (a-HPC-A) collection and transplantation are standard procedures in the treatment of patients with chemosensitive hematological malignancies. Abundant collection of a-HPC-A is assured by the combination of stem cell mobilization by growth factors (GF) and chemotherapy and apheresis collections. In case of major contamination of a-HPC-A collections by mature cells (polymorphonuclear leukocytes co-mobilized by GF) a putative detrimental effect on engraftment has been hypothesized.
Materials (or patients) and Methods: A patients series including 127 subjects suffering from lymphomas, myelomas and AML were retrospectively analyzed in platelet (plt) engraftment by an empiric algorithm that normalizes the time to plt > 20,000 after transplantation for the infused CD34 cell dose per kg. The algorithm is a simple mathematical tool which works as follows :pltEngNorm :[(pltEng*pltEng/100)+pltEng]*CD34kg/10, where pltEng is the time (days) to plt > 20,000 and CD34kg is the a-HPC-A dose in millions per kg of body weight. pltEngNorm gives, in this way, a new engraftment time that has been normalized for the CD34 dose and that may identify some other factors influencing engraftment. The application of the algorithm to our series gave access to a late engraftment series which had some common characteristics : diagnosis of non-Hodgkin lymphoma and a total nucleated cell (TNC) infused dose higher than 40 x10^9. Starting from these evidences, Cox analysis and Kaplan Meyer curves have been employed to confirm factors (CD34, TNC, gender, diagnosis, age) influencing engraftment.



Kaplan Meyer curves show the combined influence of the threshold doses of 4.5 x10^6/kg CD34+ cells and of 38.5 x10^9 total TNC in the graft on plt engraftment.

Results: Our 127 patients received a median of 5.81x10^6 CD34 cells/kg (range 2.01-13.02), experienced an actual engraftment after a median of 12 days (range 7-33) for polymorphonuclear leukocytes (PMN) and after 15 days (range 9-108) for plt. In this series, the median pltEngNorm was 10.62 (range 3.63-69.63) and 5 patients had a pltEngNorm > 30 (this threshold of pltEngNorm has been calculated adding, to the observed median value, a numeric figure calculated as the double of the observed standard deviation). As anticipated in the Method section, the patients' series with a delayed pltEngNorm had a very high dose of TNC infused with graft and, in 4 cases out 5, suffered from non-Hodgkin lymphoma. In the whole series, Cox analysis identified a dose equal to 38.5 x10^9 TNC or higher as a factor that reduces the probability of the engraftment by 50 %, while a dose equal to 4.5 CD34 x 10^6/kg CD34 or higher has been found able to increase by 50 % the likelihood of engraftment (Prob at chi^2 = 0.0010). No influence by diagnosis, age and gender has been observed. Kaplan Meyer curves plotted for these two variables show the effect of the combined influence of CD34 and TNC on plt engraftment (see figure).
Discussion: Our data indicate that an empiric algorithm is able to identify cases of autologous transplantation with an anomalous plt engraftment and may represent a useful tool to analyze the quality of graft and transplantation in an individual recipient. Cox analysis confirmed the reliability of the algorithm and has identified TNC content in the graft as a detrimental factor for plt engraftment, irrespective of CD34 cell dose infused. Future strategies to mobilize and collect a-HPC-A should take into account TNC contamination of the graft as an additional parameter able to influence engraftment, besides CD34 cell dose.
Disclosure of Interest: None Declared.

PH-P257
HLA-A, -B, -C AND -DRB1 HIGH RESOLUTION MATCHING CAN IMPROVE PATIENT' OUTCOME AFTER DOUBLE UMBILICAL ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT)
F. Malard[1,], K. Gagne[2], P. Chevallier[1], A. Devys[3], T. Guillaume[1], P. Herry[3], J. Delaunay[1], S. Castagnet[3], P. Moreau[1], M. Mohty[4], A. Cesbron[3]*
[1]Hematology department, CHU de Nantes, [2]Laboratoire HLA, EFS Pays de Loire, [3]Laboratoire HLA, EFS Pays de Loire, Nantes, [4]Hematology department, AP-HP Hopital Saint Antoine, Paris, France

Introduction: Umbilical cord blood cells (UCB) have emerged as an alternative stem cell source for allo-SCT for patients who lack a 10/10 HLA matched donor. In double UCBT (dUCBT), HLA typing is usually performed at low resolution (LR) for HLA-A and -B loci and at high resolution (HR) for HLA-DRB1 locus. However, in the setting of dUCBT, data regarding the effect of HR HLA matching between patient and UCB are scarcer. This analysis assessed whether HR matching at HLA-A, -B, -C and -DRB1 loci could be associated with improved outcome after dUCBT.
Materials (or patients) and Methods: We included 46 consecutive adult patients treated for hematological diseases. Twenty-five

patients were males (54%). The median age was 49 years (range, 16-68). Diagnoses included 20 AML, 7 MDS/MPN, 13 NHL, 2 HD, 4 ALL and 1 severe aplastic anaemia. Six patients received a myeloablative conditioning and 40 patients received a reduced intensity conditioning. HLA-A, -B, -C HR typing were retrospectively performed by sequencing on both UCB units. We compared patient' outcome according to the donor-recipient HLA-A, -B, -C and -DRB1 HR matching. We defined 2 groups, a group of 27 patients with high HLA matching, group "high" (24 UCB-recipient pairs matched at 5/8 or higher for both UCB, 3 UCB-recipient pairs matched at 4/8 for one UCB and at 6/8 or 7/8 for the second UCB) and a group of 19 patients with low HLA matching, group "low" (UCB-recipient pairs matched at 5/8 and 4/8 or less for both UCB).
Results: With a median follow-up of 30.0 months (range, 6.5-83.0), there is a trend towards improved better overall survival (OS) at 2 years in group "high" [69% (95%CI, 47-83%) versus 42% (95%CI, 18-64%) in group "low" *P*=0.07]. The estimate of progression-free survival (PFS) at 2 years was significantly higher in group "high" [62% (95%CI, 41-78%) versus 30% (95%CI, 11-51%) in group "low" *P*=0.02]. There was a trend towards a lower cumulative incidence (CI) of NRM in group "high" [12%, versus 39% in group "low" *P*=0.06]. Grade 3-4 acute GVHD and extensive chronic GVHD incidences were 11% versus 10% (*P*=0.99), and 4% versus 15% (*P*=0.25), in group "high" versus group "low", respectively. The CI of relapse was 23% in the group high versus 37% in the group low (*P*=0.07). The CI of neutrophil recovery at day 42 and platelet recovery at 6 months were 78% versus 89% (*P*=0.38) and 78% versus 84% (*P*=0.54) in group "high" versus group "low", respectively. In multivariable analysis including the most important parameters associated with outcome (patient's age at transplant, patient's sex, disease status, conditioning regimen, TNC), HLA matching (high versus low) was the only parameter with a significant impact on OS (HR=0.31 (95% CI, 0.12-0.80); *P*=0.02). Regarding impact of HLA matching at HLA-A,-B LR and –DRB1 HR, UCB-recipient pairs matched at 5/6 or higher and the other were comparable regarding OS and PFS.
Discussion: These data suggest that in contrast to standard HLA matching, HR HLA matching at HLA-A, -B, -C and –DRB1 loci affect patient outcome after dUCBT and overcome impact of cell doses regarding OS in multivariate analysis. This highlight the need to reassess the current strategy for UCB unit selection in the setting of dUCBT, where HLA-A and -B typing should be performed at the allele level and matching at HLA-C should be included.
Disclosure of Interest: None Declared.

PH-P258
EFFICACY AND FEASIBILITY OF UMBILICAL CORD BLOOD TRANSPLANTATION WITH MYELOABLATIVE NON-TBI CONDITIONING REGIMEN USING FLU180/IVBU12.8/MEL80 FOR ADULT PATIENTS WITH ADVANCED HEMATOLOGICAL DISEASES

N. Nakano[1,], A. Kubota[1], M. Tokunaga[1], S. Takeuchi[1], Y. Takatsuka[1], A. Utsunimiya[1]*
[1]Department of Hematology, Imamura Bun-in Hospital, Kagoshima, Japan

Introduction: Umbilical cord blood transplantation (UCBT) is widely practiced for advanced hematological diseases, recently. With respect to umbilical cord blood transplantation with myeloablative non-TBI conditioning regimen by fludarabine (Flu), intravenous busulufan (ivBU), and melphalan (Mel), we examined efficacy and feasibility of it for advanced hematological diseases. Similarly, we made a comparative examination of allogeneic bone marrow transplantation (BMT) with ivBU based myeloablative non-TBI conditioning regimen in the study.
Materials (or patients) and Methods: From April 2010 to September 2013, 45 adult patients (33 BMT, 12 UCBT), who were suffered from advanced hematological disease, were consecutively undergone allogeneic BMT or UCBT with myeloablative non-TBI conditioning regimens using ivBU at Imamura Bun-in Hospital. We excluded early death (<30days) from the study. Advanced hematological disease was defined as follows: a disease diagnosed as non-CR hematological malignancies and int-2 or high-risk MDS in IPSS. Conditioning regimens were conducted with ivBU 12.8/kg and CY 120 mg/kg (BU/CY) in most of allo-BMT patients. Flu 180mg/m^2, ivBU 12.8 mg/kg and Mel 80mg/m^2 (Flu/BU/Mel) were administered for all of UCBT patients. In addition, Flu/BU/Mel or Flu/BU±AraC 500 mg-2000 mg were administered to some of BMT patients. We studied days from diagnosis to HSCT, engraftment of neutrophil (EN), overall survival (OS), and incidence of transplant related mortality (TRM), respectively. OS were analyzed with Kaplan-Meir method. Cumulative incidence of EN and TRM at 100 days after HSCT was analyzed with Gray's method. Statistical significance defined *P*<0.05 in logrank test. Statistical analysis was carried out using the R commander.
Results: Median study observation period was 475 (114-1980) days. Median age at transplantation was 53 (20-65) years. Fourteen of 45 patients were AML (8 BMT, 6 UCBT), 11 MDS (11 BMT), 17 ATL (13 BMT, 4 UCBT), 2 ML (1 BMT, 1 UCBT), and 1 ALL (1 UCBT) respectively. No statistical difference was seen between BMT and UCBT in age, gender and diseases. Twenty-two patients (22 BMT) using BU/CY as conditioning regimen, 16 Flu/BU/Mel (4 BMT, 12 UCBT), and 7 Flu/BU±AraC (7 BMT), respectively. Median time from diagnosis to HSCT was 187 (114-1980) days, and UCBT was significantly shorter than BMT (215 days vs 122 days; *P*=0.026). Cumulative incidence of EN was 100% (33/33) of BMT patients and 91.7% (11/12) of UCBT. OS at 1 yr and 2yrs of each source was 80.7% vs 58.3% and 66.7% vs 27.8% (*P*=0.009), respectively. However, no statistical difference of OS was observed in AML patients (8 BMT, 6 UCBT) between BMT and UCBT (1yr OS; 87.5% vs 67.5%, 2yrs OS; 43.8% vs 50%, *P*=0.719). Incidence of grade I acute GVHD was positively contributed to OS (*P*=0.049). Cumulative incidence of TRM at day100 was 15.2% vs 22% (*P*=0.026).
Discussion: In this study, we found that UCBT was significantly shorter than BMT for days from diagnosis to transplant. Our findings suggest that UCBT with myeloablative non-TBI conditioning regimen by Flu/BU/Mel would have efficacy, especially in advanced AML patients. It was also observed that cumulative incidence of EN reached 91% and TRM at day100 was managed within 22%. Our results show that the administration of Flu/BU/Mel can be a feasible conditioning regimen for UCBT.
Disclosure of Interest: None Declared.

PH-P259
COMPARISON OF UMBILICAL CORD BLOOD AND HAPLOIDENTICAL DONOR GRAFTS IN ADULTS WITH HIGH RISK HEMATOLOGIC DISEASES AFTER FLUDARABINE CYCLOPHOSPHAMIDE AND TBI 2 GY BASED REDUCED-INTENSITY CONDITIONING REGIMEN STEM CELL TRANSPLANTATION

J. Elcheikh[1,], R. Crocchiolo[2], S. Furst[1], S. Bramanti[2], B. Sarina[2], A. Granata[1], C. Faucher[1], B. Mohty[1], S. Harbi[1], R. Bouabdallah[3], N. Vey[3], A. Santoro[2], C. Chabannon[3], L. Castagna[2], D. Blaise[1]*
[1]Transplantation, Institut Paoli Calmettes, Marseille, France, [2]Transplantation, Humanitas Cancer Center, Milan, Italy, [3]Hematology, Institut Paoli Calmettes, Marseille, France

Introduction: For patients without a suitably matched related donor, Alternative donors such as mismatched unrelated, cord blood and mismatched family donors could be searched. The aim of this retrospective study was to compare the results of graft source on outcome of patients after haploidentical related donor (Haplo), and the unrelated umbilical cord blood (UCB) transplantation in the setting of Non myeloabalative conditioning regimen (NMA).
Materials (or patients) and Methods: We retrospectively analyzed outcomes in 150 adult patients with high risk hematologic diseases who received Allo-SCT from alternative donors from two centers (Institut Paoli-Calmettes at Marseille France and Humanitas cancer center at Rozzano, Italy): These two centres have been applying common transplant approaches and procedures during the study period. 69 patients received Haplo and 81 patients received UCB. In the UCB group, the NMA regimen consisted of fludarabine (Flu), cyclophosphamide (Cy) and low dose TBI (2 Gy)

combination in the two groups. The GVHD prophylaxis consisted of Cyclosporine A (CsA) and MMF in all patients in the two groups. In the Haplo group all patients received also 50 mg/kg Cy at day 3 and 4 post transplant. Of note, supportive care was the same during the whole study period. CMV infection management was also homogeneous.
Results: With a median follow-up of 59 months (8-101) and 18 months (3-51), in the UCB group versus the Haplo group, respectively. Nine patients (11%) in the UCB and 8 patients (12%) in the Haplo group had a spontaneous autologous reconstitution subsequent to primary graft failure. The median times to neutrophil and platelet recovery were 20 d (14–39) and 29 d (14–50) after Haplo and 22 d (6–67) and 41 d (18–80) after cord blood. All supportive care measures included red blood cell, and platelet transfusions were significantly increased in cord blood transplantation group. The cumulative incidence of transplant related mortality (TRM) at one year was 23% in the UCB group versus 17% in the Haplo group (*P*=0.39). Grade 2-4 acute graft-vs.-host disease (GVHD) and extensive chronic GVHD incidences were 52% versus 29% (*P*=0.05), and 12% versus 6% (*P*<0.0001), in the UCB group versus the Haplo group, respectively. The Kaplan-Meier estimate of overall survival at 2 years was 45% (95%CI, 34-56%) in the UCB group versus 69% (95%CI, 58-80%) in the Haplo group, (*P*=0.10). The estimate of progression-free survival at 2 years was 36% (95%CI, 25-47%) in the UCB group versus 65% (95% CI, 53-77%) in the Haplo group (*P*=0.01).
Discussion: In this study, relapse and PFS was lower in Haplo group than in UCB transplants. The main difference between the 2 groups was the significantly higher incidence of acute and chronic GVHD in the UCB group. Surprisingly, this did not translate into a decrease of the cumulative incidence of OS nor increase of the TRM, which may be explained by a slightly shorter follow-up in this group. Our results suggest that haploidentical transplants are a good and promising alternative option for patients with high risk hematological diseases who lack an HLA-matched donor (sibling or unrelated donor). This should be now investigated in prospective comparative studies.
Disclosure of Interest: None Declared.

PH-P260
OUTCOMES OF OLDER PATIENTS UNDERGOING 2 STEP APPROACH TO HAPLOIDENTICAL AND MATCHED RELATED PERIPHERAL BLOOD HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT): A SINGLE INSTITUTIONAL EXPERIENCE

S. Gaballa[1,], M. Carabasi [1], J. Filicko[1], S. Alpdogan[1], J. Wagner[1], S. Rosado[1], S. Rudolph[1], M. Kasner[1], U. Martinez[1], M. Weiss[1], N. Flomenberg[1], D. Grosso[1]*
[1]Department of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University Hospital, Philadelphia, United States

Introduction: HSCT is a curative option for many patients (pts) with hematological malignancies. Significant advances in supportive care and conditioning regimens over the past decade have allowed the extension of this therapy to older individuals. Information regarding the outcomes of this older subset of pts undergoing HSCT is limited, especially those undergoing haploidentical (HI) HSCT. We report on the outcomes of pts 60 years of age or older undergoing HI and matched related (MR) HSCT.
Materials (or patients) and Methods: We did a retrospective review of pts 60 years of age or older enrolled on our 2 step HI or MR HSCT trials. Conditioning consisted of a myeloablative regimen (MA) with 12 Gy of TBI over 4 days, or a reduced intensity regimen (RIC) with Fludarabine/Cytarabine or Thiotepa followed by 2 Gy TBI. Immediately after the TBI in both regimens, a large fixed dose of allogeneic T cells were infused (2 x 10^8/kg), resulting in an alloreaction limited to pts undergoing HI HSCT, and characterized by high fevers and in some cases, rash and diarrhea. Two days later, cyclophosphamide (CY) was administered daily x 2 to eradicate alloreactive T cells and induce bidirectional tolerance. Using this method, non-alloreactive T cells remain, forming the basis for early immune reconstitution and avoiding significant GVHD A CD34 selected stem cell product was infused after CY.
Results: Of 182 pts undergoing HSCT on one of the 2 step protocols, we identified 62 pts (40 males, 22 females) who were ≥ 60 years of age. The majority of pts received a HI HSCT (90%) with 34 pts ≥ 65 years old (55%). The median age of the donors was 41 years (range: 19-70). The median CD34 cell dose infused was 4.4 x 10^6/kg. Twenty-Eight pts (45%) underwent MA HSCT and 34 pts (55%)(mostly >65 years, *n*=26, 77%) underwent RIC HSCT. Forty Four pts had active disease at the time of HSCT [AML/MDS=29 (47%), NHL=13 (21%), other=2 (3%)]. After a median follow-up of 8 months (range 1-74), 57% of pts were alive with a relapse rate of 21%. Non-relapse mortality rate was 26% [infection (8%), regimen related toxicity (13%) and GVHD (5%)] while relapse related mortality rate was 18%. Factors that correlated with favorable survival (spearman, *P*=0.05) included KPS (≥90%), HCT-CI score (<3), and absence of active leukemia at the time of HSCT. Pts with active lymphoma at the time of transplant had a probability of survival of 76% at 60 months compared with 22% in pts with active AML or MDS. No rejections or engraftment failures were observed. GVHD was controlled in all cases with steroids and/or photopheresis.

	MA	RIC
Median Recipient Age (range)	63 (60-68)	68 (60-78)
KPS 90-100%	19 (68)	22 (65)
HCT-CI= >3	15 (54)	19 (56)
aGVHD III-IV	0	3 (9)
cGVHD	3 (10)	0
Diagnosis: AML/MDS	23 (82)	20 (59)
Diagnosis: ALL	1 (4)	1 (3)
Diagnosis: NHL	4 (14.3)	11 (32)
Diagnosis: Other	0	2 (6)

Discussion: HI or MR HSCT on the 2 step approach is associated with acceptable outcomes in older pts. Age and lack of a MR donor should not be barriers to HSCT if pts are fit. Patients with lymphoma and controlled myeloid malignancies fared better in this older population.
Disclosure of Interest: None Declared.

PH-P261
IS HLA-G -725 G/C/A POLYMORPHISM ABLE TO PREDICT THE LEVEL OF SOLUBLE HLA-G ? PERSPECTIVES IN CORD BLOOD TRANSPLANTATION

I. Sbarsi[1,], P. Bergamaschi[1], C. Badulli[1], V. Genovese[1], A. Marchesi[1], A. Pasi[1], B. Romano[1], C. Tinelli[2], M. Zorzetto[3], M. Martinetti[1], L. Salvaneschi[1]*
[1]Servizio di Immunoematologia e Medicina Trasfusionale, [2]Unità di Biometria e Statistica, [3]Laboratorio di biochimica e genetica delle malattie dell'apparato respiratorio, Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy

Introduction: HLA-G is a non-classical HLA class I molecule which plays a crucial role in induction and maintenance of tolerance in pregnancy as well as in the transplant setting. In keeping its function essential for life, HLA-G gene is poorly polymorphic in coding sequences, while the unexpected extraordinary rate of variation in the 5'upstream regulatory region indicates a great importance of HLA-G cell surface expression and protein release. The HLA-G -725 G/C/A polymorphism (rs1233334) is located in the promoter region of HLA-G gene closely flanking an IRF (interferon response factor-1) binding motif. Several authors have previously hypothesized differences in the transcriptional properties of the HLA-G -725 G and -725 C alleles although with no functional data supporting this.
Materials (or patients) and Methods: HLA-G -725 G/C/A polymorphism genotyping was performed on 88 DNA samples from cord blood (CB) units stored at the Pavia CBB (Cord Blood Bank) by the means of PCR-SBT (PCR - Sequence Based Typing).

HLA-G -725 G/C/A	N	Conc_sHLA-G (ng/ml) mean	sd	
AC	1	33,48	.	
AG	12	36,54233	24,34642	
CC	2	27,93	6,646804	
GC	24	37,96375	26,19727	
GG	49	44,3898	29,09805	
Total	88	41,06907	27,20953	

sHLA-G levels were determined, by ELISA test using a commercial kit (Exbio, Praha, Czech Republic), in the plasma's samples of the corresponding CB units.
Results: HLA-G -725 G/C/A allelic frequencies determined in CB units from the Pavia CBB were comparable with those from Caucasian controls described in previous papers (*T. Vauvert et al, J Hum Imm, 2005*) thus sanctioning the presence of the third variant "A allele" in the population of our region. Data referring to sHLA-G levels in CB's plasma, splitted up by the means of the HLA-725 G/C/A polymorphism genotype, are reported in Table1 (sd= standard deviation).
Discussion: In this work, even if preliminary, we demonstrate the existence of a correlation between the HLA-G -725 G/C/A promoter polymorphism and levels of sHLA-G detected in CB units stored at Pavia CBB. In particular, combining our molecular data on HLA-G -725 polymorphism and serological ones on sHLA-G levels we could report differences in HLA-G gene transcriptional properties in individuals carrying the -725CC genotype compared with those characterized by the -725GG one. This is probably due to the introduction of an additional methylated cytosine on a CpG nucleotide in presence of the -725C allele which may down regulate transcription of the HLA-G gene itself. CB is an alternative hematopoietic stem cell source for allogeneic transplantation in patients affected by hematological and non hematological diseases. HLA-G is physiologically expressed throughout pregnancy and is contained in CB and may play a crucial role of immunomodulator in the transplant setting. To this, genotyping of the CB units for the investigated polymorphism could be useful to predict the content of sHLA-G in CB and have an impact on donor selection.
Disclosure of Interest: None Declared.

PH-P262

FIRST APPLICATION OF THE EBMT RISK SCORE IN DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION FOR HEMATOLOGICAL MALIGNANCIES: SIGNIFICANT IMPACT ON DIFFERENT OUTCOMES

H. Labussière[1,], M. Sobh[1], M. Robin[2], N. Fegueux[3], S. Furst[4], M. Mohty[5], I. Yakoub-Agha[6], E. Marry[7], V. Rocha[2], D. Blaise[4], N. Milpied[8], F. E Nicolini[1], M. Michallet[1]*

[1]Hematology, Centre Hospitalier Lyon Sud, Pierre Bénite, [2]Hematology, Saint-Louis Hospital, Paris, [3]Hematology, Hôpital Lapeyronie, Montpellier, [4]Hematology, Institut Paoli Calmettes, Marseille, [5]Hematology, Hopital Saint-Antoine, Paris, [6]Hematology, CHRU Lille, Lille, [7]France Greffe de Moelle, Agence de la biomédecine, St-Denis, [8]Hematology, University Hospital of Bordeaux, Bordeaux, France

Introduction: The European Group for Blood and Marrow Transplantation (EBMT) risk score has never been applied in double umbilical cord blood transplantation (dUCBT). We recently published results of 136 patients who underwent dUCBT reported to the SFGM-TC registry from 23 centers between 2005 and 2007 (Labussière *et al.*, Exp. Hem. 2013). After having recorded the full data on CB units including HLA and sex, we decided to test the validity of the EBMT risk score and check its application in dUCBT.
Materials (or patients) and Methods: There were 88 males and 48 females with a median age of 41 years (range: 18 – 66), 42 AML, 27 ALL, 5 secondary AL and 2 Biphenotypic AL; 8 with chronic leukemias (5 lymphoid and 3 myeloid), 10 with MDS, 24 with Hodgkin and non Hodgkin lymphomas, 13 with multiple myelomas and 5 with other myeloproliferative diseases. Median interval between diagnosis and transplantation was 20.5 months (range: 3-385). At time of allo-HSCT, 41 patients were in CR1, 40 in ≥CR2 and 55 less than CR. Forty-six patients received MAC and 90 RIC. Age was categorized as <20 years (0 pt; *n*= 6), 20 to 40 years (1 pt; *n*= 59) and >40 years (2 pts; *n*=71). For disease status we distinguished patients in CR1 at transplantation, (0 pt; *n*=41) from patients in CR >1 (1 pt; *n*=40) and patients not in CR (2 pts; *n*=55). Time from diagnosis to transplant was categorized into ≤12 months (0 point (pt); *n*=26) and >12 months (1pt; *n*=110). The adaptation of the new risk score concerned HLA and sex matching. HLA matching showed patients with at least 4/6 HLA matching on HLA-A, -B and DRB1 without any mismatch on DRB1 alleles between the recipient and each UCB unit and the 2 units together (0 pt; *n*=77); patients with at least 4/6 HLA matching on HLA-A and -B between the recipient and each unit but without complete matching on DRB1 or with less than 4/6 HLA matching between the 2 units (1 pt; *n*=51), and finally patients with more than 2 mismatches on the three loci (2 pts; *n*=8). Donor-recipient sex combination separated all others (0 pt; *n*=73) from the male recipient with one female and one male cord blood unit (1pt; *n*=45) and male recipient with two female cord blood units (2 pts; *n*=18). Hence, we found 5 patients (4%) in score 1, 12 (9%) in score 2, 23 (17%) in score 3, 26 (19%) in score 4, 30 (22%) in score 5, 26 (19%) in score 6, 11 (8%) in score 7 and 3 (2%) in score 8. We pooled patients with scores 1-2; 3-4 and 5-8 as their outcomes were found to be similar.
Results: After a median follow-up of 49.5 months, the 3 years probabilities of overall and progression-free survivals for the whole population were 41% and 35% respectively. with a two-year cumulative relapse incidence of 28% (24.0 – 31.8). Interestingly patients with score 1-2 showed better overall survival rates than those with score 3-4 and 5-8 with a 3 years probability of 77%, 42% and 32% respectively (*P*=0.002); this was associated with a 3 years relapse incidence of 19%, 35% and 55% respectively (*P*=0.021), while the 3 years transplant related mortality (TRM) was 6%, 39% and 36% respectively (score 1-2 versus all others, *P*=0.02).
Discussion: In conclusion, we found that the EBMT risk score can perfectly be applied to double UBCT with a significant impact on different outcomes; its use enables a better selection of patients who will benefit the more from this treatment strategy.
Disclosure of Interest: None Declared.

PH-P263

DETERMINANTS OF ENGRAFTMENT AND SINGLE-UNIT PREDOMINANCE AFTER DOUBLE UMBILICAL CORD BLOOD (DUCB) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN ADULTS: HHV-6 REACTIVATION DURING APLASIA, LOWER UNIT-UNIT HLA MATCHING AND YOUNGER UCB UNIT AGE MAY REPRE

A. Le Bourgeois[1,], T. Guillaume[1], D. Jacques[1], Y. Foucher[2], B. Tessoulin[1], F. Malard[1], C. Precupanu[1], P. Peterlin[1], S. Derenne[3], P. Herry[3], A. Cesbron[4], K. Gagne[3], L. Lode[5], M. Illiaquer[6], B.-M. Imbert-Marcille[7], S. Le Gouill[8], P. Moreau[1], M. Labopin[9], M. Mohty[9], P. Chevallier[1]*

[1]Hématologie Clinique, Centre Hospitalier et Universitaire (CHU), [2]INSERM U1064, Université de Nantes, [3]Laboratoire d'histocompatibilité immunogénétique, EFS Pays de la loire, [4]Laboratoire d'histocompatibilité immunogénétique EFS, CHU, [5]Laboratoire d'hématologie, Centre Hospitalier et Universitaire (CHU) de Nantes, [6]laboratoire de virologie, 1Centre Hospitalier et Universitaire (CHU) de Nantes, [7]Laboratoire de virologie, Centre Hospitalier et Universitaire (CHU), [8]Hématologie Clinique, 1Centre Hospitalier et Universitaire (CHU), Nantes, [9]hématologie clinique, Hôpital saint Antoine, Paris, France

Introduction: Only few factors that predict engraftment or single-unit predominance after double umbilical cord blood (dUCB) allogeneic stem-cell transplant (allo-SCT) have been reported so far. Our main objective for this study was to determine new factors associated with engraftment and single-unit predominance after dUCB allo-SCT.

Materials (or patients) and Methods: This retrospective study included 77 patients (male: *n*=40; median age: 52 years, myeloid diseases *n*=43; complete remission at transplant *n*=46, previous autograft *n*=20) who had received a dUCB allo-SCT between June 2006 and December 2012 at the CHU of Nantes (France). The majority of patients received a reduced-intensity conditioning regimen (*n*=69) and the median interval between diagnosis and transplant was 11 months. Cumulative incidence (CI) of engraftment was 78±4% at day 60.

Results: With a median follow up of 40 months for survivors, 3-year OS, DFS, RI and TRM were 55+-6%, 44+-6%, 33+-5% and 23+-4%, respectively. CI of grade II-IV and III-IV acute GVHD were 27+-5% and 9+-3%, respectively. 3-year CI of chronic GVHD was 26+-5% (limited *n*=14; extensive *n*=5). In multivariate analysis, HHV6 reactivation during aplasia (HR=2.63; 95% CI: 1.64-4.17; *P*<0.001), younger recipient age (< 53 years; HR=1.97; 95% CI: 1.16-3.35; *P*=0.012) and lower HLA matching between the 2 units (3/6 or 4/6, HR=2.09; 95% CI: 1.22-3.59; *P*=0.013) were the three factors independently associated with graft failure. Also, in multivariate analysis, factors predicting the losing unit were the younger age of the CB unit (continuous, HR=1.01; 95% CI: 1-1.02; *P*=0.03), a lower CD34+ cell doses contained in the CB unit after thawing (<=0.8 10^5/Kg; HR=2.55; 95% CI: 1.05-6.16; *P*=0.04) and the presence of an ABO incompatibility between the CB unit and the recipient (OR: 2.53; 95% CI: 1-15-5.53; *P*=0.02).

Discussion: Thus, HHV-6 reactivation during aplasia, lower unit-unit HLA matching, and younger cord blood age, as new unfavorable predictive factors, may represent parameters to take into account after dUCB allo-SCT. These results have to be confirmed prospectively as they may influence units selection and outcomes of patients.

Disclosure of Interest: None Declared.

PH-P264

THE NUMBER OF TOTAL NUCLEATED CELLS AND CD34 CELLS IN BONE MARROW HARVESTS ARE BETTER PREDICTORS FOR ENGRAFTMENT THAN NUCLEATED CELL COUNTS IN BONE MARROW GRAFTS AFTER CELL CONCENTRATION AND/OR VOLUME REDUCTION

J. Overdevest[1], A. Lankester[2], P. von dem Borne[3], Y. Rozier[1], H. Bakker-Steenveld[2], J. J. Zwaginga[1], T. Netelenbos[1,]*

[1]immunohematology and blood transfusion, [2]Pediatric Stem Cell Transplantation, [3]Hematology, LUMC, Leiden, Netherlands

Introduction: In contrast to peripheral blood stem cell grafts, number of total nucleated cells (TNC) per kg is used internationally when a request for a bone marrow graft is made, because TNC has been shown to be an important prognostic factor for bone marrow transplantation. At our stem cell laboratory two techniques are used to concentrate cells or reduce volume from bone marrow harvests: 1) manual concentration (centrifugation of flasks) or 2) mechanical concentrating by the COBE® Spectra Apheresis System. The first method is used for small volumes (<500 ml) in combination with a small recipient. The second method is used for large volume grafts or major ABO-incompatibility. The manual concentration method hardly influences the amount of TNC, whereas using the mechanical concentration method results in enrichment of mononuclear cells (MNC). We questioned whether MNC counts post-processing would be superior to TNC with respect to prediction of engraftment and would therefore be a better parameter for quality control of the processing procedure.

Materials (or patients) and Methods: We retrospectively analyzed all allogeneic bone marrow harvests processed in our lab between January 2011 and November 2013. The following graft parameters were collected: volume, TNC, TNC/kg (before and after processing), MNC, MNC/kg CD34, CD34/kg, and engraftment data in the recipients: days of neutrophil recovery > 0.5 x 10^9/l and platelet recovery > 20 and > 50 x 10^9/l.

Results: Eighty patients (73 children and 7 adults) were transplanted with bone marrow grafts. In 54% bone marrow was manually concentrated. The total TNC count was reduced from a median of 8.4 before to 7.1 x 10^9 after concentration. Using mechanical concentration the TNC decreased on average with 58.9% from a median of 13.1 to 5.1 x 10^9; the MNC decreased with 12% and CD34 cells with 9.3%. TNC and CD34 cells in the harvest correlated well (Pearson correlation coefficient 0.75; *P*<0,0001).

In the evaluable patients (*n*= 73) 4 died before engraftment. Two non-engraftments were observed. All other patients engrafted with a median neutrophil recovery of 22 days. In 8 patients platelet recovery (> 20 x 10^9/l) was not reached. Of these, 5 received another transplant. The median platelet recovery in the remaining patients was 26 (> 20 x 10^9/l) and 31 days (>50 10^9/l).

The graft TNC and MNC/kg counts after processing were found not to correlate with engraftment. However, pre-processing TNC/kg showed correlation with platelet recovery: when TNC was >= 5,5 x10^8/kg (26% of patients), 100% reached platelet recovery > 20 x 10^9/l compared to 82% in the group with TNC counts < 5,5 x 10^8/kg. This was also shown with CD34 cells using a cut-off value of 3.5 x 10^6/kg infused.

Discussion: We show that the amount of TNC in the graft is greatly reduced when mechanically concentrated, whereas MNC and CD34 are hardly affected. However, post-processing TNC and MNC/kg are not correlated with engraftment parameters. The pre-processing TNC counts and CD34/kg are the only predictors for platelet engraftment. Therefore, we recommend to use pre-processing TNC counts as quality parameter of the bone marrow harvest, as is current practice and to use CD34 pre- and post-processing as quality control for the concentration procedures.

Disclosure of Interest: None Declared.

PH-P265

ENGRAFTMENT AND OUTCOME AFTER ASCT WITH PLERIXAFOR-MOBILZED STEM CELLS IN POOR MOBILIZERS

D. Nachbaur[1,], O. Angelova[1], A. Ditlbacher[1], S. Schmidt[1]*

[1]Internal Medicine V, Hematology & Oncology, Innsbruck Medical University, Innsbruck, Austria

Introduction: The CXCR4 chemokine receptor antagonist plerixafor (Mozobil®) is a novel stem cell mobilizer which is indicated for use in combination with G-CSF to enhance mobilization of hematopoietic stem cells to the PB for collection and subsequent ASCT in adults with lymphoma or MM who are poor mobilizers.

Materials (or patients) and Methods: Between Nov 2008 and Oct 2013, 71 patients (median age 51 y; 65% male pts.) were mobilized with plerixafor in conjunction with G-CSF (7.5 µg bid alone (*n*=46) or after chemotherapy plus G-CSF (*n*=25). Diagnoses were MM and lymphoma (*n*=31 each), solid tumor (*n*=6) and three patients with acute lymphocytic leukemia. Indications for

Plerixafor use were (i) a CD34 cell count ≤ 20/μL in PB on day 4 of mobilization with G-CSF alone (ii) a CD34+ cell count ≤20/μL after chemomobilization despite administration of G-CSF for at least 4 days and a rise in WBC ≥5.0 G/L (iii) a CD34+ cell count ≤1x10^6/kg collected after the 1st leukapheresis. Apheresis targets were 2x10^6/kg CD34+ cells in pts. with lymphoma, solid tumor and ALL, and ≥4x10^6/kg in pts. with MM. Ninety-four pts. with lymphoma or MM who were successfully collected w/o plerixafor receiving a 1st ASCT between Nov 2008 and Sept 2013 served as control.
Results: Apheresis targets were reached in 68/71 (96%) pts. with a 4.9-fold increase in the PB CD34+ cell count after the first plerixafor administration resulting in a median CD34+ cell number collected of 5.19x10^6/kg. Until Oct 2013, 53/71 (75%) plerixafor-mobilized pts. received a 1st ASCT. The median transplanted CD34+ cell number was 4.02 (range, 1.6-29.34) x 10^6/kg in the plerixafor group and 4.01 (range, 1.69-43.69) x 10^6/kg in the control group (*P*=n.s.). The median time to neutrophil engraftment was 11 days in both groups (range, 8-20 in the plerixafor group and 8-14 days in the control group, *P*=n.s.). The median time to PLT engraftment was 12 days in both groups (range, 9-35 days in the plerixafor group and 8-35 days in the control group, *P*=n.s.). The median number of red cell transfusions was 2 in both groups (range, 0-24 in the plerixafor group and 0-14 in the control group, *P*=n.s.). The median numbers of PLT transfusions were 3 (range, 0-54) in the plerixafor group and 2 (range, 0-18) in the control group (*P*=n.s.). After a median observation time of 11 (range, 1-59) months in the plerixafor group and 21 (range, 1-58) months in the control group EFS at three years was 20% (0-44, 95% CI) in the plerixafor group and 47% (35-59, 95% CI) in the control group (*P*=n.s., log-rank). OS at three years was 79% (66-91, 95% CI) in the plerixafor group and 71% (59-83, 95% CI) in the control group (*P*=n.s., log-rank).
Discussion: This retrospective single center analysis demonstrates that the use of plerixafor-mobilzed HSC for ASCT results in similar engraftment kinetics as well as similar postransplant outcomes in poor mobilzers compared with normal controls. Long-term FU is needed to assess the risk of therapy-related secondary malignancies.
Disclosure of Interest: None Declared.

PH-P266
UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION (UCBT) IN ADULTS: SINGLE CENTRE EXPERIENCE USING CORD BLOOD UNITS SOURCED FROM UK CORD BANKS

S. Tulpule[1,], L. Watson[1], L. Pearce[1], H. Kaur[1], S. Gomez[2], E. Das-Gupta[1], J. Byrne[1], N. Russell[1]*
[1]Nottingham University Hospital, [2]Anthony Nolan Cord Bank, Nottingham, United Kingdom

Introduction: Umbilical cord blood (UCB) as an alternative stem cell source for transplantation is well established.In addition to being available 'off the shelf', HLA matching is less restrictive.However its limited stem cell dose can have an impact on engraftment, TRM and OS. The use of 2 cord units has successfully overcome this limitation. RIC has extended this procedure to elder patients and those with comorbidities. In the UK, UCBT has been used in adults since 2000. Efforts were made to put in place a national cord blood programme. Querol *et al* suggested an inventory of 50,000 units of more than 9 x10^8 TNC in order to provide up to 6 potential units with a 5/6 HLA match for 75% of the patients with a median body weight of 50 kg. National guidelines were published in 2009. We describe our experience of UCBT in adults using units sourced from UK cord banks (NHSBT and Anthony Nolan CBB).
Materials (or patients) and Methods: 9/10 patients received the Minnesota RIC regimen (Flu 200mg/m^2, Cyclo 50mg/kg, TBI 2Gy) with CyA and MMF for GVHD prophylaxis.1 patient had MA conditioning. Cord blood units were selected as per national guidance being at least 4/6 matched at low resolution for HLA-A, -B and at least 1 allelle matched for DRB1. Whole blood chimerism was performed at days 14,21,28,45,60,100,180,270,360 and 3 monthly thereafter post transplant.
Results: Median age was 63.5yrs (range:45–67). Disease indications-AML-8,FL-1 and CLL-1.Median total cell dose infused (cord 1+2) for TNC was 5.5 x10^7/kg and for CD34 was 2.3 x 10^5/kg. The 'winning' unit had a median unit:recipient match of 5/6 (range 4-5/6), with a median TNC of 2.35x10^7/kg (range 1.42-3.50) and CD34 of 1.3x10^5/kg (range 0.37-3.20).The 'losing' unit had a median unit:recipient match of 4/6 (range 4-5/6), with a median TNC of 2.25x10^7/kg (range 1.67-3.70) and CD34 of 0.97x10^5/kg (range 0.28-2.00). The degree of HLA matching was statistically significant for the winning unit (*P*= 0.033, paired t test).Median duration of follow up is 11 mnths (range 4-11). All patients engrafted. ANC recovery to >0.5 x 10^9/L occurred at a median of 21.5 (range 10-28) days and platelets to >20 x10^9/L at a median of 39 (range 25-49) days. 7/10 patients developed mild grade1-2 acute GvHD. Of these 2/7 developed grade 1-2 acute GvHD of the gut controlled with topical (1/2) and oral (1/2) steroids. All 7/7 patients received topical steroid to control skin GvHD. Only 3/10 patients had CMV reactivation requiring treatment.Chimerism analysis revealed that the winning unit could be predicted as early as D14. By D21 all 10/10 patients were 100% donor chimeric. However 2/10 patients remain dual chimeric with both cord units until last time point 9 months and 3 months post transplant respectively.
Discussion: This is the first report of adult UCBT in the UK who have received cord units from UK cord banks.
1. The winning unit was clearly evident by day 14 with the losing unit contributing towards engraftment.
2. The winning units were better matched suggesting a role for underlying immunogenetic factors in engraftment
3. Most patients developed grade 1-2 GvHD with no disease relapse demonstrating a good allogeneic response and GvL effect.
4. Factors governing the emergence of stable dual chimerism and its relevance are still unclear and need further investigation
5. The good engraftment times provide support to the quality initiatives of the UK CBBs.
Disclosure of Interest: None Declared.

PH-P267
NO SIGNIFICANT ADVANTAGE OF PERIPHERAL BLOOD STEM CELLS OVER BONE MARROW FOR MYELOABLATIVE CONDITIONING UNRELATED DONOR TRANSPLANTATION IN ADULTS WITH HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA

S. Lee[1,], K.-S. Eom[1], Y.-J. Kim[1], J.-A. Kim[1], S.-K. Park[2], J.-Y. Kwak[3], N.-G. Chung[1], H.-J. Kim[1], C.-K. Min[1], D.-W. Kim[1], J.-W. Lee[1], W.-S. Min[1], C.-W. Park[1]*
[1]Catholic BMT Center, College of Medicine, The Catholic University of Korea, Seoul, [2]Soonchunhyang University Medical School, Bucheon, [3]Chonbuk National University Medical School, Chonju, Republic Of Korea

Introduction: During the last decade, peripheral blood stem cells (PBSC) has become the most common graft source in unrelated donor stem cell transplantation (URD-SCT). However, few studies are available that compares the outcomes of PBSC vs. bone marrow (BM) transplants in the setting of URD-SCT. Furthermore, an analysis of the long-term outcomes of URD-SCT according to the graft source has not yet to be reported in adults with acute lymphoblastic leukemia (ALL). In order to determine which graft source is superior in adults with high-risk ALL, we compared the long-term outcomes of URD-SCT between PBSC and BM. The strengths of this study include sufficient follow-up duration and restriction to a single disease with a uniform pre- and post-transplantation treatment strategy.
Materials (or patients) and Methods: To determine which graft source is superior in adult high-risk ALL, we analyzed the long-term outcomes of 106 consecutive transplants from 8/8-matched or 7/8-matched URD (38 PBSC vs. 68 BM). All patients received a uniform strategy of pre-transplant chemotherapy (modified hyper-CVAD regimen), myeloablative conditioning (total body irradiation plus cyclophosphamde), and an identical graft-versus-host disease (GVHD) prophylaxis (tacrolimus plus methotrexate).
Results: The median patient age was 23 years (range, 15-48 years). All patients had at least one high-risk factor. Eighty-three patients

(78.3%) underwent transplantation in CR1 (34 PBSC, 49 BM). The median follow-up of survivors was 61 months (range, 38 to 83 months) for PBSC transplants and 104 months (range, 49 to 149 months) for BM transplants. At 5 years, PBSC transplants showed higher incidence of chronic GVHD than that of BM transplants (74.3% vs. 46.7%, *P*=0.001). PBSC transplants showed outcomes comparable to those of BM transplants in relapse (23.7% vs. 28.1%), non-relapse mortality (18.4% vs. 25.0%), disease-free survival (57.9% vs. 46.9%), and overall survival (57.9% vs. 50.0%). In a separate comparison of outcomes between the two graft sources according to the presence of Philadelphia chromosome, no significant advantage of PBSC over BM was found in both subgroups of patients.
Discussion: Our data suggest that the outcomes of URD-SCT are similar between PBSC and BM in adult high-risk ALL. Whether PBSC should be the preferred graft source for a specific subgroup of adult ALL needs to be further investigated.
Disclosure of Interest: None Declared.

PH-P268
TOTAL BODY IRRADIATION-FLUDARABINE-CYTARABINE CONDITIONING REGIMEN FOR UNRELATED DOUBLE CORD BLOOD TRANSPLANTATION IN ADULTS WITH HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA IN KOREA

S. Lee[1,], K.-S. Eom[1], Y.-J. Kim[1], J.-A. Kim[1], S.-K. Park[2], J.-Y. Kwak[3], N.-G. Chung[1], H.-J. Kim[1], C.-K. Min[1], D.-W. Kim[1], J.-W. Lee[1], W.-S. Min[1], C.-W. Park[1]*
[1]Catholic BMT Center, College of Medicine, The Catholic University of Korea, Seoul, [2]Soonchunhyang University Medical School, Bucheon, [3]Chonbuk National University Medical School, Chonju, Republic Of Korea

Introduction: Unrelated umbilical cord blood (CB) has been shown to be a valuable alternative source of hematopoietic stem cells for transplantation in patients who lack a suitable related or unrelated voluntary donor. Graft cell dose is an important determinant of outcomes following CB transplantation (CBT), limiting the use of this strategy for low body weight patients. To overcome this barrier, infusion of two partially HLA-matched CB units was adopted as a new strategy (double CBT). Here, we report the results of "total body irradiation-fludarabine-cytarabine conditioning double CBT" for 20 consecutive adults with high-risk acute lymphoblastic leukemia (ALL) in Korea (median age, 32 years [range, 16-58 years]; median weight, 67 kg [range, 45-84 kg]).
Materials (or patients) and Methods: Graft selection algorithm required that each CB unit to be ≥4/6 HLA matched with the recipient (HLA class I antigens [A and B] considering the antigen level and class II antigen [DRB1] considering allele level resolution DNA typing). The combined minimum total nucleated cell dose was $\geq 2.5\times10^7$/kg of recipient's body weight with 1 unit having a cell dose $\geq 1.5\times10^7$/kg. All patients had one or more high-risk features, including 9 Philadelphia chromosome-positive ALL. Sixteen patients (80.0%) were transplanted in first comple remission. All patients received the same conditioning (total body irradiation [12 Gy] + fludarabine [150 mg/m^2] + cytarabine [9 g/m^2]) and GVHD prophylaxis (tacrolimus + mycophenolate mofetil).
Results: The median cell doses infused were 3.99×10^7 nucleated cells/kg (range, 2.62-7.88), 1.69×10^5 CD34/kg (range, 0.65-5.07), and 8.11×10^7 CD3/kg (range, 5.60-14.20). All but one patient achieved a successful engraftment. Neutrophil recovery occurred at a median of 25 days (range, 16-109 days), and platelet recovery occurred at a median of 39 days (range, 8-185 days). Acute GVHD was observed in 11 patients (9 grade II, 2 grade III). Six of the 19 evaluable patients had chronic GVHD (4 mild, 2 moderate). After a median follow-up of 33 months (range, 3-89 months), 14 patients are alive with a leukemia-free status, while the other 6 patients have died of relapse (*n*=3) or transplant-related complications (*n*=3; 2 infections, 1 hemorrhage). Cumulative incidence of relapse and non-relapse mortality at 3 years were 11.9% and 20.6%, respectively, and the 3-year disease-free survival and overall survival rates were 69.4% and 64.8%, respectively.
Discussion: Our data suggest that "total body irradiation-fludarabine-cytarabine conditioning double CBT" is a feasible approach for adults with high-risk ALL who lack a suitable related or unrelated voluntary donor.
Disclosure of Interest: None Declared.

PH-P269
LOW CD34 CELL DOSE IS ASSOCIATED WITH HIGHER NON-RELAPSE AND OVERALL MORTALITY AFTER REDUCED INTENSITY CONDITIONING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROME

J. Törlén[1,], O. Ringdén[2], J. Le-Rademacher[3], M. Battiwalla[4], J. Chen[5], V. T. Ho[6], P. Kebriaei[7], C. A. Keever-Taylor[8], T. L. Kindwall-Keller[9], H. M. Lazarus[10], M. Laughlin[11], M. C. Lill[12], T. O´Brien[13], M.-A. Perales[14], V. Rocha[15], B. N. Savani[16], D. Szwajcer[17], D. Valcarcel[18], M. Eapen[5] and Center for International Blood and Marrow Transplant Research (CIBMTR)*
[1]Centre for Allogeneic Stem Cell Transplantation, Karolinska University Hospital, [2]Division of Therapeutic Immunology, Karolinska Institutet, Stockholm, Sweden, [3]Center for International Blood & Marrow Transplantation Research, Medical College of Wisconsin, Milwaukee, WI, [4]Heart, Lung, and Blood Institute, Hematology Branch, National Institutes of Health, Bethesda, MD, [5]Center for International Blood & Marrow Transplant Research, Medical College of Wisconsin, Milwaukee, WI, [6]Dana-Farber Cancer Institute, Boston, MA, [7]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX, [8]Division of Hematology & Oncology, Medical College of Wisconsin, Milwaukee, WI, [9]University of Virginia Medical Center, Charlottesville, VA, [10]Seidman Cancer Center, University Hospitals Case Medical Center, [11]Cleveland Cord Blood Center, Cleveland, OH, [12]Cedars-Sinai Medical Center, Los Angeles, CA, United States, [13]Centre for Children's Cancer & Blood Disorders, Sydney Children's Hospital, Sydney, Australia, [14]Memorial Sloan-Kettering Cancer Center, New York, NY, United States, [15]Cancer and Haematology Centre, Churchill Hospital, Oxford, United Kingdom, [16]Vanderbilt University Medical Center, Nashville, TN, United States, [17]CancerCare Manitoba, University of Manitoba, Winnipeg, MB, Canada, [18]Hospital Infantil Vall d'Hebron, Barcelona, Spain

Introduction: We investigated the role of cell dose for outcome after reduced intensity conditioning (RIC) in patients who underwent allogeneic hematopoietic stem cell transplantation (HCT) for acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).
Materials (or patients) and Methods: We studied CD34 cell dose in RIC HCT in 1057 patients aged 45-75 years with AML or MDS between 2000 and 2011. All received peripheral blood progenitor cells (PBPC). Study data was retrieved from the CIBMTR-registry. Primary outcome was non-relapse mortality (NRM). Secondary outcomes were hematopoietic recovery, graft failure, incidence of acute and chronic graft-versus-host disease (GVHD), relapse and overall mortality (OM). Characteristics of low and high CD34 cell doses were compared using the X^2 test for categorical variables and the Wilcoxon two-sample test for continuous variables. A model was built in which we tested the effects of CD34 cell dose on each outcome, using a forward stepwise selection method. The proportionality assumption of the Cox model was tested by adding a time-dependent covariate for each covariate factor including the main effect.
Results: In HLA-matched sibling HCT (AML *n*=301; MDS *n*=69) a CD34 dose $<4\text{x}10^6$/kg was associated with a higher risk of NRM (HR 2.03, *P*=0.004) and OM (HR 1.48, *P*=0.008), and the likelihood of neutrophil (HR 0.76, *P*=0.03) and platelet (HR 0.76, *P*=0.03) recovery were lower. In MUD HCT (HLA 8/8 or 7/8; AML *n*=558; MDS *n*=130) a CD34 cell dose $<6\text{x}10^6$/kg was associated with a marginally higher risk for NRM (HR 1.38, *P*=0.02) and OM (HR 1.20, *P*=0.05). CD34 cell dose was not associated with acute or chronic GVHD or relapse, neither in HLA-matched sibling nor MUD HCT.
Discussion: We only found a significant correlation between the cut-point of CD34 cells in the graft and the speed of hematopoietic recovery in the HLA-matched sibling group. Previous studies have

shown that a higher number of CD34 cells infused leads to a more rapid engraftment. However, as long as CD34 cell doses infused were above the cut-point associated with more rapid engraftment, we were not able to discern specific advantages of doses higher than that threshold. Relapse was not affected by CD34 cell dose in any group. This is in contrast to a previous CIBMTR study of HLA-identical sibling MAC HCT, where CD34 cell doses >6x10^6/kg correlated to decreased relapse risk. We have no explanation for this discrepant finding, since a high CD34 cell dose is expected to be more important in RIC than in MAC HCT. Despite this, CD34 cell dose was of importance of OM. Earlier studies of RIC HCT have shown a correlation between high cell doses and better survival, but at cost of more chronic GVHD. In this study we did not find a CD34 cell dose above which acute or chronic GVHD risks were higher. However, threshold levels of CD34 cells associated with increased GVHD incidence in previous studies have been high, >107 cells/kg. Hence, it is possible that such high cell doses have been avoided in many centers in recent years.
To conclude, in RIC HCT for AML or MDS, the PBPC CD34 cell dose should be delivered in excess of 4x10^6/kg for HLA-matched siblings, and in excess of 6x10^6/kg using MUD. However, no data on accessory cells infused were analyzed in this study, which may hamper the interpretation of the results.
Disclosure of Interest: None Declared.

PH-P270
HAPLOIDENTICAL T-REPLETE TRANSPLANT WITH POST-TRANSPLANT HIGH DOSE CYCLOPHOSPHAMIDE (PT-HDCY) IN PATIENTS OLDER THAN 60 YEARS: EARLY OUCOMES COMPARED TO HLA MATCHED RELATED (MRD) OR UNRELATED (MUD) TRANSPLANTS

D. Blaise[1,], L. Castagna[2], S. Fürst[1], R. Crocchiolo[2], R. Devillier[3], S. Bramanti[2], J. El Cheikh[1], A. Granata[1], B. Sarina[2], S. Harbi[1], B. Mohty[1], C. Lemarie[4], C. Chabannon[4], A. Santorro[2]*
[1]Unité de Greffe, Institut Paoli-Calmettes, Marseille, France, [2]humanitas, Milan, Italy, [3]Institut Paoli-Calmettes, Marseille, France, [4]Thérapie Cellulaire, Institut Paoli-Calmettes, Marseille, France

Introduction: The identification of a donor has always limited the extent of allogeneic HSCT. Recently it has been shown by different teams that a haploidentical donor could be a valid option to perform allo HSCT given adapted immunosuppression. Notably the use of PT-HDCy), after T-replete HSCT following reduced intensity (RIC) or non-myeloablative (NMAC) conditioning, has been associated with promising results. However little data exist concerning elderly population when this population is characterized by a lack of HLA matched sibling and a higher incidence of severe GVHD and non-relapse mortality.
Materials (or patients) and Methods: Using this strategy in a merged program between our two institutions we recently transplanted 30 patients over the age of 60 years (70% received the strict Baltimire conditioning while 30% were prepared with more aintense treatment. all received the same PTHDcy schedule. they were compared with patients of the same age transplanted from a MRD or MUD prepared with our sandard Fluda (5 days) -Iv Bu(2 days) and ATG (2 days).
Results:

	Haplo (*N*=30)	MRD (*N*=34)	MUD (*N*=42)
Age	64 (60-73)	63 (60-72)	64 (60-71)
HCT-CI > 2	16 (53%)	11 (32%)	16 (42%)
Myeloid malignancies: N (%)	15 (50%)	20 (58%)	18 (43%)
High or Very High DRI	13 (43%)	14 (41%)	8 (19%)
2-4 / 3-4 aGVHD	17% / 7%	23% / 18%	45%/ 21%
Total /ext cGVHD	9% / 0%	27% / 15%	36% / 19%
Evaluable patients at 6 mths	17	30	42
NRM prior to 6 mths (%eval)	3 (17%)	3 (10%)	10 (24%)
Rel prior to 6 mths (%eval)	4 (24%)	6 (20%)	3 (7%)

DRI: disease risk index; NRM: non relapse mortality; OS: overall survival; PFS: progression free survival

Patients with haplo had a trend for higher HCT-CI and DRI. They received more often a NMAC (P<0.05). Grade 3-4 and ext cGVHD were less frequent after haplo (P<0.05). Early NRM and Relapse occurrences were similar after haplo and MRD transplants. Early relapse was lower after MRD but population had better DRI.
Discussion: In conclusion haplo HSCT based on HD-PTCy is associated with lower severe GVHD incidences and no superior NRM and relapse occurrences. Longer follow-up is needed to fully evaluate this strategy and will be presented.
Disclosure of Interest: None Declared.

PH-P271
PERIPHERAL STEM CELL TRANSPLANT WITH CD34+ SELECTION AND CD3 ADD-BACK FOR HIGH RISK HEMATOLOGICAL PATIENTS

D. Damiani[1,], A. Sperotto[2], A. Michelutti[1], A. Geromin[1], M. Cerno[1], M. Battista[1], F. Patriarca[1], R. Fanin[1]*
[1]division of hematology, Udine, Italy, [2]bone marrow transplant, division of hematology, Udine, Italy

Introduction: CD34 selected stem cell transplantation is associated with sustained engraftment and low-risk graft – versus – host disease (GVHD), but limited by delayed immune reconstitution and increased risk of infection. The optimal dose of donor T cells to prevent graft failure and minimize risk of early opportunistic infection and post-transplant lymphoproliferative disorder (PTLD), while avoiding severe GVHD, remains unknown.
Aim. To analyze the efficacy of CD34+ selected peripheral blood stem cell transplantation with fixed dose of CD3+ add back.
Materials (or patients) and Methods: Thirty – one high – risk haematological patients were submitted to peripheral stem cell transplant with CD34+ selection, T cells were added back to achieve total dose 1.0x10^{7}CD3+/kg. Twenty – eight (90.5%) were mieloablative transplant. GVHD prophylaxis consisted of cyclosporine and methotrexate. Twenty – six (84.0%) were HLA-ID sibling transplant. Twelve (39.0%) patients were transplanted with advance disease.
Results: Twenty – nine (93.5%) patients engrafted. Probability of grade II–IV acute GVHD, limited chronic GVHD and extensive GVHD were 18.0%, 20.0% and 18% respectively. One – year infection–related mortality was 5.0%. T cell-immunoreconsitution was delayed. Three – year overall survival and progression – free survival was 55% and 49.5% respectively with a median follow-up of 24 months.
Discussion: CD34 selected peripheral stem cell transplant using a defined dose of T cell add-back resulted in high rated of engraftment and low risk of severe acute GVHD, early transplantation related mortality, and extensive chronic GVHD.
Disclosure of Interest: None Declared.

PH-P272
CLINICAL GRADE PROCESSING OF BONE MARROW HARVESTS IN A FULLY AUTOMATED SYSTEM

B. Mazzanti[1,], S. Dal Pozzo[1], S. Urbani[1], M. Santosuosso[1], L. Lombardini[1], A. Bosi[1], R. Saccardi[1]*
[1]Hematology, Careggi University Hospital, Florence, Italy

Introduction: Processing of intermediate/large volume of bone marrow (BM) harvests is requested for different clinical situations, including the management of ABO mismatching in allogeneic HSC transplantation and manipulation of the graft in regenerative medicine. Harvested volume and final product characteristics may vary largely, according to the clinical target. We present a monocentric experience using a fully automated, closed system (Sepax, Biosafe, Ch).
Materials (or patients) and Methods: From 2003 to 2013 50 procedures for 24 patients with hematological malignancies were performed in the following conditions: 1. ABO mismatching in allogeneic HSCT (n=19, major in 11, minor in 4 and double in 4 patients, respectively). 2. Autologous BM volume reduction before cryopreservation in case of PBSC mobilization failure (n=5). In the same time period the following procedures were carried

Patients	n	sw	Initial volume (ml)	Final volume (ml)	Volume reduction (%)	TNC recovery (%)	CD34+ recovery (%)
Allo-HSCT	19	V100 V300	1221 (773-1727)	200 (120-300)	84.9 (75.3-86.9)	86 (76-104)	96 (81-134)
Auto-HSCT	5	V100	1047 (1021-1129)	150 (119-153)	86.4 (85.3-88.4)	74.0 (54.1-76.3)	NA
BC	54	V100	210 (150-300)	30 (22-45)	86 (79-90)	51 (23-84)	NA
DGS	13	DGBS V126	300 (200-360)	42 (27-51)	86 (73-90)	26 (7-35)	69 (36-124)

out within trials of regenerative medicine: 3. volume reduction by a simple buffy-coat (BC) separation for a protocol of bone regeneration in the orthopaedic setting (*n*=54) and 4. density-gradient mononuclear cells separation in a trial for the treatment of critical limb ischemia (CLI) (*n*=13). For each protocol specific software (table) have been used and/or settled to different clinical targets.
Results: Initial and final volume, volume reduction, recovery of both Total Nucleated Cells (TNC) and CD34+ cells are reported below (median and range). Data are divided for Allo-HSCT, Auto-HSCT, BC for regenerative medicine and density-gradient separation (DGS). No side effects were reported in the clinic; in particular, no transfusional reactions were reported in the first group except for one patient who developed a reaction after the infusion of 20 mL of the product, possibly due to the presence of irregular Abs. In the latter case, the graft was rescued by MNC separation with DGS protocol and the infusion was carried out without any side effect. In case of very large volumes, the product was split in two or three aliquots and processed in parallel in different devices, in order to spare time. Time to engraftment of transplanted patients was in the normal range. No microbiological contamination due to the manipulation process was reported.
Discussion: The relatively low volume of the separation chamber required multiple processing of large volume products. However all the procedures were run in a fully automated setting through specific software aimed to different clinical targets, resulting in a modest time involvement of the operators. Clinical efficacy of the graft was in line with historical controls. BM manipulation with Sepax was shown effective, operator independent and could be applied for a broad range of clinical applications.
Disclosure of Interest: B. Mazzanti: None Declared, S. Dal Pozzo: None Declared, S. Urbani: None Declared, M. Santosuosso: None Declared, L. Lombardini: None Declared, A. Bosi: None Declared, R. Saccardi Conflict with: Funded by Biosafe.

PH-P273
STERILITY TESTING OF CORD BLOOD: VALIDATION OF MINIMAL VOLUME OF INOCULUM TO DETECT THE GROWTH OF BOTH AEROBIC AND ANAEROBIC MICRORGANISMS

P. Bergamaschi[1,], P. Cambieri[2], I. Sbarsi[1], V. Genovese[1], A. Marchesi[1], B. Romano[1], S. De Vitis[1], P. Marone[2], L. Salvaneschi[1]*
[1]Servizio di Immunoematologia e Medicina Trasfusionale, [2]Laboratorio di Microbiologia, Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy

Introduction: Cord blood (CB) is widely employed as alternative stem cell source for allogeneic transplantation in patients affected by hematological diseases. CB units may be contaminated by a variety of environmental contaminants, despite measures are in place to reduce contamination such as validated protocols for disinfection of the cord, manipulation in laminar flow cabinets, etc. For this reason sterility testing is done on the final product (FP) and unrelated CB units demonstrating microbial growth are not listed for transplantation. Directed allogeneic units that are culture-positive may be retained and infused if antimicrobial sensitivities are determined. In our policy we perform sterility testing on CB after processing and prior to cryopreservation: on the final product (FP) with cryoprotectant. A small volume of FP is obtained after plasma depletion, therefore very small specimens are available for inoculating culture vials.
Materials (or patients) and Methods: Culture media selected in this validation study included BD BACTEC Plus Aerobic/F and Plus Anaerobic/F for adults' specimens (recommended seeded volume 10-8ml) and Peds Plus/F for pediatric samples (recommended seeded volume 3-1ml). BD media were chosen because intended for testing aerobic and anaerobic bacteria and fungi. Plus Aerobic/F and Plus Anaerobic/F need a larger inoculum volume but allow the growth of anaerobic microorganisms, while Peds Plus/F allows seeding of small volumes but is for aerobic microorganisms only. We evaluated the adequacy of the sample, the inoculum volume and the BD media ability to detect the growth of aerobic/anaerobic bacteria and fungi in our setting. For this purpose, we inoculated fresh CB with low bioburden (1CFU/ml) of the following microbial isolates: E.Coli, Group B streptococcus and S.aureus (aerobic bacteria), B. fragilis, C.perfrigens and P.acnes (anaerobic bacteria), C.albicans and A.niger (fungi). Then CB was processed according to current practice and after cryoprotectant addition, 10, 5, 2.5 and 1ml FP were inoculated into Plus Aerobic/F and Plus Anaerobic/F and 3 and 1ml FP into Peds Plus/F medium, respectively. Incubation time was 30 days.
Results: CPD, cryopreservation solution and CB nucleated cells (various densities) were also inoculated in the BD media (10 ml/vial) for testing their ability to give false positive signals and resulted negative. BACTEC Plus Aerobic/F was able to detect the growth of tested aerobic bacteria for all four volumes FP, and so did BACTEC Anaerobic/F when an anaerobic bacterium was involved. The growth of aerobic bacteria was also detected by BACTEC Peds Plus/F for both 3 and 1ml of inoculated FP. Concerning the growth of fungi, BACTEC Peds Plus/F demonstrated to be superior than Plus Aerobic/F for 1ml samples.
Discussion: As in our policy the culture inoculum is obtained from the FP (prior to cryopreservation with cryoprotectant), the volume is necessarily minimal (1 ml/medium). Here we demonstrated that both the sample and volume are adequate to detect aerobic and anaerobic bacteria and fungi in CB at the end of processing (FP) if BACTEC Anaerobic/F is combined with BACTEC Peds Plus/F.
Disclosure of Interest: None Declared.

PH-P274
UMBILICAL CORD BLOOD FOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN 23 PATIENTS: ACCEPTABLE TOXICITY PROFILE AND EXCELLENT TREATMENT OPTION FOR PATIENTS WITH GRAFT FAILURE

M. Bojic[1,], M. Troch[1], N. Worel[1], P. Schellongowski[1], W. Reinhard Sperr[1], I. Schwarzinger[1], G. Mitterbauer-Hohendanner[1], G. Fischer[1], K. Dieckmann[1], A. Schulenburg[1], H. Theresia Greinix[1], P. Kalhs[1], W. Rabisch[1]*
[1]Medical University of Vienna, Vienna, Austria

Introduction: The use of umbilical cord blood (UCB) in haematopoietic stem cell transplantation (HSCT) is associated with a

longer time to engraftment and with a higher risk of graft failure, but it enables patients with haematological malignancies lacking an HLA-matched related or unrelated donor to receive a potentially curative treatment.
Materials (or patients) and Methods: In this retrospective analysis we investigated the efficacy and safety of UCB-transplantation (UCBT) at our centre.
Results: Between June 2009 and October 2013 we performed 25 UCBT on 23 patients at our centre. Median age at transplantation was 40 years (range 19-70). All patients had either leukaemia or lymphoma as underlying disease. UCBT was used as second transplantation after previous HSCT in 10 cases. Reason for use of UCB was lack of adequate donor in 18 cases and graft failure after previous HSCT in 7 cases. A myeloablative conditioning regimen was used in 8 patients; conditioning with reduced intensity (RIC) was used in 17 patients. GVHD prophylaxis consisted of cyclosporine and mycophenolate mofetil in 24 patients and cyclosporine and methotrexate in one patient. All patients received at least two units of UCB with a median of $1.36x10^5$/kg CD34+ cells and $2.83x10^7$/kg nucleated cells. Incidence and severity of treatment related toxicity were low with nausea/vomiting (WHO grade 1-2: *n*=6) and diarrhoea (WHO grade 1-2: *n*=5; WHO grade 3-4: *n*=1) being the most frequent ones. Three patients developed haemorrhagic cystits. Median time to neutrophil engraftment was 21 days after UCBT (range: day 13-32). Donor haematopoiesis was confirmed by chimerism-analysis. Four patients experienced graft failure. All of these had received RIC. Two of these patients were consecutively successfully transplanted with UCB again. Thirteen patients developed acute GVHD of the skin (grade 1: *n*=5; grade 2: *n*=3; grade 3: *n*=3) and of the gastrointestinal tract (grade 2: *n*=2; grade 3: *n*=2), whereupon 4 patients also developed chronic GVHD. After a median follow-up time of 10.8 months (range: 0.8-52.1 months) five patients relapsed. Eight patients died. Cause of death was relapse in 5 and transplant related events in 3 patients, respectively. Median overall survival and progression free survival have not been reached yet.
Discussion: From our data we conclude that UCBT is an alternative treatment modality for patients lacking an adequate related or unrelated matched donor. Furthermore, UCB is an excellent treatment option for patients with graft failure after previous HSCT.
Disclosure of Interest: None Declared.

PH-P275
CELL COMPOSITION OF DONOR LYMPHOCYTE INFUSION DISPLAYS DISTINCT ROLES IN DIFFERENT TYPES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
X. Zhao[1,], Y. Wang[1], C. Yan[1], X. Huang[1]*
[1]Peking University Institute of Hematology, Beijing, China

Introduction: Donor lymphocyte infusion (DLI) is frequently used to harness the graft-versus-leukemia (GVL) activity in patients who relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, whether the cell composition of this G-CSF mobilized DLI would play an essential role in the outcomes of patients after mDLI still remained unclear. In this study, in this study, our purpose was to elucidate the clinical significance of cell composition of mDLI in different type of allo-HSCT including HLA-identical and haploidentical transplant.
Materials (or patients) and Methods: 194 patients with hematological malignancies undergoing allo-HSCT from January, 2007 to March, 2011 were enrolled in this study and received mDLI for various clinical reasons. The infused cellular components of mDLI were examined by flow cytometry. We analyzed the possible risk factors of patient clinical outcomes and focused on the diverse infused cells in mDLI.
Results: The objectives in this study was composed of patients undergoing HLA-identical (*n*=60) and haploidentical allo-HSCT (*n*=134). The median of MNCs infused in mDLI was 1.0×108 (range: 0.75-2.18×108) cells/kg. The results showed that infusion of a higher number of CD34+ cells was associated with a lower DLI-related mortality (DRM, *P*=0.038) and a better overall survival (OS, *P*=0.002) in all patients. In HLA-identical transplant patients infused a lower number of CD14+ cells ($<0.33x10^8$/kg) was the independent risk factor of the occurrence of II-IV°aGVHD (HR=0.104, *P*=0.032). In addition, a dose of CD14+ cells less the 50th percentile was associated with a lower incidence of hematological relapse after mDLI (HR=0.193, *P*=0.007). However, in contrast to the results of HLA-identical transplant. It showed that a higher number of CD14+ cells was the independent risk factor of II-IV°aGVHD (HR=1.758, *P*=0.034) in haploidentical allo-HSCT.
Discussion: Recently, major studies about CD14+ cells focused on a new population of myloid-derived suppressor cells (MDSCs) which had the capacity to regulate alloreactive T-cell responses. Due to the immunosuppressive effect of MDSCs, several recent studies proved the correlation between the occurrence of aGVHD and MDSCs. Another surprising finding of our study was that a higher number of CD14+ cells in mDLI were associated with the lower incidence of relapse. Nausch *et al*. has demonstrated that CD14+HLA-DRlow/neg cell levels correlated with activated CD25+ NK-cell proportions and a ligand for activating NKG2D receptor on NK-cells termed as RAE-1 was expressed on murine MDSCs. Based on these data, this monocytic CD14+ cells would also help to enhance the graft-versus-leukemia (GVL) effect through modulating the activity of NK cells. While, in haploidentical transplant the infused CD14+ cells in mDLI displayed the opposite role and they were associated with a higher incidence of aGVHD. We postulated that maybe in haploidentical transplant the balance between these two subsets CD14+ cells was different from that of HLA-identical transplant. CD14+HLA-DR+ monocytes might take the lead role in this mismatched allo-HSCT. In conclusion, the fact that CD14+ cells would act as distinct regulators in different types of transplants might provide a new sight for the cellular therapy through manipulating the component of infused cells.
Disclosure of Interest: None Declared.

PH-P276
EVALUATION OF DIFFERENT ALTERNATIVE GRAFT SOURCES IN THE TREATMENT OF HEMATOLOGICAL DISORDERS: COMPARISON OF OUTCOMES OF HLA-MISMATCH UNRELATED DONOR, UNRELATED UMBILICAL CORD BLOOD, OR HLA-HAPLOIDENTICAL RELATED DONOR TRANSPLANTATION
A. Hausmann[1,2,], T. Abdu-Hamdeh[1], R. Paul Laubender[3], N. Engel[1], S. Fritsch[1], D. Prevalsek[1], R. Reibke[1], A. Katharina Zoellner[1], C. Schulz[1], W. Hill[1], G. Ledderose[1], C. Schmid[4], H. J. Kolb[5], J. Tischer[1]*
[1]Medizinische Klinik III, University of Munich (LMU), Klinikum Grosshadern, [2]Klinik für Hämatologie, Onkologie, Immunologie,Pall iativmedizin, Infektiologie und Tropenmedizin, Klinikum Schwabing, [3]Institute of Medical Informatics, Biometry and Epidemiology (IBE), LMU-Klinikum Grosshadern, Munich, [4]Medizinische Klinik II, Klinikum Augsburg, Augsburg, [5]Medicine Faculty of Munich, Munich, Germany

Introduction: Graft sources such as an HLA-mismatched unrelated donor (MMUD), unrelated umbilical cord blood (UBC), or an HLA-haploidentical related donor (haplo-RD) are viable alternative treatment options for patients who lack an HLA-matched donor. Here, we evaluated the outcome of these different graft sources, while making a distinction between the use of a T-cell replete graft (TCR) and a combination of a T-cell replete and a T-cell deplete graft (cTCR/TCD) in haplo-RD transplantation.
Materials (or patients) and Methods: Between August 2000 and March 2011 alternative donor graft sources were used in 183 patients, while 196 transplantations were conducted at our institution. Graft source was a MMUD in 75, UCB in 23 and a haplo-RD in 98 recipients, respectively. In the HLA-haploidentical setting a TCR graft followed by high dose cyclophosphamide (Cy) was used for 24, while a combined T-cell replete and deplete graft (bone marrow on day 0 plus CD6+ deplete PBSCs on day +6) was used for 74 transplantation procedures. Median age was 43 years. Acute leukemia was the most frequent underlying disease (MMUD: 73%; UCB: 87%; haplo-RD: cTCR/TCD: 78%; haplo-RD TCR: 64%), while B-cell lymphoma was diagnosed in 5% (MMUD), 9% (UCB), 16% (cTCR/TCD) and 25% (TCR). In haplo-RD transplantation disease stage was advanced in 100% of the TCR group including 42%

second allo-transplantations, whereas in only 16%, 9% and 8% of the cTCR/TCD, UCB and MMUD group a second allo-transplantation was performed. In the TCR group 75% had adverse cytogenetics in AML (46% cTCR/TCD; 40% UCB and 44% MMUD).
Results: With a median follow up of 33 (MMUD), 17 (UCB), 48 (cTCR/TCD), and 6 (TCR) months estimated one-year OS and DFS were 55% and 48% for the MMUD, 54% and 49% for the UCB, 44% and 43% for the cTCR/TCD, and 51% and 35% for the TCR recipients. One-year OS was 79% for patients receiving a first allo-transplantation in the TCR group. CI of acute GvHD was 76% for the MMUD, 47% for the UCB, 46% for the cTCR/TCD and 46% for the TCR group, while CI of chronic GvHD was 52% after MMUD, 22% after UCB, 34% after cTCR/TCD, and also 34% after TCR haplo-RD transplantation, respectively. One-year CI of NRM was 36% (MMUD), 30% (UCB), 30% (cTCR/TCD) and 18% (TCR) with no significant difference. Despite remarkable differences in risk contributions within the four groups, in particular regarding the TCR group including more high risk features, OS and DFS were not significant different between the groups. CI of acute GvHD was significant higher in the MMUD group compared to the other groups (P=0.00001), and cGvHD was significant lower for the UCB compared to MMUD recipients (P=0.04). Patients with diagnosis of lymphoma, AML with adverse cytogenetics, time from initial diagnosis to transplantation >1 year and second allo-transplantation had a significant lower OS (P=0.01; P=0.02; P=0.03 and P=0.01). Using a cTCR/TCD graft was associated with a high incidence of PTLD (25%).
Discussion: Our results confirm the utility of alternative donor graft sources, suggesting that UCB and haplo-RD transplantation, in particular using T-cell replete grafts, is equivalent to the use of MMUD grafts, while resulting in less GvHD. However, in our cohort a longer follow up for the TCR group is needed.
Disclosure of Interest: None Declared.

PH-P277
INTRABONE INFUSION OF POSITIVELY SELECTED CD34+ CELLS FROM HAPLOIDENTICAL DONORS TO REDUCE THE RISK OF GRAFT REJECTION

M. Zecca[1,], A. Tolva[1], M. E. Bernardo[2], C. Perotti[3], P. Comoli[1], C. Del Fante[3], E. Bergami[1], T. Mina[1], R. Raschetti[1], P. Merli[2], F. Introzzi[1], L. Strocchio[1], G. Viarengo[3], F. Locatelli[2,4]*
[1]Pediatric Hematology/Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, [2]Pediatric Hematology/Oncology, Ospedale Pediatrico Bambino Gesù, Roma, [3]SIMT, Fondazione IRCCS Policlinico San Matteo, [4]University of Pavia, Pavia, Italy

Introduction: Haploidentical T-cell depleted hematopoietic stem cell transplantation (haploHSCT) is an effective treatment for patients with both malignant and non-malignant disorders lacking an HLA-compatible donor. Extensive T-cell depletion, obtained with positive selection of CD34$^+$ cells, can prevent GVHD. Nevertheless, the infusion of highly purified stem cells together with a very low number of donor T lymphocytes or other accessory cells can increase the risk of graft rejection or poor engraftment. Rejection can be overcome by infusing a very high number of CD34$^+$ cells. Nevertheless, in the case of donors that are poor mobilizers (10-20% of cases), the target number of CD34$^+$ cells after T-cell depletion could be unattainable.
In the setting of cord blood transplantation (CBT), the problem of low cellularity was brilliantly solved by the intrabone injection of CB stem cells, with good engraftment even in adult patients. In cases of poor haplo-donor stem cell mobilization and insufficient number of CD34$^+$ available, we decided to adopt the same strategy of intrabone injection of part of the positively selected CD34$^+$ cells.
Materials (or patients) and Methods: From September 2009 to April 2013 14 children affected by malignant or non-malignant hematological disorders and given aploHSCT received part of the CD34$^+$ stem cells as intrabone injection because of a low stem cell dose collected from the donor (11 cases) or in case of a second haploHSCT after a previous graft rejection (3 cases).
The intrabone infusion was carried out at the patient bedside under general anesthesia as previously described (*Frassoni F. et al. Lancet Oncol 2008; 9:831-39*). For patients receiving the intrabone injection as part of a first aploHSCT the median number of CD34$^+$ cells infused was 9.7 x 10^6/kg (range, 5-12) and the median number of CD3$^+$ lymphocytes was 0.7 x 10^4/kg (0.3-11). For children receiving the intrabone injection as part of a second aploHSCT after a first rejection the median number of CD34$^+$ cells infused was 20 x 10^6/kg (12-31) and the median number of CD3$^+$ lymphocytes was 1.8 x 10^4/kg (0.8-2.8).
About 1/3 of the stem cell inoculum, corresponding to a total volume of 20 ml, was given intrabone, while the remaining stem cell portion was infused intravenously.
Results: No complication occurred during or after the intrabone injection.
Eleven of the 14 patients achieved complete donor engraftment, while 3 patients rejected the transplant. The median time to neutrophil recovery was 13 days (range, 12-20), while the median time to achieve platelet engraftment was 14 days (range, 13-40). Only 1 patient developed grade II acute GVHD and only 1 limited chronic GVHD. Three patients died, 2 for transplant related causes and 1 for disease progression. The overall survival probability was 76% (52-100), and the cumulative incidence of transplant-related mortality was 14% (4-51).
Discussion: Our data suggest that the intrabone infusion of positively selected CD34$^+$ cells, in the context of an haploHSCT, can be safely used in case of poor donor mobilization and low number of available stem cells, with the aim of minimizing the risk of graft rejection or poor engraftment. Our data need to be confirmed in a larger number of patients and compared with those obtained with conventional intravenous administration of comparable numbers of cells.
Disclosure of Interest: None Declared.

PH-P278
UK EXPERIENCE OF UNRELATED CORD BLOOD TRANSPLANTATION (UCBT) IN ADULTS: A RETROSPECTIVE ANALYSIS ON BEHALF OF EUROCORD AND THE BRITISH SOCIETY OF BLOOD AND MARROW TRANSPLANTATION (BSBMT)

J. Snowden[1,2,], R. Danby[3], A. Ruggeri[4], D. Marks[5], R. Hough[6], A. Pagliuca[7], M. Potter[8], N. Russell[9], C. Craddock[10], A. Clark[11], P. Miller[8], E. Gluckman[4], G. Cook[12], B. Shaw[8], V. Rocha[13] and British Sociey of Blood and Marrow Transplantation (BSBMT) and Eurocord*
[1]Department of Haematology, Sheffield Teaching Hospitals NHS Foundation Trust, [2]Department of Oncology, University of Sheffield, Sheffield, [3]Department of Haematology, University of Oxford, Oxford, United Kingdom, [4]Eurocord International Registry, Hopital Saint-Louis, Paris, France, [5]Department of Haematology, University Hospitals NHS Foundation Trust, Bristol, Bristol, [6]Department of Haematology, University College Hospital, [7]Department of Haematological Medicine, King's College Hospital NHS Foundation Trust, London, [8]Haemato-oncology Unit, Royal Marsden Hospital, Sutton, [9]Centre for Clinical Haematology, Nottinham University Hospital, Nottingham, [10]Centre for Clinical Haematology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, [11]Department of Haematology, The Beatson West of Scotland Cancer Centre, Glasgow, [12]St James's Institute of Oncology, St James's University Hospital, Leeds, [13]Cancer and Haematology Centre, Churchill Hospital, Oxford, United Kingdom

Introduction: Unrelated cord blood transplantation (UCBT) has been used increasingly in adults in the UK for over a decade. Two national prospective clinical trials have been recruiting (RIC UCBT and MAC UCBT) and national consensus guidelines have been produced (Shaw *et al*, Bone Marrow Transplantation 2009;44:7-12). As UK experience has not been appraised outside of these trials, we conducted a retrospective analysis to summarise demographic data and outcomes in pts >18 years treated with UCBT using Eurocord and BSBMT databases.
Materials (or patients) and Methods: From 2000 to 2012, there were 176 registrations with corresponding cord blood bank data

from 23 centres. 28 pts in national prospective clinical trials were excluded from further analysis. Outcomes were analysed for 148 pts with acute leukaemia (*n*=81), myeloproliferative disorders/ myelodysplastic syndrome (MDS) (*n*=42), lymphoproliferative diseases/myeloma (*n*=20) or aplastic anaemia (*n*=5).
Results: Pts had a median age of 40.8 years (18-72) and weight 69.5 kg (38.5-130). Various conditioning regimens were used, with 52% receiving a reduced intensity conditioning regimen. Most (74%) received double cord blood units (dCBU) and the remainder a single CBU. Recorded median total cell dose infused was 3.58 x10^7/kg (0.41-34.35) for total nucleated cell count (TNC) and 1.55 x10^5/kg (0.13-14.97) for CD34+ cells. Engraftment of neutrophils to >0.5 x10^9/L occurred at a median of 22 days (3-52) and platelets to >20 x10^9/L at a median of 38 days (1-118). Overall survival at 1 year was 48.4% (CI 40.9-57.2%) and 3 years was 38.2% (CI 31.0-47.3%), with an overall median survival of 49.5 months (4.2-97.6). The incidence of grade II-IV acute graft-versus-host disease (GVHD) was 29.4% (CI 19.7-40.4%) and chronic GVHD at 3 years was 26.3% (CI 17.1-36.3%). In patients treated for malignant disease with remission status available (*n*=139), cumulative incidence of relapse was 30.4% (CI 22.7-38.3%) at 3 years. Treatment related mortality was 23.0% (CI 16.4-30.3%) at 100 days, 33.1% (CI 25.4-41.0%) at 1 year and 36.3% (CI 28.3-44.4%) at 3 years. In univariate analysis, overall survival (OS) at 4 years was strongly related to stage of disease; 51% for early (CR1, chronic phase, MDS subtype-RA, good remission) versus 42% for intermediate (CR2, accelerated phase, MDS transformation, PR) versus 22% for advanced (non-remission, other subtypes of MDS) (*P*=0.0005). Use of serotherapy in the conditioning regimen impacted negatively on 4 year OS (24% versus 42%, *P*=0.008). There was no significant impact on OS of gender, age, diagnosis, conditioning regimen intensity, CBU number, TNC or CD34+ dose, HLA or ABO matching, or year (2000-08 versus 2009-12). In a subgroup analysis of acute leukaemia, the relationship between 4 year OS and disease status was stronger; 56% for early versus 38% for intermediate versus 0% for advanced (*P*=0.00007). Half the activity was between 2000-2008 and half between 2009-2012, reflecting a greater than doubling of activity in recent years.
Discussion: This retrospective national analysis supports the evolution of UCBT as an effective treatment in adults with comparable outcomes to unrelated blood and marrow transplantation. Selection of pts with good and intermediate risk disease and avoidance of serotherapy in the conditioning regimen is associated with improved survival.
Disclosure of Interest: J. Snowden: None Declared, R. Danby: None Declared, A. Ruggeri: None Declared, D. Marks: None Declared, R. Hough: None Declared, A. Pagliuca: None Declared, M. Potter: None Declared, N. Russell: None Declared, C. Craddock: None Declared, A. Clark: None Declared, P. Miller: None Declared, E. Gluckman Conflict with: Cord use, Conflict with: Gamida, G. Cook: None Declared, B. Shaw: None Declared, V. Rocha: None Declared.

PH-P279
SALVAGE HAPLOIDENTICAL TRANSPLANTATION USING NON MYELOABLATIVE CONDITIONING FOR PRIMARY GRAFT FAILURE IN CORD BLOOD TRANSPLANTATION

B. Tang[1,], X. Zhu[1], H. Liu[1], C. Zheng[1], L. Geng[1], W. Yao[1], J. Tong[1], K. Song[1], Z. Sun[1]*
[1]Hematology, Anhui Provincial Hospital, Hefei, China

Introduction: Graft failure (GF) is a major concern after cord blood transplantation (CBT). Primary graft failure (pGF) in CBT is associated with considerable morbidity and mortality. Salvage hematopoietic stem cell transplantation(HSCT) can rescue pGF patients. However, the stem cell source and the preconditioning regimen in salvage transplantation are yet to be detemined. Because the patients who received CBT almost always lack both matched-related and -unrelated donors during the clinically relevant period, haploid donor is considered to be suitable as stem cell source for salvage transplant.
Materials (or patients) and Methods: In this study,we retrospectively analyzed 17 patients with hematologic malignancies who receiced haploidentical transplant with non myeloablative conditioning as salvage therapy for pGF in CBT.At the first transplant, 12 patients received single unit CB, while 5 patients received double units CB. The first transplant was administered with myeloablative conditioning. The median interval between the two transplants were 36 days. The non-myeloablative conditioning regimen for salvage transplant consisted of Flu(total 120mg/m^2), antithymocyte globulin (ATG, total 10mg/kg), cyclophosphamide (50mg/kg.d for one day) and low dose TBI (3Gy). G-CSF mobilized PBSCs and BM were transfused intravenously to the recipients just after the completion of the collection on days 0 and 2 of transplantation, respectively.The median number of infused total nucleated cells dose was 8.02 ×10^8/kg (range, 6.25-26.56×10^8/kg), and CD34+ cells was 5.23×10^6/kg (range, 2.98-27.36 ×10^6/kg).
Results: Neutrophil and platelet engraftment occurred in 14 (82.4%) and 13 (76.4%) patients . The median times to neutrophil recovery and platelet recovery were 12 days and 17 days, respectively. Three patients(19%) suffered from a second pGF. 7 patients died from treatment-related causes: 3 from a second pGF, 2 from severe acute GVHD, 1 from infection and 1 from uncontrolled cGVHD. 6 patients developed aGVHD (>grade II = 3). 5 patients developed chronic GVHD (limited = 1, extensive = 4). With a median follow-up of 1146 days (range, 727-2810 days), all 10 patients have currently had a leukemia-free survival. The 2-year overall survival and leukemia-free survival rate was 58.8%.
Discussion: These results indicate that a salvage transplant with non-myeloablative conditioning using haloid G-PBSCs and BM as the donor after pGF in CBT is feasible and effective.
Disclosure of Interest: None Declared.

PH-P280
RAPID AND SUSTAINED ENGRAFTMENT OF A SINGLE ALLOGENEIC *EX-VIVO* EXPANDED CORD BLOOD UNIT (CBU) AFTER REDUCED INTENSITY CONDITIONING (RIC) IN ADULTS. PROOF OF REALITY

N. Milpied[1,], B. Dazey[2], Z. Ivanovic[2], S. Vigouroux[1], R. Tabrizi[1], A. Pigneux[1], M.-s. Dilhuydy[1], K. Bouabdallah[1], T. Leguay[1], M. Sauvezie[1], P. Duchez[2], X. Lafarge[2], G. Marit[1], J.-m. Boiron[2]*
[1]university hospital Bordeaux, [2]efsal, Bordeaux, France

Introduction: Engraftment rate and speed of a single CBU in adults remains unsatisfactory. Until now, attempts at using *ex-vivo* expanded CBU were unsuccessful to promote long term engraftment. We report the results achieved in 16 Pts included in a PCT of transplantation of a single *ex-vivo* expanded allogeneic CBU. Eudract 2008-006665-81, Clinicaltrials.gov NCT 01034449.
Materials (or patients) and Methods: Adults patients with an indication for SCT and unable to tolerate MAC were included after inform consent if no Id sibling, no MUD 9 (C or DQ mismatch) to 10/10 HLA matches and no CBU fulfilling the HLA matching (≥ 4/6) and richness (≥ 3 to 4 x 10^7 TNC/kg before thawing) were available. RIC: Flu (40 mg/m^2/d x 5d), Cyclophosphamide (50 mg/kg x 1d) ICT 2 Gy. GVHD prevention: MMF (d-3 to d28) and CSA from d-3. Graft engineering: 1 CBU with > 2x10^7 TNC/kg and< 4 and 4 to 6 HLA compatibilities was thawed, CD34+ cells were selected (Miltenyi) and submitted to *ex-vivo* expansion in SF medium (HPO1-Macopharma) supplemented with SCF, Flt3l, G-CSF and TPO for 12 days, from d-12. CD34- cells were cryopreserved. On d0, exp cells were washed and suspended in HSA 4% and upon viability and sterility injected to the pt. Cd34- cells thawed and injected 3 h later.
Results: From 03/2010 to 09/2013 16 pts were included, age 52 (26-64) with AL: 9, HD: 2, MDS: 3. CMML: 2. Pts had received 0 to 3 lines of Tx (med:1). For 1 pt the exp product was contaminated, a rescue unmanipulated CBU engrafted, he is AW at 37 m, 1 pt died of MOF before RIC while expansion was on-going. The median fold expansion of CD34+ and TNC was 45 (22-77) and 363 (95-528) leading to a graft content of 0.6-12.8x10^6 CD34+ cells/kg (median:1.8) and 2.4-92x10^6 TNC/kg (median:18). The CD34- counterpart con-

tained a med of 2.3x10^6 CD3+/kg (1.2-5) and of 0.7x10^6 CD19+/kg (0.1-1.5). Twelve/14 pts achieved a complete donor engraftment from d15 to d42 (med d15). The med time to 500 PMN's and to 20 000 plat was 9 days (2-30) and 23 days (15-45). Two pts with CMML failed to engraft the exp product as well as further unmanipulated CBU. AGVHD grade II, III, IV was observed in 3, 1, 1 pts. Two pts died of TRM (1 with RIC neuro tox, 1 grade IV AGVHD). Five pts had relapsed (2/2 who did not engraft and 3/12 who engrafted exp CBU). One pt rejected the exp CBU at 3 m and is AW after rescue.Overall, after a med FU of 20 m (3-47 m) the 2y OS and DFS is 50%. Full donor chimerism maintained until relapse, death of TRM or last FU in 11/14 pts who received the exp graft.
Discussion: *Ex-vivo* expansion of a single CBU is feasible and reproducible. Transplantation of the exp CBU with the CD34- counterpart of the same CBU produces rapid, complete and sustained donor engraftment after RIC in adults.
Disclosure of Interest: None Declared.

PH-P281
THE SAFETY AND EFFICACY OF THE USE OF INFANTS AS HAEMATOPOIETIC STEM CELL DONORS FOR THEIR MATCHED SIBLINGS

R. Raj[1,], D. munirathinam[1], V. kumar[1], H. doss[1]*
[1]Paediatric Blood and Marrow Transplantation, Apollo Speciality Hospital, Chennai, India

Introduction: Haematopoietic stem cell donation from sibling donors carries significant saftey and ethical issues. We present here, our experience in the use of infants as stem cell donors for their older siblings.
Materials (or patients) and Methods: Between the years 2002 and 2013, we have performed a total of 248 haematopoietic stem cell transplantation in children at our centre. Fifteen of our donors were under 1 year of age. The parents were counselled on multiple occasions before proceeding with transplantation. Issues discussed were related to the risk due to general anaesthesia in an infant, the need for transfusion for the donor as the amount harvested for an older sibling would necessitate a top up transfusion and difficult iliac crest punctures in an infant as the posterior crest is mostly cartilaginous at that age. The children were anaesthetised by an experienced paediatric anaesthetist at our centre. All transfusions for the donor came from directed donations from the families and the samples were screened using nucleic acid testing in addition to antibody screening. The harvests in these infants were all performed by paediatric transplant physicians directly to ensure safety of the procedure. The posterior crest punctures were done using marrow harvest needles that were changed after 15 punctures.
Results: All 15 children tolerated the general anaesthesia without any complications. They received top up transfusions during their donation. An average of 5 to 7 ml per kilogram of the recipient body weight of bone marrow was harvested from each of these donors. The volumes were low compared to the usual harvests done in older children. The total nucleated cell count and CD34 counts per ml of bone marrow were far higher in infant donors. The average yield was 7.5 x 10^6/kg with a range from 4 to 23 x 10^6/kg of the recipient body weight. There was no haematoma at puncture sites and the babies regained their mobility soon after recovery from anaesthesia.
Discussion: Our series highlights that the process of haematopoietic stem cell donation is safe for an infant in a paediatric unit. The stem cell yield is extremely high resulting in rapid and engraftment in all recipients.
Disclosure of Interest: None Declared.

PH-P282
EVALUATION OF CELL DOSE TO ACHIEVE ENGRAFTMENT IN UNRELATED CORD BLOOD TRANSPLANTATION

H. Yabe[1,], K. Tabuchi[2], Y. Takahashi[3], K. Kudo[4], K. Kato[5], H. Sakamaki[6], K. Kawa[7], R. Suzuki[8], H. Kanamori[9], S. Mori[10], M. Murata[11], Y. Kanda[12]*
[1]Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara, [2]Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital, Tokyo, [3]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, [4]Division of Hematology and Oncology, Shizuoka Children's Hospital, Shizuoka, [5]Division of Hematology/Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, [6]Division of Hematology, Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital, Tokyo, [7]Japanese Red Cross Kinki Block Blood Center, Osaka, [8]Department of HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, [9]Kanagawa Cancer Center, Yokohama, [10]St. Luke's International Hospital, Tokyo, [11]Nagoya University, Nagoya, [12]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan

Introduction: Cord blood (CB) is increasingly used as an alternative hematopoietic stem cell source and the results of unrelated CB transplantation (CBT) have improved in the last decade. However, graft rejection has been a major concern in unrelated CBT. Although it is common practice to select the cord unit with the greatest total nucleated cell (TNC) counts within a given HLA-match grade, the essential cell dose ensuring engraftment with acceptable probability was not established. We analyzed the impact of prefreeze TNC dose, CD34-positive cell (CD34C) dose based on per body, per actual (ABW), or per standard body weight (SBW) upon engraftment after unrelated CBT.
Materials (or patients) and Methods: A total of 5668 patients who received single-unit CBT between 2000 and 2011 were analyzed based on the data reported to the Japan Society for Stem Cell Transplantation registry. A crude analysis was performed in whole cohort and then 787 patients were selected according to the following criteria: (1) AML in 1CR or ALL in 1CR; (2) CBT were performed between 2006 and 2011. Predictive potentialities of the parameters including TNC/ABW, TNC/SBW, CD34C/ABW, and CD34C/SBW for neutrophil engraftment were compared by time-dependent receiver operating characteristic analysis (time-dependent ROC; Biometrics 2010; 66: 999-1011). Only death was used as competitive risk. Cumulative incidence (CI) was used to compare the neutrophil engraftment between more and less than cut-off point of cell dose. The optimal cut-off point to predict neutrophil engraftment was determined by the Youden index. Engraftment rate was calculated according to each cut-off point in both AML and ALL who received CBT between 2009 and 2011.
Results: In whole cohort, area under the curves (AUC) of TNC/body, TNC/ABW, TNC/SBW, CD34C/body, CD34C/ABW, CD34C/SBW were 0.557, 0.522, 0.520, 0.560, 0.530, 0.530, respectively. CI of neutrophil engraftment at +60 day in patients who received equal or more than 37.7 x 10^5 CD34C/body was 77.0% and that in patients with less than 37.7 x 10^5 CD34C/body was 72.9% (P<0.001; Gray's test). In 426 AML patients, AUC of TNC/body, TNC/ABW, TNC/SBW, CD34/body, CD34/ABW, CD34/SBW were 0.528, 0.645, 0.651, 0.490, 0.562, 0.549, respectively. CI of neutrophil engraftment at +60 day in patients who received equal or more than 2.35 x 10^7 TNC/SBW was 85.0% and that in patients with less than 2.35 x 10^7 TNC/SBW was 78.5% (P=0.009; Gray's test). In 361 ALL patients, AUC of TNC/body, TNC/ABW, TNC/SBW, CD34/body, CD34/ABW, CD34/SBW were 0.482, 0.559, 0.608, 0.507, 0.568, 0.573, respectively. CI of neutrophil engraftment at +60 day in patients who received equal or more than 3.55 x 10^7 TNC/SBW was 95.3% and that in patients with less than 3.55 x 10^7 TNC/SBW was 86.5% (P<0.001; Gray's test). The lowest doses of TNC/SBW achieving engraftment in more than 80% of recipients in AML and in ALL were 1.99 x 10^7/kg and 1.86 x 10^7/kg, respectively.
Discussion: CD34C/body was the best predictor of engraftment in whole cohort, however, AUC was only 0.560. TNC/SBW was the best predictor of engraftment in patients with AML/1CR or ALL/1CR, and the AUC rose to 0.651 or to 0.608. A number of patients

may obtain engraftment with smaller cell dose than previously recommended.
Disclosure of Interest: None Declared.

PH-P283
CLINICAL EVALUATION OF AN AUTOMATIC DILUTION/ WASHING METHOD OF CORD BLOOD UNIT ADMINISTRATION AND OUTCOMES IN PATIENTS WITH MALIGNANT AND NO MALIGNANT HEMATOLOGICAL DISEASES

N. Castillo[1,], I. Garcia-Cadenas[2], C. Canals[3], C. Diaz Heredia[1], P. Barba[1], I. Elorza[4], T. Olive[1], R. Martino[5], C. Azqueta[6], C. Ferra[7], I. Badell[2], R. Duarte[7], D. Valcarcel[1], S. Querol[8]*
[1]Hematology, Hospital Universitari Vall Hebron, [2]Hematology, Hospital Santa Creu i Sant Pau, [3]Banc Sang i Teixits, [4]Hematology, Hospital Vall Hebron, [5]Hematology, Hospital de la Santa Creu i Sant Pau, [6]Banc Sang Teixits, [7]Hematology, Institut Catala Oncologia, [8]Banc de Sang i Teixits, Barcelona, Spain

Introduction: Umbilical cord blood (UCB) is a well established hematopoietic progenitor cell (HPC) source for patients requiring allogeneic transplantation. Severe adverse reactions (SAR) involving UCB infusion have been described related to graft composition and thawing methodology. To minimize SARs, we developed an automatic method that ensure appropriate cell yield after graft dilution and DMSO washing. We retrospectively evaluated the immediate complications and the engraftment of UCB stem cell infusion in our transplant programs.
Materials (or patients) and Methods: Between January 2005 and December 2010, all consecutive patients with hematological and non-hematological malignancies underwent UCB transplant from an unrelated donor at four different institutions using cord blood units thawed at (HPC) Banc de Sang i Teixits (BST). Median follow-up for survivors was 54 months (3-103). According to BST protocol, Cord was reconstituted with 1:1 dilution with a hyperosmolar solution of 7.5% Dextran/albumin using the Sepax device. Immediate infusional-related events were evaluated after infusion. In addition, we analyzed engraftment and main clinical outcomes.
Results: A total of 145 patients underwent a single UCBT during the study period (median age 18 years, range 1-56) Most of patients received myeloablative conditioning regimen based on busulfan (6.4 mg/kg iv), thiotepa (10 mg/kg), fludarabine (150 mg/m^2) and anti-thymocyte globulin (6-10 mg/kg). The diagnosis were: AML (*n*=47, 32%), ALL (*n*=47, 32%), NHL (*n*=14, 10%), primary immunodeficiency diseases (*n*=12, 8%) and other (*n*=25, 18%). Among 130 patients with malignancies diseases, at UCBT 42 patients had early, 42 intermediate and 46 advanced disease. Median (percentile 25-75%) cellularity infused for TNC and CD34+ was 3.22 (2.08-6.13) and 1.45 (0.94-2.66) in overall population. The median (percentil 25-75) of TNC and CD34+ cells recovered after thawing procedures compared to the values of the origin CB bank was 89% (83-98) and 85% (76-95). Immediate CB infusional events were reported in 15 (10.3%) of infusions, in 12 patients, all of the events were grade I/II. Primary graft failure was observed in 17 patients. At a median of 28 and 60 days the cumulative incidence (CI) of myeloid engraftment was 54% and 87% for neutrophils and 18% and 72% for platelets. The CI of acute and chronic GVHD grade II-IV were 39% and 19% respectively. At 3 years, the CI of non-relapse mortality and relapse was 42% and 14% being opportunistic infections and primary graft failure the two major causes of death. The probability of disease-free (DFS) and overall survival were 43% (95% CI: 35.3%>51%) and 44% (95% CI: 36%>52%). Factors associated with rapid neutrophil recovery and improved DFS in univariate analysis were CD34+ and TNC cell dose infused higher than 1.2 x10^5/kg (*P*=0.04) and 2 x10^7/kg (*P*=0.02).
Discussion: Our automatic dilution/washing method of Cord blood units is efficacious, rendering a high percentage of CD34 and TNC recovery and is safe with a very low of adverse infusional-related events. Furthermore our results support that the CD34 and TNC cell count are 2 of the main variables related with engraftment and DFS.
Disclosure of Interest: None Declared.

PH-P284
STEM CELL MOBILIZATION FOR AUTOLOGOUS TRANSPLANT IN HEMATOLOGIC MALIGNANCIES. THREE-YEAR EXPERIENCE AT A SINGLE INSTITUTION

S. Capria[1,], S. Mallano[1], M. Shafii Bafti[1], S. Maria Trisolini[1], G. Annechini[1], M. Canichella[1], F. Gentilini[1], P. Paisano[1], A. Polino[1], U. La Rocca[1], S. Mohamed[1], L. Quattrocchi[1], A. Vitale[1], R. Foà[1], G. Meloni[1]*
[1]Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy

Introduction: High dose chemotherapy followed by autologous stem cell transplant (ASCT) represents the standard of care for several hematologic malignancies. The failure in peripheral blood stem cell (HSC) mobilization is a crucial issue, because it results not only in the impossibility of carrying out the transplant, but also in an increased toxicity and risk of infectious complications following the mobilization attempts. Purpose of our study was to retrospectively analyze the results of HSC mobilization in patients with hematological malignancies, at our Center since 2011, focusing in particular on factors that may have influenced the mobilization.
Materials (or patients) and Methods: We analyzed 272 patients who underwent a HSC mobilization process. Two were affected by amyloidosis, 30 by Hodgkin's lymphoma, 94 by non-Hodgkin's lymphoma, 50 by acute myeloid leukemia, 15 by acute lymphoid leukemia and 81 by multiple myeloma. The median age was 51 years (3-72) and the M/F ratio 137/135. The primary outcome was CD34+ cell yield; secondary outcomes included number of aphereses, proportion of failures, possible factors negatively affecting the mobilization. Among the 272 patients, 63 met the criteria for the definition of predicted poor mobilizer according to the GITMO proposal; in particular, 56 satisfied major criteria and 7 met 2 minor criteria, while 209 had no adverse prognostic factors for mobilization. The mobilization strategies were: chemotherapy combined with G-CSF in 90% of cases, G-CSF alone in 3% and Plerixafor (PLX) with G-CSF in 7%.
Results: In the entire patients' population, the median CD34+ cell yield was 7.1 x 10^6/kg (range 2-21) and the median number of aphereses required to reach the target was 1 (range 1-3). According to the algorithm employed at our Institution, the median White Blood Cell (WBC) count to start the apheretic procedure was 13. x 10^9/L (range 2-81) and the median number of CD34+ cells/μL in the peripheral blood was 43 (range 12 -232).
The failure rate was 14% and the main negative prognostic factors for HSC mobilization in univariate analysis were: AML diagnosis (*P*=0.001), use of cytotoxic compounds such as melphalan and fludarabine or clofarabine at least in one of the previous therapeutic regimens (*P*<0.001), more than 2 previous therapeutic lines (*P*<0.001).
Among the 63/272 patients defined as predicted poor mobilizers, 28 were actually proven poor mobilizers, because they reached a median peak of 1.45 CD34/μL (0-13) and 12x10^9/L WBC (0.2-45) at a median of 10 days from the start of a mobilizing procedure based on chemotherapy and G-CSF in 64% of cases, G-CSF alone in 7% and PLX with G-CSF in 29%; in 35 patients, a median of 4.5 x 10^6/kg CD34+ cells (range 2-11) were collected using the following methods: chemotherapy and G-CSF in 77% of cases, G-CSF alone in 3% and PLX with G-CSF in 20%.
Discussion: HSC collection is a challenge in patients eligible for an ASCT. In our experience, HSC mobilization was successful in the majority of patients (86%) and a considerable percentage of those failing with standard procedures could be successfully rescued with PLX. For these patients, the identification of reliable predictors of mobilization failure is mandatory, with the aim of determining which patients might benefit from the pre-emptive addition of PLX and in order to optimize the cost-effectiveness of the procedure.
Disclosure of Interest: None Declared.

PH-P285

IMPACT OF THE HEMATOPOIETIC STEM CELL SOURCE IN ALLOGENEIC HSCT IN PAEDIATRIC ALL: A RETROSPECTIVE STUDY ON BEHALF OF THE EBMT PAEDIATRIC DISEASES WORKING PARTY

M. Simonin[1,], M. Zecca[2], A. Mouhab[3], A. Baruchel[4], A. Chybicka[5], A. Biondi[6], L. Volin [7], M. Labopin [8], J.-H. Dalle[1], C. Peters[9] and EBMT working party*

[1]Pediatric Hematology, Robert Debré, Paris, France, [2]Pediatric Hematology, San Mateo, Pavia, Italy, [3]Pediatric Hematology, King Faisal, Riyadh, Saudi Arabia, [4]Pediatric Hematology, Hôpital Robert Debré, Paris, France, [5]Pediatric Hematology, Wrocław Medical University, Wrocław, Poland, [6]Pediatric Hematology, Clinica Pediatrica, Monza, Italy, [7]Pediatric Hematology, Helsinki University Central Hospital, Helsinki, Finland, [8]Biostastistics, Saint Antoine, Paris, France, [9]Pediatric Hematology, St. Anna Children's Hospital, Vienna, Austria

Introduction: We evaluated the outcome of allogeneic hematopoietic stem cell transplantation (HSCT) in paediatric ALL depending on the stem cell source, either bone marrow (BM) or peripheral blood stem cells (PBSC).

Materials (or patients) and Methods: We analyzed the outcome of 2744 children who received a first HSCT for ALL and were registered in the EBMT database from 2003 to 2012. 1882 patients (69%) received BM and 31% received PBSC (*n*=862). Median age: 9.8 years, 1810 males, 1051 females. HSCT in 45% CR1, 46% CR2 and 9% CR3.

Results: The two groups were similar regarding disease status before transplantation, patient and donor gender and CMV status. Both, patients and donors of the PB group were older than the BM group: 11.4 vs 9.1 years and 30.7 vs 20.9 years ($P<10^{-3}$).

Total Body Irradiation (TBI) during the conditioning regimen was more frequent in the BM group (80 vs 66%; $P<10^{-3}$) and there were more unrelated donor (UD) in the PB group (67 vs 52% ; $P<10^{-3}$).

Engraftment rate was higher in the BM group (98.8 % vs 97.3%; *P*= 0.006) whereas interval from transplantation to reach neutrophil counts of 0.5 x 10^9/L was shorter in PBSC group (15 days versus 19 days; $P<10^{-3}$).

The 5-year overall survival (OS) and the 5-year leukemia free survival (LFS) in PB group were poorer than BM group in univariate analysis (respectively 61 ± 2% vs 67 ± 1% $P < 10^{-3}$ and 53 ± 1% vs 59 ± 1% $P < 10^{-3}$) but not significant in multivariate analysis : hazard ratio (HR) 1.14 (95% CI 0.96-1.35) and HR 1.14 (95% CI 0.97-1.33).

In multiple regression 5 risk factors were independently associated with poorer OS including: unrelated donor (HR 1.20 (95% CI 1.02-1.40) *P*=0.03, CR2 at transplantation : HR 1.37 (95% CI 1.16-1.61) *P*=0.0002, or CR3 : HR 1.88 (95% CI 1.47-2.83) $P <10^{-4}$, patient CMV seropositivity : HR 1.24 (95% CI 1.06-1.44) *P*=0.01, and age > median : HR 1.37 (95% CI 1.16-1.61).LFS was higher for patients who underwent HSCT after 2007: HR 0.84 (95% CI 0.73-0.96) *P*=0.01. TBI was also associated with better outcome: HR 0.55 (95% CI 0.47-0.64) $P < 10^{-4}$.

Treatment-related mortality was higher in the PBSC group: HR 1,40 (95% CI 1.09-1.80) *P*=0,01 and for patients with CMV seropositivity : HR 1.70 (95% CI 1.32-2.19) $P < 10^{-4}$. TBI was associated with less TRM: HR 0.61 (95% CI 0.47-0.81) $P < 10^{-3}$. Acute GVHD> grade 2 was not statistically different between PB and BM groups: respectively 41 vs 38 % *P* = 0.19 (univariate) and HR 0.97 (95% CI 0.78-1.2) *P* = 0.76 (multivariate).The 5-years cumulative incidence of chronic GVHD was significantly higher in PBSC group in both univariate and multivariate analysis (32 ± 2% vs 20 ± 1% $P < 10^{-3}$ and HR 1.64 (95% CI 1.33-2.02) $P < 10^{-3}$ respectively.

The cumulative incidence of relapse at 5 years was not statistically different between PBSC and BM group in univariate and multivariate analysis; (25 ± 2% vs 29 ± 1% *P*=0.12) and HR, 0.99 (95% CI 0.81-1.21).CR2 or CR3 disease status at transplant were independently associated with higher risk of relapse: HR 1.42 (95% CI 1.18-1.78 *P* = 0.0002) and HR 2.15 (95% CI 1.63-2.83 $P < 10^{-4}$).

Discussion: In paediatric HSCT the use of PBSC should be avoided, since this source of stem cell yield more chronic GVHD and TRM without statistical significant gain on overall survival, nor relapse and engraftment rates.

Disclosure of Interest: None Declared.

PH-P286

HIGHER COUNTS OF PLASMACYTOID DENDRITIC CELLS IN THE ALLOGRAFT ARE ASSOCIATED INCREASED RISK OF ACUTE GVHD AFTER STEM CELL TRANSPLANTATION

M. Vescovi Goncalves[1,], M. Yamamoto[1], E. Kimura[1], V. Antonio Renzi Colturato[2], M. Valeria Ikoma[3], M. Souza[2], M. Mauad[2], P. G. Alvarenga Oliveira[1], A. P. Vaz Carvalho[1], F. Rodrigues Kerbauy[1], N. Hamerschlack[4], Y. Novis[5], V. Cortez Ginani[6], A. Seber[6], V. Rocha[7], A. Orfao[8], C. Arrais Rodrigues[1]*

[1]Hematology and Tranfusion Medicine, UNIFESP, Sao Paulo, [2]Bone Marrow Transplantation, [3]Laboratory of Flow Cytometry, Hospital Amaral Carvalho, Jaú, [4]Bone Marrow Transplantation, Hospital Israelita Albert Einstein, [5]Bone Marrow Transplantation, Hospital Sirio Libanes, [6]Bone Marrow Transplantation, Instituto de Oncologia Pediatrica - GRAACC - UNIFESP, Sao Paulo, Brazil, [7]Eurocord, Paris, France, [8]Centro de Investigación del Cáncer (CIC, IBMCC USALCSIC); Servicio General de Citometría, Universidad de Salamanca, Salamanca, Spain

Introduction: Recent studies suggest that higher circulating dendritic cell counts after hematopoietic stem cell transplantation (HSCT) may be associated with lower risk of graft versus host disease (GVHD) and mortality. Plasmacytoid dendritic cells (pDC) have tolerogenic properties and may explain such results. However, only a few studies with conflicting results analyzed pDC counts in the allograft, mainly in the related HSCT setting.

Objectives: To compare pDC counts among different cell sources [umbilical cord blood (UCB), bone marrow (BM), and peripheral blood (PBSC)] and to correlate the pDC content on the graft with main HSCT outcomes.

Materials (or patients) and Methods: Plasmacytoid dendritic cells (pDC: lineage negative, HLA-DR+ and CD123+) were quantified by multiparametric flow cytometry in the graft just before its infusion. Overall, 77 patients (49 male; median age 21y, range 1-74y) receiving UCB (*n*=26), BM (*n*=34) or PBSC (*n*=17) HSCT from unrelated (*n*=67) or related donors (*n*=10) were studied. The most common diagnosis was acute leukemia (ALL, 30 cases; AML, 22). Most patients received myeloablative conditioning regimens (*n*=47, 61%). Antithymocyte globulin was used in 30 patients (39%) and total body irradiation in 41 (53%). Median follow up time was 15 months (4-33).

Results: Median time to neutrophil engraftment was 19 days (range: 11-49) and 35 days to platelet engraftment (range 2-176). The median percentage of pDC on the graft was 0.20% of non erythroid nucleated cells (range: 0.02-0.67) and no differences were noticed among sources. Cumulative incidence (CI) of grade II-IV acute GVHD at 100 days was higher on patients receiving grafts with higher percentages of pDC (22% for patients with less than 0.15%pDC graft content *vs*. 52% for patients receiving grafts with higher counts, *P*=0.026). There was no impact of graft pDC content on mortality, relapse, chronic GVHD or overall survival. In a multivariate analysis, graft pDC content remains an independent risk factor for acute GVHD [HR: 3.0, CI (95%): 1.15-7.8].

Discussion: Higher pDC graft percentages were associated with increased risk of acute GVHD, but had no impact on non-relapse mortality or survival. The precise role of pDC on immunity after HSCT deserves further investigation and might be related not only to pDC biology itself but to the chronology of the pDC interaction with host antigens.

Disclosure of Interest: None Declared.

PH-P287

PERIPHERAL BLOOD STEM CELLS COLLECTION IN PEDIATRICS PATIENTS USING THE CELLULAR SEPARATOR SPECTRA OPTIA

T. Baptista[1], M. Coutinho[1], F. Bordalo[1], S. Lopes[1], A. Bernardo[1], F. Amado[1], S. Ferreira[1], S. Roncon[1,]*

[1]Cellular Therapy Service, Instituo Português de Oncologia do Porto Francisco Gentil, EPE, Oporto, Portugal

Introduction: We intend to analyze the collection of peripheral blood stem cells (PBSC) with a new cellular separator Spectra

Optia since the beginning of its utilisation in our oncology institute. Due to its particular features, we choose pediatric patients proposed for autologous hematopoietic transplant.
Materials (or patients) and Methods: We observed retrospectively harvests by leukapheresis since January 2012 until November 2013. We study all the patients' clinical process and then we performed a descriptive analysis using Microsoft Excel 2007.
Results:
Population Characteristics: Our population is composed by 16 patients (9 male / 7 female), with a median age of 7 years (1-18) and a median weight of 30 Kg (8-91). All of them had inserted a central venous catheter.
The main diagnosis were: Medulloblastoma 7 cases, Neuroblastoma 4 cases, Ewing`s Sarcoma 2 cases, Primitive Neuroectodermal Tumor (PNET) 1 case and Hodgkin`s Disease 1 case.
Until that moment, 13 patients had already been transplanted with autologous PBSC.
Mobilization and Collection Characteristics: Mobilization protocol was done with granulocyte colony-stimulating factor (G-CSF) 10 ug/kg/day.
All the pediatric patients were monitorized for clinical parameters and laboratorial tests during the leukapheresis.
In 12 patients, we had to perform blood priming; so, we used a leukocyte depleted erythrocyte concentrate, irradiated, compatible and with the hematocrit adjusted to the patient. For the anticoagulation of the extracorporeal circuit, we planned to use Heparin+Citrate (ratio 14:1) in 12 patients and Citrate (ratio 25:1) in 4.
The median number of leukapheresis performed was 1.5 (1-3); the median number of blood volemias processed was 3 (2-5); and the median number of CD34+ cells collected was 3.7 (0.44-22.6) x 10^6/kg of patient body weight.
After the first mobilization, 11 children done 25 leukapheresis and 4 weeks later 5 bad mobilizers performed more 11 procedures.
The most frequent adverse reaction described with this type of PBSC collection is oral paresthesias; so, in order to prevent symptoms associated with hypocalcemia in the most small child (*N*=8), we administered Calcium Gluconate in continuous perfusion during 1 hour. Five patients needed platelets transfusion at the end of the apheresis.
Discussion: We can conclude that harvesting PBSC with Spectra Optia proved to be a safe and effective technique, even in patients with a body weight less than 10 kg. Most of them did the collection with just one apheresis in a out-patient regimen and without needing the support of pediatrician or specialist in intensive care.
Disclosure of Interest: None Declared.

PH-P288
COMPOSITION OF PBPC ALLOGRAFTS: CONTENT OF ALLOREACTIVE CELLS AND HEMATOPOIETIC PROGENITOR CELLS IS DEPENDENT ON THE APHERESIS DEVICE
J. Christian Fischer[1,], T. Robitzsch[1], J. Maria Rox[1]*
[1]Institute for Transplantation Diagnostics and Cell Therapeutics, Heinrich-Heine-University Düsseldorf, Medical Faculty, Düsseldorf, Germany

Introduction: Mobilized peripheral blood stem cells (PBPC) are the preferred stem cell source for allogeneic transplantation. Beside PBPC, transplants also contain further therapeutic active cells, e.g. NK cells. For collection mostly the Cobe spectra apheresis device (SPECTRA) was used. Recently its successor (Spectra Optia MNC apheresis system (OPTIA)) was introduced. Instead of continuous collection of the buffy coat during low spin centrifugation, the OPTIA collects target cells into an intermediate elutriation chamber during hard spin centrifugation. After processing a certain volume of peripheral blood, the chamber is flushed into the final product. During flushing no substantial further blood is processed. The peripheral blood volume processed between two flushes (BVpbF) can vary, flushing itself is either triggered automatically or manually by the operator. We questioned whether the performance of the devices and the composition of the allogeneic grafts differ between SPECTRA and OPTIA.
Materials (or patients) and Methods: At the 5th day of 10µg Lenograstim/kg BW PBPC were collected, starting apheresis 2h after the last G-CSF injection. 245 PBPC SPECTRA and 64 OPTIA runs (2010- 2013) could be analyzed for NK cells (CD56+CD3-), T helper cells (T4), cytotoxic T cells (T8), B cells by flow cytometry. Full differential blood as well as the PBPC count was obtained from PB before apheresis (as well as from the product itself. For platelets (plt) and PBPC collection efficiency (CE2) was calculated by yield/(amount pre apheresis x processed blood volume). Further performance measurements were target cell collection rate (CR, 10^6) per fold processed total blood volume and kg BW donor and throughput (TP2) (PBPC yield/[kg] BW donor/harvest time [min]/PBPC pre apheresis [/µl]). Significance level at $P < 0.02$ was chosen.
Results: OPTIA showed higher CE2 for CD34 (51% vs 46%) and plt (23% vs 20%). Lower amounts of T (CR: 87.4 vs 102.4), T4 (55.5 vs 64.7), T8 (25.2 vs 28.8), B (19.3 vs 22.5) and NK cells (8.8 vs 10.5) were collected by OPTIA, with most striking relative difference for NK cells. Also lower CD34-TP2 (346 vs 475) was observed in Optia. In OPTIA BVpbF (796 +/– 235ml (mean per donor), range 500 – 1750 ml) influenced the performance: lower BVpbF resulted in higher CR for T cells ($r^2 = 0.29$) T4 (0.32), B (0.15), NK cells (0.21), CE2_CD34 (0.17) but also in higher platelet loss in the donor (0.42). T8 numbers as well as CD34_TP2 showed only weak linear correlation to BVpbF. In Optia donations with a mean BVpbF lower than 750ml (median split, *n*=32), CR for T (T4, T8), B and NK cells did not differ from Spectra, higher CD34_CE2 (54%) as well as higher platelet loss (CE2_plt 27%) was observed.
Discussion: Performance of the devices and resulting composition of the graft, including alloreactive potentially graft vs. leukemia inducing cells like NK cells, differ. In OPTIA composition of the graft as well as collection efficiency is dependent on BVpbF: lower BVpbF results in higher collection efficiencies of the device, but for the sake of a higher platelet loss in the donor. Low BVpbF in OPTIA enables graft compositions (including alloreactive cells) comparable to the SPECTRA. Due to the missing PBPC processing during flushing periods in OPTIA frequent flushes do not translate into substantial higher PBPC throughput.
Disclosure of Interest: None Declared.

PH-P289
PREDICTORS DETERMINING THE EFFICACY OF CORD BLOOD HARVESTING AS A BIOLOGICAL MATERIAL FOR THE STEM CELL TRANSPLANTATION AND THERAPY
D. Gladysz[1], I. Marszalek[1], K. Poteralska[1], T. Oldak[1], K. Pawelec[1,2], J. Baran[1], D. Boruczkowski[1,]*
[1]Medical Department, The Polish Stem Cell Bank, [2]Department of Paediatrics, Haematology and Oncology, Medical University, Warszawa, Poland

Introduction: The study aimed to predict cord blood cell yield before harvesting procedure as the cell dose is the main limiting factor concerning therapy with stem cells derived from umbilical cord blood (UCB).
Materials (or patients) and Methods: A retrospective analysis of medical records of 11742 UCB units collected from January 1, 2010, to March 31, 2013 and qualified for cryopreservation (net volume ≥20mL) was carried out. Multiple logistic regression analyses were performed to examine effects of each studied factor on unit TNC, $CD34^+$/µL and cell viability. The univariate analyses were made with Pearson's r or Kendall's tau correlations and differences between groups were examined with Kruskal-Wallis test or Mann-Whitney U test as appropriate. A multivariate linear regression analysis was carried out to predict cell yield before harvesting. Knowing that collected volume is significantly correlated with number of total nucleated cells (TNC, r=0.71), CD34+ cell count (r=0.15) and cell viability (r=0.12), we included in the model a median UCB net volume (63 mL) as a constant.
Results: In univariate analysis we have found significant (P<0.0001) however weak correlations between TNC and older gestational

age (r=0,11) and single gestation pregnancy (r=0,12). Only negligible small correlations were found concerning $CD34^+$/μL and cell viability. In multivariate analysis TNC was affected by older gestational age, single-gestation pregnancy, female fetal gender (all with $P<0.0001$) and natural delivery route ($P=0.02$). The $CD34^+$/μL was connected with multiple-gestation pregnancy ($P<0.0001$) and male fetal gender ($P=0.005$). The cell viability, apart from younger gestational age, cesarean delivery (both with $P<0.0001$) and male fetal gender ($P=0.007$), was affected by maternal age ($P<0.0001$).
Discussion: Identifying variables having influence on cell yield before harvesting procedure should help in a quick decision concerning banking in an obstetric ward during, for example, preterm labor as well as optimize UCB donors for public banking and minimalize the costs. With many prejudices concerning cord blood collection from preterm or multiple births, we have proved that those factors should not immediately disqualify from harvesting procedure especially having in mind developing stem cell therapies in neonatal diseases.
Disclosure of Interest: D. Gladysz Conflict with: Polish Stem Cell Bank, I. Marszalek Conflict with: Polish Stem Cell Bank, K. Poteralska Conflict with: Polish Stem Cell Bank, T. Oldak Conflict with: Polish Stem Cell Bank, K. Pawelec Conflict with: Polish Stem Cell Bank, J. Baran Conflict with: Polish Stem Cell Bank, D. Boruczkowski Conflict with: Polish Stem Cell Bank

PH-P290
SIGNIFICANT INCREASE OF CELL QUANTITY IN UMBILICAL CORD BLOOD UNIT DERIVED FROM SINGLE DONOR

A. Orczykowska[1], T. Oldak[1], M. Murzyn[1], J. Placzkowska[1], J. Piskula[1], K. Zagorska[1], K. Poteralska[1], I. Marszalek[1], I. Czaplicka[1], A. Olkowicz[1], J. Baran[1], I. Balogh[2], J.-M. Pujal[3], R. Brasov[4], D. Boruczkowski[1,]*
[1]Medical Department, The Polish Stem Cell Bank, Warszawa, Poland, [2]KRIO Institute ZRt., Budapest, Hungary, [3]Sevibe Cells S.L., Girona, Spain, [4]Biogenis S.C., Bucarest, Romania

Introduction: At present, the umbilical cord blood-derived hematopoietic stem cells (UCB-HSC) are already well known as an effective transplant in the treatment of various disorders. For potential transplantations the number of nucleated cells (NC) and CD34+ cells play a significant role. To overcome the problem of insufficient number of cells derived from single umbilical cord blood unit, for effective transplantation - especially in adults, we developed a new strategy of obtaining additional pool of NC and CD34+ cells.
Materials (or patients) and Methods: After parents' informed consent, we obtained and compared 100 UCB units using specially designed separate set for both: umbilical and placental blood collected from the same patients, during natural deliveries and caesarean sections. After visual inspection of delivered placenta, placental vessels were rinsed with anticoagulant solution which resulted in the flow out of placental blood (second fraction of UCB) into the collection sack. The number of CD34+ cells, total nucleated cells (TNC) and their viability, as well as microbial contamination in placental blood and UCB units were determined and analyzed.
Results: The presence of CD34+ UCB-HSC in the second fraction of UCB was confirmed by flow cytometry. A significant increase of TNC and CD34+ cells was observed (27% and 21% on average, respectively). The viability of placental blood cells reminded on high level (95% and higher). Additional cell collection procedure did not result in increased level of microbial contamination.
Discussion: This report indicates that placental blood is a good complementary source of UCB-HSC, which can be isolated, examined and cryopreserved. Results presented in our studies suggest that collected second fraction of umbilical cord blood (placental blood) might offer an additional portion of cells, allowing to increase the potential of transplantable single-donor unit.
Disclosure of Interest: A. Orczykowska Conflict with: Polish Stem Cell Bank, T. Oldak Conflict with: Polish Stem Cell Bank, M. Murzyn Conflict with: Polish Stem Cell Bank, J. Placzkowska Conflict with: Polish Stem Cell Bank, J. Piskula Conflict with: Polish Stem Cell Bank, K. Zagorska Conflict with: Polish Stem Cell Bank, K. Poteralska Conflict with: Polish Stem Cell Bank, I. Marszalek Conflict with: Polish Stem Cell Bank, I. Czaplicka Conflict with: Polish Stem Cell Bank, A. Olkowicz Conflict with: Polish Stem Cell Bank, J. Baran Conflict with: Polish Stem Cell Bank, I. Balogh Conflict with: KRIO Institute ZRt., J.-M. Pujal Conflict with: Sevibe Cells S.L., R. Brasov Conflict with: Biogenis S.C., D. Boruczkowski Conflict with: Polish Stem Cell Bank.

PH-P291
ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION

I. Baltadakis[1,], D. Economopoulou[1], F. Panitsas[1], E. Xenou[1], A. Kesari[1], A. Komitopoulou[1], I. Tzannou[1], S. Vasileiou[1], M. Garofalaki[1], Z. Poulopoulou[1], M. E. Karatza[1], I. Tziotziou[1], I. Bika[1], M. Papageorgiou[1], S. Gigantes[1], I. Apostolidis[1], N. Harhalakis[1], D. Karakasis[1]*
[1]BMT Unit, Evangelismos Hospital, Athens, Greece

Introduction: Double umbilical cord blood transplantation (DUCBT) offers access to allogeneic stem cell transplantation (alloSCT) for almost all adult patients who lack an appropriately matched volunteer donor. Despite superior rate of engraftment, DUCBT has been associated with higher incidence of graft-versus-host disease (GvHD) compared to single-unit UCBT. In a cohort of patients who underwent DUCBT at our center, we analyzed possible risk factors for the development of acute and chronic GvHD, its clinical features and impact on the outcome.
Materials (or patients) and Methods: Between 2006 and 2013, 42 patients underwent DUCBT at a median age of 38.5 years (range, 16-60) for various myeloid ($n=30$) or lymphoid ($n=12$) hematologic malignancies. The conditioning regimen was myeloablative in 34 (81%) patients. Antithymocyte globulin was not administered during conditioning, with the exception of one case. GvHD prophylaxis consisted of mycophenolate mofetil in combination with either cyclosporine ($n=21$) or tacrolimus ($n=21$). Most units (60/84, 71.4%) were 4/6 antigen matched to recipient at HLA-A, -B, and -DRB1 loci, and the remaining were 5/6 matched. By retrospective high-resolution typing for class I HLA alleles, histocompatibility was demoted in 61% of units. By additional allele-level typing at HLA-C and -DQB1 loci, the degree of compatibility varied from 8/10 to 3/10, with 80.5% of the units being ≤6/10 matched to the patient. The median dose of cryopreserved total nucleated cells (TNC) per unit was 2.58×10^7/kg (range, 1.09-5.76). At infusion, patients received in total a median of 4.55×10^7 TNC/kg (range, 2.65-9.43) and 1.75×10^5 CD34+ cells/kg (range, 0.6-5.13).
Results: The cumulative incidence (CI) of neutrophil engraftment was 92.9%. Non-relapse mortality (NRM) was 26.4% at 100 days, and 42% at 12 months. Overall survival (OS) and disease-free survival (DFS) were 35.8% and 33.7% at 2 years. The CI of grade II-IV acute GvHD was 90.5%, with median time of onset of 19 days (range: 5-57). Grade III-IV acute GvHD occurred at a CI of 26.4%. Major sites of involvement were skin (71%), and gastrointestinal (GI) track (upper: 52.6%, lower: 44.7%), whereas liver was involved in 29% of cases. Acute GvHD responded to steroids in the majority of patients (92%), but recurrence was observed in 59%. Of note, 9/11 patients with grade III-IV acute GvHD died of NRM. The development of aGvHD did not correlate with age, intensity of the conditioning regimen, TNC or CD34+ cell dose, and the degree of HLA match of the highest mismatched unit or the engrafting unit (by either low- or high-resolution typing at 6 or 10 loci). The CI of chronic GvHD was 39% (extensive: 24%,), with median time of onset of 6 months (range: 3.5-9). Affected organs included skin (73%), GI (40%), liver (20%), mouth (20%), serosae (13%), and eyes (6.7%). The occurrence of extensive chronic GvHD was associated with less than 6/10 HLA match of the engrafting unit (HR: 4.0, $P=0.05$). Among the 15 patients with chronic GvHD, 8 have discontinued immunosuppression, 2 are still under treatment, and 5 died of complications related to GvHD ($n=4$) or relapse ($n=1$).
Discussion: A considerable incidence of grade III-IV acute GvHD was observed in DUCBT with an associated adverse effect on NRM.

Despite multiple HLA disparities, development of chronic GvHD after DUCBT was less frequent compared to the reported incidence in alloSCT from adult matched unrelated donors.
Disclosure of Interest: None Declared.

Tolerance, Chimerism and Immune Reconstitution

PH-P292
ACUTE GRAFT-VS-HOST-DISEASE IMPAIRS A CXCR4-RELATED INCREASE OF CIRCULATING HUMAN LYMPHOID PROGENITORS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

S. Glauzy[1], I. andre-Schmutz[2], J. Larghero[3], S. Ezine[4], R. Peffault de Latour[5], H. Moins-Teisserenc[1], S. Servais[5], M. Robin[5], G. Socié[5], E. Clave[1,], A. Toubert[1]*
[1]INSERM UMRS940, Univ Paris Diderot, Sorbonne Paris Cité, [2]INSERM U768, Université Paris Descartes, Sorbonne Paris Cité, Institut Imagine, [3]Unité de Thérapie Cellulaire et CIC de Biothérapies, Hôpital St Louis, [4]Unite 1020, INSERM, [5]Service d'Hématologie-Greffe de Moelle, Hôpital Saint-Louis, AP-HP, PARIS, France

Introduction: Immune- and especially T-cell-recovery after profound lymphopenia is a major challenge in many clinical situations, such as allogeneic hematopoietic stem cell transplantation (allo-HSCT). Recovery depends on hematopoietic lymphoid progenitors emerging from the bone marrow (BM).
Materials (or patients) and Methods: In this study, we characterized $CD34^+Lin^-CD10^+$ lymphoid progenitors in the peripheral blood of 39 patients by flow cytometry. T-cell potential of FACS sorted cells was assessed in 9 patient using OP9-DL1 differentiation assay in limiting dilution and their gene expression in 21 patients using RT-qPCR. Finally chemokine and cytokine concentration was assessed using Luminex or ELISA assay on plasma from 40 patients.
Results: Our data demonstrate a strong recovery of this population 3 months after transplantation. This rebound was abolished in patients who developed acute graft-versus-host disease (aGVHD). A similar recovery profile was found for both $CD24^+$ B-cell committed progenitors and $CD24^-$ circulating Thymus-Seeding Progenitors (TSP). We found a similar T cell potential for TSP isolated either from patients or from normal Healthy Donors (HD). Quantitative expression profile of 22 genes involved in T-cell differentiation and homing, in TSP from patients with aGVHD did not differ from those from patients without GVHD or HD. However, TSP from patients not presenting aGVHD had reduced CXCR4 gene expression, consistent with enhanced egress from the BM. CCR7 gene expression was reduced in all patients after allo-HSCT, as were its ligands CCL21 and CCL19. This reduction was particularly marked in patients with aGVHD, suggesting an impaired T-cell homing potential.
Discussion: Thus, the data presented here identify the TSP population as an important target when seeking to enhance immune reconstitution in humans.
Disclosure of Interest: None Declared.

PH-P293
FAVORABLE IMPACT OF NATURAL KILLER CELL RECONSTITUTION ON CHRONIC GRAFT-VERSUS-HOST DISEASE AND CMV REACTIVATION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

V. David Kheav[1], R. Peffault de Latour[2], M. Busson[1], C. Scieux[3], P. Haas[1], G. Maki[1], A. Xhaard[2], M. Carmagnat[1], M. Robin[2], P. Ribaud[2], D. Charron[1], M.-C. Mazeron[3], N. Dulphy[1], G. Socié[2], H. Moins-Teisserenc[1], A. Toubert[1,]*
[1]Immunology, Hôpital Saint-Louis, INSERM, Université Paris Diderot, AP-HP, [2]Service d'Hématologie-Greffe de Moelle, [3]Virology, Hôpital Saint-Louis, AP-HP, Paris, France

Introduction: NK cells are the first lymphocytes to recover after allo-HSCT and play a major role in innate immunity. This recovery follows the NK differentiation pattern with an early expansion of the NK $CD56^{bright}$ subset followed by NK $CD56^{dim}CD16^+$. In addition, relatively small cohort studies suggest that the kinetics of NK-cell reconstitution and cytomegalovirus (CMV) reactivations are closely intertwined. NK-cells that express the activating receptor NKG2C seem crucial in the resolution of CMV episodes.
Materials (or patients) and Methods: We investigated prospectively in a single center the kinetics and profiles of NK cell reconstitution in a large cohort of allo-HSCT adult patients (n=439) treated (2005-2011) with non T-cell depleted stem cells from bone marrow (76%) or peripheral blood (24%) and reduced intensity (61%) or myeloablative conditioning (39%). NK-cell subsets were analyzed at months M3, M6, M12 and M24 post-transplantation on freshly collected blood samples.
Results: Data were analyzed with respect to conditioning regimen, source of stem cells, underlying disease, occurrence of Graft-versus-Host Disease (GvHD), and profiles of CMV reactivation. We showed, from multivariate analysis, that acute GvHD impaired reconstitution of total and $CD56^{dim}$ NK cells at M3 (P=0.006 and 0.002 respectively). An efficient NK cell reconstitution at M3 was associated with a lower incidence of chronic GvHD, independently of a previous episode of acute GvHD and stem cell source. With regard to CMV, $CD56^{dim}$ and total NK counts were lower at M3 in the group of patients reactivating CMV between M0 and M3 (P=0.03 and 0.01). Whereas there was no statistical difference between counts of total NK, $CD56^{bright}$ and $CD56^{dim}$ at M3 and a CMV reactivation detected during the M3-M6 period, NKG2C expressing total NK cells and the $CD56^{dim}$ NK subset were significantly increased at M3 in patients who did not experience further reactivation (P=0.011 and 0.007). This favors a direct role of NKG2C expressing NK cells in the early control of CMV reactivation.
Discussion: These data highlight the interest to monitor NK cells at the subset rather than at the level of the whole population and highlight in a large cohort of adult patients the beneficial impact of a potent early NK reconstitution, especially of the $CD56^{dim}$ NK subset.
Disclosure of Interest: None Declared.

PH-P294
QUANTITATIVE CHIMERISM:AN INDEPENDENT ACUTE LEUKEMIA PROGNOSIS INDICATOR FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Q. Xiaoying[1,], L. Guoxuan[1], Q. Yazhen[1], H. Xiaojun[1]*
[1]Peking University People's Hospital, Peking University Institute of Hematology, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing, China

Introduction: To evaluate the prognostic significance of quantitative chimerism for monitoring minimal residual disease and predicting relapse in patients with acute leukemia (AL) following allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Materials (or patients) and Methods: the quantitative chimerism levels of 129 AL patients were measured using real-time quantitative PCR based on 29 sequence polymorphism(SP) markers at designed time points after allo-HSCT. All patients were divided into different groups based on the quantitative chimerism levels and intervention application.

Results: The ROC curve results indicate that the optimal cutoff point to predict an inevitable relapse is 1.0%, When this tentative cutoff value was used, chimerism levels had a sensitivity of 96.2% and specificity of 79.6%, the relapse rate of patients with chimerism >1.0% at two years was 55.0%, while the relapse rate of the patients with chimerism <1.0% was 0%, *P*=0.000,quantitative chimerism>1.00% was indicative of a higher probability of relapse.The Cox multivariate analysis indicated only quantitative chimerism >1.00% were associated with lower disease-free survival(hazard ratio(HR)=10.825; 95% confidence interval (CI) =4.704–24.912, *P*=0.000) and lower overall survival (HR=8.681; 95% CI=3.728–20.212, *P*=0.000). Of the 47 patients with quantitative chimerism >1.00%, 24 patients received modified donor lymphocyte infusion (mDLI) immunotherapy, This group had a significantly lower relapse rate (37.5%) than the 9 patients who did not receive mDLI immunotherapy (100%; *P* =0.001).
Discussion: the quantitative chimerism is an independent prognostic factor that can predict clinical outcomes for AL patients after HSCT and provide a guide for suitable interventions. Dynamically monitoring the level of quantitative chimerism after HSCT, however, is more important for determining whether intensive intervention should be performed.
Disclosure of Interest: None Declared.

PH-P295
FUNCTIONAL ANALYSIS OF IMMUNE RECONSTITUTION FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

M. Waterhouse[1,], L. Konukiewitz[1], H. Bertz[1], J. Finke[1]*
[1]Freiburg University Medical Center, Freiburg, Germany

Introduction: Immune status assessment after allogeneic hematopoietic stem cell transplantation (HSCT) relies primarily on complete blood counts and immunophenotypic analyses of T and B lymphocytes. However, this kind of approach does not analyze their functional capacity. Characterization of potential functional immune defects following allogeneic transplantation may contribute to predict clinical complications after transplant.
Materials (or patients) and Methods: . Blood samples from a cohort of 77 transplanted patients were collected at fixed time points (30, 100 and 365 days) after HSCT. Patient's samples were analyzed for lymphocytes subsets (CD3, CD4, CD8, CD56) T cell proliferation, CD4 intracellular ATP content, T cell cytotoxicity, neutrophil phagocytic capacity and granulocyte oxidative burst. High risk acute leukemia was the main indication for HSCT (61% of the patients). Eighty percent of the patients received a non-manipulated matched unrelated graft. As preparative regimen, 66 patients received a fludarabine-based toxicity-reduced regimen; while the rest of the patients, a conventional high-dose conditioning protocol was used. For graft versus host (GvHD) prophylaxis, in addition to cyclosporine, all the patients with unrelated donor received either alemtuzumab or rabbit anti-T lymphocyte globulin.
Results: The GvHD (grade III and IV) and relapse incidence was 5 and 31 percent respectively. Complete blood count was reduced below normal limits until day 100 and normalized one year after transplantation. All lymphocytes subsets were below normal levels one year after HSCT. CD3 proliferation and CD4 intracellular ATP content was significantly reduced at day 30 and 100 following HSCT. CD4 intracellular ATP content remained reduced for one year after transplant. T cell degranulation, assessed as CD107 expression, did not show significant changes at all time points tested. Neutrophil phagocytic capacity remained unchanged during the first year after transplantation, while the oxidative burst was significantly reduced until day 100 returning to normal levels one year after transplantation. Patients (*n*=12) with low and stable levels of CD4 intracellular ATP content between day 30 and 100, showed an increased incidence of post-transplant complications (CMV reactivation, infections and relapse) in comparison with patients that increased the CD4 intracellular ATP levels in the same time period. Reduced T cell proliferation was not associated with low CD4 ATP intracellular ATP content. Transplanted patients with reduced T cell proliferation and low oxidative burst activity did not showed an increase in the incidence of post-transplant complications such as infections or relapse.
Discussion: CD4 ATP intracellular ATP content might be a predictive biomarker for post-transplant complications. Predictive value should be established in a larger patient cohort.
Disclosure of Interest: None Declared.

PH-P296
KINETICS AND QUALITY OF GAMMA/DELTA T-CELL RECONSTITUTION AFTER TCR-ALPHA/BETA/CD19-DEPLETED HAPLOIDENTICAL HSCT

I. Airoldi[1], I. Prigione[1], A. Bertaina[2], A. Zorzoli[1], D. Pagliara[2], G. Barbarito[1], S. Rutella[2], R. M Pinto[2], V. Pistoia[1], P. Merli[2], L. Brescia[2], B. Lucarelli[2], L. Moretta[1], F. Locatelli[2,3,]*
[1]Ospedale Gaslini, Genoa, [2]IRCCS Bambino Gesù Children's Hospital, Rome, [3]University of Pavia, Pavia, Italy

Introduction: We recently developed a new method of graft manipulation based on the physical removal of $\alpha\beta^+$ T cells and $CD19^+$ B cells for HLA-haploidentical hematopoietic stem cell transplantation (HSCT). This approach permits to leave in the graft mature, donor natural killer cells and $\gamma\delta^+$ T cells, which can both exert a graft-versus-leukemia effect and reduce the risk of infection.
Materials (or patients) and Methods: We characterized circulating $\gamma\delta^+$ T cells from 38 pediatric patients (pts, 32 with malignancies and 6 with non-malignant disorders) receiving this type of allograft. *Ex vivo* assays of $\gamma\delta^+$ T cells were performed weekly until Hospital discharge and monthly until 6 months after HSCT. Phenotype of $\gamma\delta^+$ T cells was analysed using flow-cytometry; the following mAbs (CD3, CD45, pan-γδ, anti-Vδ1, -Vδ2, -Vγ9, CD45RO, CD45RA, CD27, CD16, CD56) were used to allow the identification of the main $\gamma\delta^+$ T-cell subsets (*naïve*, central memory CM, effector memory EM, and terminally differentiated TD). Functional studies were performed using $\gamma\delta^+$ T cells both shortly after collection from pts and after *in vitro* expansion with zoledronic acid (ZOL) and IL-2. The cytotoxic activity of $\gamma\delta^+$ T cells was tested against primary leukemia cells; for these experiments, we used the CD107a degranulation assay and/or standard ^{51}Cr-release assay.
Results: Until day +30 after HSCT, circulating T cells were consistently $\gamma\delta^+$ (>90%); subsequently, $\alpha\beta^+$ T cells began to increase over time. $\gamma\delta^+$ T cells included both $V\delta2^+V\gamma9^+$ and $V\delta1^+V\gamma9^{+/-}$ cells, as well as, marginally, the $V\delta1^-V\delta2^-V\gamma9^-$ population. Of note, the $V\delta1^+$ subset was significantly higher than the $V\delta2^+$ population in pts with cytomegalovirus (CMV) reactivation ($P<0.01$). On day +20 after HSCT, 10.5% of $V\delta2^+$ cells on average were *naïve*, 53% CM, 23.5% EM and 13% TD. Similarly, 22.3% of $V\delta1^+$ cells were *naïve*, 49% CM, 5.6% EM and 23% TD. The proportion of the different $V\delta2^+$ and $V\delta1^+$ T cell subsets changed significantly over time, when comparing samples collected on day +20 with those obtained on day +90 or +180 after HSCT. In particular, within both $V\delta2^+$ and $V\delta1^+$ T-cell subsets, *naïve* and TD increased, whereas CM decreased over time; EM cells did not change. In pts experiencing CMV reactivation, $\gamma\delta^+$ T cells were mostly of the $V\delta1^+$ phenotype (49% were TD, 10% EM, 21% CM and 20% *naïve*). We investigated the effect of exposure to ZOL and IL-2 on gd T cells in 21 pts; $\gamma\delta^+$ T cells consistently expanded *in vitro*, the resulting Vγ9Vδ2 population mainly comprising EM cells. These Vγ9Vδ2 cells lysed primary leukemia cells of both myeloid and lymphoid origin (as shown in a classical chromium-51 release assay in 8 pts); this effect was even more pronounced when leukemia targets were pre-treated with ZOL. Such activity was unambiguously TCRVγ9-mediated and dependent on phosphoantigen recognition. Finally, $\gamma\delta^+$ T cells obtained from 30 pts showed CD107a degranulation when challenged *in vitro* with primary blasts obtained from 12 pts with either lymphoid or myeloid acute leukemia.
Discussion: We provide the first detailed characterization of circulating $\gamma\delta^+$ T cells reconstituting in children given this type of T-cell-depleted haplo-HSCT. We demonstrate that $\gamma\delta^+$ T cells

are important effector cells, which can be expanded and activated after exposure to bisphosphonates and IL-2 with the aim of improving their killing capacity against leukemia cells.
Disclosure of Interest: None Declared.

PH-P297
GRAFT-VS-HOST-DISEASE AFFECTS NEW B CELL PRODUCTION AND PROLIFERATION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

J. Soret[1], S. Glauzy[1], R. Peffault de Latour[2], G. Maki[1], M. Robin[2], H. Moins-Teisserenc[1], G. Socié[2], E. Clave[1,], A. Toubert[1]*
[1]INSERM UMRS940, Univ Paris Diderot, Sorbonne Paris Cité, [2]Service d'Hématologie-Greffe de Moelle, Hôpital Saint-Louis, AP-HP, PARIS, France

Introduction: B-cell recovery after Allogeneic Hematopoietic Stem Cell Transplantation (aHSCT) is characterized by an early rise of naïve B cells and a long term deficit in memory B cells. Adverse reactions such as chronic Graft-versus-Host Disease (GvHD) could delay this reconstitution. Homeostasis of the naïve and memory B cell compartments is critical for both response to infection and the reestablishment of B cell tolerance.
Materials (or patients) and Methods: We used here molecular markers based on the rearrangement of immunoglobulin genes during B-cell differentiation. Quantification of DNA excision circles generated during the deletion of the Kappa constant region (sjKREC for signal joint Kappa deleting element Rearrangement Excision Circle) and its genomic "coding joint" counterpart (cjK) allowed to estimate the B-cell neogenesis (sjKREC) and the B-cell proliferative history (cjK/sjKREC ratio). We quantified by real time PCR, absolute number of sjKREC and cjK in the peripheral blood of 40 healthy donors and 255 adult aHSCT patients transplanted between 2006 and 2009. Patient blood was collected before graft and at 3, 6 12 and 24 months after graft. Additionally, absolute number of CD19+CD27- or CD27+ was assessed by flow cytometry.
Results: In normal controls, cjK copy numbers correlated with CD19+ cell counts. The percentage of naïve (CD27-) B cells inversely correlated with the cjK/sjKREC ratio indicating a lower proliferation rate in this population. Moreover, neither cjK, sjKREC nor their ratio were affected by age.
In aHSCT patients, recipient conditioning (RIC vs. MAC), stem cell source (BM vs. PBMC mobilized stem cells) and the primary disease (malignant vs. others) did not affect significantly B-cell excision circles reconstitution. Recovery of both absolute numbers of cjK and sjKREC were significantly delayed in case of acute and chronic GvHD, from month 3 and month 6 respectively to month 24 after graft. Noteworthy, cjK/sjKREC ratios as proliferative indexes, were significantly higher at month 3 (median 10.1 vs 3.6 divisions, P=0.0016) in case of acute and at months 6 and 12 (median 3.6 vs 2.5, P=0.0463 and 5.4 vs 3.1, P= 0.0002, respectively) in chronic GvHD.
Discussion: So, in this large cohort of patients, cjK and sjKREC quantification provided a new insight in the kinetics of B-cell homeostasis during GvHD, showing a delay in B-cell recovery despite an increased B-cell proliferation rate.
Disclosure of Interest: None Declared.

PH-P298
ULTRA LOW-DOSE IL-2 MEDIATED EXPANSION OF REGULATORY T CELLS AS GVHD PROPHYLAXIS FOR RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELLS

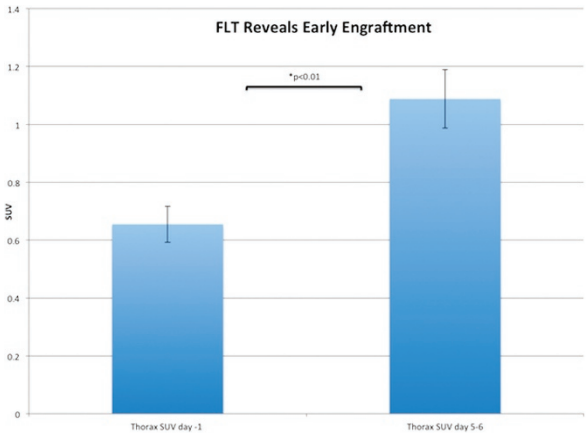
C. Bollard[1,], A. Kennedy-Nasser[2], A. J. Barrett[3], S. Ito[3], E. Yvon[4], R. Krance[2], K. Leung[2], H. E Heslop[2], M. K Brenner[2]*
[1]Childrens National Medical Center and The GW University, Washington, [2]Baylor College of Medicine, Houston, [3]National Institutes of Health, Bethesda, [4]MD Anderson Cancer Center, Houston, United States

Introduction: Graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (alloSCT) has been associated with low numbers of circulating $CD4^+CD25^+FoxP3^+$ regulatory T-cells(T_{regs}). Because T_{regs} express high levels of the IL-2 receptor, they may selectively expand *in vivo* in response to doses of IL-2 insufficient to stimulate T-effector T-cell populations, thereby preventing GvHD.
Materials (or patients) and Methods: We prospectively evaluated the effects of ultra low-dose (ULD) IL-2 injections on T_{reg} recovery in pediatric patients after alloSCT and compared this recovery with T_{reg} reconstitution post alloSCT in patients without IL-2. Sixteen recipients of related(n=12) or unrelated(n=4) donor grafts received ULD-IL-2 post HSCT (100,000-200,000 IU/m^2 3xweekly), starting <day30 and continuing for 6-12weeks.
Results: No grade 3/4 toxicities were associated with ULD-IL-2. $CD4^+CD25^+FoxP3^+$ T_{regs} increased from a mean of 4.8% (range, 0-11.0%) pre IL-2 to 11.1%(range,1.2-31.1%) following therapy, with the greatest change occurring in the recipients of MRD transplants. No IL-2 patients developed grade II-IV aGvHD, compared to 4/33(12%) of the comparator group who did not receive IL-2. IL-2 recipients retained T-cells reactive to viral and leukemia antigens, and in the MRD recipients, only 2/13(15%) of the IL-2 patients developed viral infections versus 63% of the comparator group (P=0.022).
Discussion: Hence, ULD IL-2 is well-tolerated, expands a $CD4^+CD25^+FoxP3^+$ T_{reg} population *in vivo*, and may be associated with lower incidence of GvHD and viral infections.
Disclosure of Interest: None Declared.

PH-P299
NOVEL IMAGING REVEALS EARLY ENGRAFTMENT AND STEM CELL HOMING AFTER HSCT

K. M Williams[1,], J. L Holter Chakrabarty[2], L. Lindenberg[3], J. Gea-Banacloche[4], B. Blacklock Schuver[4], D. Hickstein[4], J. Kochenderfer[4], J. Wilder[4], K. Kurdziel[3], S. Steinberg[5], H. Khuu[6], D. H. Fowler[4], D. Halverson[4], D. N. Avila[4], G. Selby[2], T. N. Taylor[4], J. Mann[4], J. Hsu[4], S. Z. Pavletic[4], C. M. Bollard[7], P. Choyke[3], R. E. Gress[4]*
[1]BMT, Children's National Medical Center/ETIB/NCI/NIH, Washington, [2]BMT, University of Oklahoma, Oklahoma, [3]Molecular Imaging Branch, [4]ETIB/National Cancer Institute, NIH, [5]Biostatistics and Data Management Section, OCD, CCR, NCI, NIH, [6]Department of Transfusion Medicine, NIH, Bethesda, [7]BMT, Children's National Medical Center, Washington, United States

Introduction: Although rates of engraftment have improved since the advent of hematopoietic stem cell transplantation (HSCT), graft failure confers high mortality due to diagnostic delay. Therefore, novel approaches to predict engraftment are of critical importance. We have shown that a novel nuclear medicine test FLT PET/CT could predict engraftment in rats; we hypothesized that this strategy would predict engraftment after HSCT.
Materials (or patients) and Methods: Thirteen adult patients with acute leukemia received myeloablative conditioning with TBI/CY followed by (7/8 or 8/8) SD or UD BM or PBSC allogeneic HSCT. FLT imaging was performed peri-HSCT (at day -1 and day +5-12) (on NCT01338987). Analysis of images was performed using MIM Software, with standardized regions of interest of 10mm size used to capture standardized uptake value (SUV) in medullary spaces.
Results: All 13 patients engrafted (ANC >500) at a median of 15 days for recipients of PBSC grafts and a median of 20 days for BM recipients. There were no toxicities attributable to FLT. FLT revealed a consistent pattern of marrow activity, first to thorax, followed by cervical/lumbar, then sternum, and finally to extremities. This was shown by slope changes in SUV between day -1 and day +5-9 of: 0.1, 0.06, 0.04, and 0.03 respectively. Using thoracic peak SUV for those without relapse (n=10), FLT SUV inversely correlated with days to ANC >500 as a log slope of -0.29 (R2 of 0.822), predicting engraftment 3 to 25 days before ANC>500. The magnitude of FLT SUV distinguished between non- and early engraftment with at day -1 and at 5-6 days post-HSCT, of 0.65 and 1.1 respectively (P<0.01). Although all patients engrafted, 1 experienced secondary graft failure on day 60 and FLT SUV predicted the recovery without additional HSC infusion (0.93, outside the range of myeloablation).



Discussion: Hence, these data reveal that the kinetics of engraftment may be defined using FLT imaging as early as 5 days post-HSCT. In addition, these data provide unique insights into stem cell biology and homing post HSCT. In summary, our data support the safety and feasibility of FLT imaging for sensitive diagnosis of engraftment early after HSCT, thereby providing support for a future study in cord blood recipients.
Disclosure of Interest: None Declared.

PH-P300
IMMUNE RECONSTITUTION OF DENDRITIC CELLS SUBPOPULATIONS IN THE PERIPHERAL BLOOD AFTER PAEDIATRIC ALLOGENEIC STEM CELL TRANSPLANTATION

O. Ciocarlie[1], M. Christian Elze[2], A. Heinze[3], S. Kloess[1], L. Arseniev[1,], T. Klingebiel[3], M. Serban[4], J. Hutton[2], U. Koehl[1]*
[1]Institute of Cellular Therapeutics, GMP-DU, IFB-Tx, Medical School Hannover, Hannover, Germany, [2]Department of Statistics, University of Warwick, Coventry, United Kingdom, [3]Paediatrics Department, University Hospital Frankfurt, Goethe University, Frankfurt am Main, Germany, [4]Paediatrics Department, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

Introduction: Dendritic cells (DC) are highly specialized antigen presenting bone marrow (BM) derived leukocytes which play an important role in linking innate and adaptive immunity. Data on immune reconstitution of DC after allogeneic stem cell transplantation (allo-SCT) are mostly in adults and limited for paediatric patients. Therefore, we evaluated DC subsets immune recovery after paediatric allo-SCT and the impact of various factors on the pattern of DC recovery and patients survival.
Materials (or patients) and Methods: Paediatric patients (media*n*=13.2 yr) with a regular follow-up interval (media*n*=1816 days) were enrolled for evaluation. Single platform, 5 color flow cytometryc analysis of myeloid DC (mDC) and plasmacytoid DC (pDC) in the peripheral blood (PB) of patients (*n*=54) diagnosed with acute leukemia (*n*=38) and solid tumor (*n*=16) after allo-SCT (+30, +60, +90, +120, +150, +180, +240, +270, +360 days) were performed and compared to DC normal values in children (*Ciocarlie et al., Klin Padiatr, 2013*). Stem cell sources are CD3/19 depleted peripheral blood stem cells (PBSC) (*n*=38) and unmanipulated BM (*n*=16).
Results: Most patients did not reach normal DC values in the PB during the 1st yr after allo-SCT with no significant difference between mDC and pDC pattern of recovery. However, ranges of mDC are overlapping the lower limit of mDC in the PB of healthy children at the end of the 1st yr after allo-SCT, while pDC show a kinetic of recovery with levels below the reference ranges during the entire 1st yr. Although BM grafts have significantly less pDC (p≤0.0001) compared with CD3/19 depleted PBSC grafts, values in the PB after allo-SCT were higher for mDC and pDC in patients with BM transplantation. Evaluation of various factors impact on immune recovery of DC after allo-SCT resulted in no significant differences in relation to diagnoses, relapse, various degrees of acute graft versus host disease (aGvHD) or concomitant GvHD and relapse. However, investigation of the impact of aGvHD on DC at different time points revealed at an early time point after allo-SCT a significant difference with regard to the immune recovery of mDC (*P*=0.015) and pDC (*P*=0.003) with faster immune recovery for the patients developing aGvHD. Survival rates for patients with a slow DC immune recovery (mDC<5/µl; pDC<4/µl) and the ones with a fast pattern of immune reconstitution (mDC≥5/µl; pDC≥4/µl) after allo-SCT were investigated by using Kaplan-Meier survival curves. Myeloid DC recovery in the PB differed significantly between patients with a slow and those with a fast recovery on day +120 (*P*=0.0463) and day +180 (*P*=0.0128), indicating better survival rates for the latters.
Discussion: Knowledge about immune recovery pattern of DC subsets after allo-SCT, especially in comparison with DC in the PB of healthy children might contribute as an additional piece of the immunological puzzle in order to improve risk stratification for patients who underwent allo-SCT for malignant diseases. Future analysis should also include monoclonal antibodies for HLA typing in order to allow a fast flow cytometric chimerism analysis between donor and recipient DC origin.
Disclosure of Interest: None Declared.

PH-P301
REDUCED INTENSITY ALLOGENEIC TRANSPLANTS OUTCOMES FOR HIGH RISK AML ARE FAVOURED BY ACQUISITION OF EARLY FULL DONOR T CELL CHIMERISM:SINGLE CENTER EXPERIENCE

A. Gudger[1,], H. Hussein[1], F. Seymour[1], P. Dyer[1], J. Khwaja[1], J. Patel[1], K. Holder[1], L. Baker[1], L. Bratby[1], B. Kishore[1], R. Lovell[1], S. Paneesha[1], D. Milligan[1], E. Nikolousis[1]*
[1]Haematology, Heart of England NHS Trust, Birmingham, United Kingdom

Introduction: Reduced intensity conditioning (RIC) T-deplete regimens are now widely used as a platform to deliver a potentially curative T cell mediated graft-versus-leukaemia (GVL) effect in patients with haematological malignancies. T cell chimerism has been shown to correlate with the risk of both GVHD and disease relapse in T replete RIC AML allografts but it remains unclear whether such a correlation exists in patients undergoing an alemtuzumab based allograft for intermediate and high risk AML.
Materials (or patients) and Methods: We have therefore correlated T cell chimerism on D+60 and D+180 in 67 patients undergoing an alemtuzumab based RIC allograft for AML(high risk=30, intermediate risk=37) in CR1 with transplant outcomes. The conditioning used was Fludarabine 30mg/m^2 for 5 days, Melphalan 140mg/m^2 for 1 day and Alemtuzumab 30mg IV for 1 day for sibling transplants and 50mg IV over 2 days for unrelated donor transplants. The median age of the patients was 54 years (range 42-62 years) and median follow up was 48 months (range 6 – 108 months). 28 patients received a graft from a sibling donor and 39 from an unrelated fully matched donor.
Results: The 3 year overall survival was 49% on the intermediate risk group and 42% on the high risk group. Overall 33% of patients relapsed in both groups.
T cell full donor chimerism at D+60 and D+180 was significantly associated with better overall survival (50 months vs 19 months p:0.04), 2 years disease free survival of (53% vs 32% p:0.03) and higher incidence (82%) of acute GvHD (p:0.01) and chronic GvHD (p:0.02) in the high risk AML group on both univariate and multivariate analysis. In the intermediate risk group though full donor chimerism was associated with improved disease survival on univariate analysis but this wasn't confirmed on multivariate analysis and this was likely secondary to the small number of patients. In both univariate and multivariate analysis patients with full donor chimerism at 60 days were almost 80% more likely to have acute GVHD than patients with fully donor chimerism (*p*=0.04) and patients with full donor chimerism at D+180 were almost 85% more likely to achieve chronic GvHD (p:0.02).

Preemptive DLI infusion for mixed chimerism post D+180 was given to patients without prior severe GvHD after stopping Ciclosporin and was associated with improved disease free survival and a trend for improved overall survival for both risk groups by potentially augmenting the GvL effect. The 2 year incidence of graft-versus-host disease post DLI was only 29%.
Discussion: Although the small number of patients included can be a limiting factor it seems that high risk AML group of patients will benefit from acquisition of full donor T cell chimerism. In the intermediate risk group this effect was not demonstrated although molecular risk stratification with flt-3 and NPM-1 is needed to draw safe conclusions.
Disclosure of Interest: None Declared.

PH-P302
IMPACT OF HLA-DISPARITY DEGREE ON B-CELL RECONSTITUTION AFTER UNRELATED STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA

M. Medeot[1,], A. Sperotto[1], D. Damiani[1], A. Michelutti[1], M. Cavallin[1], M. Tiribelli[1], M. Cerno[1], M. Lisa Battista[1], R. Fanin[1], F. Patriarca[1]*
[1]Division of Hematology and BMT, Azienda Ospedaliero-Universitaria di Udine, Udine, Italy

Introduction: Post-transplant immune reconstitution is a complex and still poorly characterized process; this is particularly true for B-cell recovery.
The aim of this study was to assess the immune recovery of B cells over a period of 24 months after allogeneic stem cell transplantation (HSCT) from an unrelated donor for acute leukemia.
Materials (or patients) and Methods: Ninety-one patients (median age 51 years, range 22-71) were enrolled in the study, including 64 with acute myeloid leukemia (AML) and 27 with acute lymphoblastic leukemia (ALL).
Donors and recipients were typed at HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 at the allele level. According to HLA compatibility, 30, 31 and 30 donor-recipient pairs (D/R) showed no (FULL), 1 (MM1) or ≥2 (MM2) mismatches at the antigenic or allelic level, respectively. Namely, in the MM2 subset, 23 D/R had 2 HLA-mismatches, 4 D/R had 3, and 3 D/R had 4.
Eighteen patients received donor bone marrow stem cells and 73 received donor peripheral stem cells. Preparative regimens included conventional myeloablative conditioning (60 patients) and different reduced-intensity regimens (31 patients). No patient received rituximab as part of the conditioning; in patients reactivating Epstein-Barr virus (EBV) (*n*=17), B-cell reconstitution was censored at the time of first rituximab administration.
Flow-cytometry was used to prospectively assess $CD19^+$, $CD20^+$ B-cell reconstitution on fresh blood samples before and at 1, 2, 3, 6, 9, 12 and 24 months after HSCT.
Results: B-cell counts were already decreased before HSCT (median 16 cells per ml blood, range 0-343) and dropped even further within the first month post-transplant (median 1/ml, range 0-297). Starting from the second month (median 4/ml), B-cell counts slowly but steadily increased (6/ml at 3 months, 68/ml at 6 months, 143/ml at 9 months, 151/ml at 12 months) to reach values in the normal range by month 24 (median 258/ml, range 22-625).
Subsequently, we tried to identify factors influencing B-cell recovery at the different time-points. Patient and donor age, hematologic malignancy (AML ALL), stem cell source, conditioning regimen (conventional myeloablative *vs* other), CD34+ and CD3+ graft content did not correlate with B-cell reconstitution at any time point. According to the degree of HLA-matching (FULL *vs* 1MM *vs* 2MM), there was no difference in B-cell counts at the earlier time points (months 1, 2 and 3 post-transplant). Conversely, when compared to FULL and 1MM, MM2 D/R had lower B-cell counts starting from month 6 throughout all the period of observation. Namely, B-cell values for FULL, MM1 and MM2 D/R were as follow: 62 *vs* 119 *vs* 50/ml at month 6 (*P*=0.026), 195 *vs* 191 *vs* 31/ml at month 9 (*P*=0.003), 195 *vs* 300 *vs* 122/ml at month 12 (*P*=0.09) and 304 *vs* 375 *vs* 66/ml at month 24 (*P*=0.03). Incidence of grade 2 to 4 acute GVHD and of CMV reactivation, which mostly occurred in the first 3 months after transplant, were similar in the 3 groups. Impaired B-cell recovery in MM2 recipients was associated with a trend for higher incidence of chronic GVHD, as 18 out of 27 (67%) evaluable MM2 patients developed cGVHD, compared to 13 out of 25 (52%) FULL and 12 out of 26 (46%) MM1 patients (*P*=0.15).
Discussion: We conclude that high-degree HLA-mismatching between donor and recipient correlates with an impaired B-cell recovery, which is likely to be associated with increased risk of cGVHD development.
Disclosure of Interest: None Declared.

PH-P303
LOW COUNTS OF NATURAL KILLER CELLS CD56 BRIGHT CD16 NEGATIVE AFTER ENGRAFTMENT ARE ASSOCIATED WITH WORSE SURVIVAL IN PATIENTS RECEIVING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

M. Vescovi Goncalves[1,], M. Yamamoto[1], E. Kimura[1], V. Antonio Renzi Colturato[2], M. Valeria Ikoma[3], M. Souza[2], M. Mauad[2], P. G. Alvarenga Oliveira[1], T. X. Carneiro[1], F. Rodrigues Kerbauy[1], N. Hamerschlack[4], Y. Novis[5], V. Cortez Ginani[6], A. Seber[6], V. Rocha[7], A. Orfao[8], C. Arrais Rodrigues[1]*
[1]Hematology and Tranfusion Medicine, UNIFESP, Sao Paulo, [2]Bone Marrow Transplantation, [3]Laboratory of Flow Cytometry, Hospital Amaral Carvalho, Jaú, [4]Bone Marrow Transplantation, Hospital Israelita Albert Einstein, [5]Bone Marrow Transplantation, Hospital Sirio Libanes, [6]Bone Marrow Transplantation, Instituto de Oncologia Pediatrica-GRAACC-UNIFESP, Sao Paulo, Brazil, [7]Eurocord, Paris, France, [8]Centro de Investigación del Cáncer (CIC, IBMCC USALCSIC); Servicio General de Citometría, Universidad de Salamanca, Salamanca, Spain

Introduction: Natural Killer Cells are innate immune system cells important in host defenses against viruses and tumor cells. Two subpopulations are well described: NK CD56bright CD16neg (NK56++16-, lower frequency on peripheral blood-PB, high cytokine production) and NK56dim16pos (NK56+16+, higher frequency on PB, high cytotoxic activity). They are activated through a balance between signals given from activating and inhibitory receptors (KAR and KIR, respectively). The ligands of KIRs are the MHC molecules and in the absence of compatible MHC, NK cells are activated. In the allogeneic hematopoietic stem cell transplantation (HSCT), recent studies showed that NK cells recovery is important on infection control and, in the presence of a KIR-MHC mismatch, they may be important on graft versus host disease(GVHD) and graft versus leukemia effects. However few studies evaluated NK subpopulations recovery and HSCT endpoints.
Materials (or patients) and Methods: NK (CD3-,CD56+) subpopulations (NK56++16- and NK56+16+) were quantified by multiparametric flow cytometry at 9 sequential time points (before conditioning, at engraftment, and at days 3, 7, 14, 21, 60, 100 and 180 after engraftment). Overall, 111 patients, from 4 HSCT centers (65% male, median age 17 years, range1-74), receiving bone marrow (BM, 46%), umbilical cord (UCB, 32%) or peripheral blood (PB, 22%) from unrelated (*n*=90) or related donors (*n*=21) were studied. The most common diagnosis was acute leukemia (AML 36%, ALL 31%, MDS 9%, CML 9%, Aplastic anemia 8%). Most patients received myeloablative conditioning (MAC) regimens (60%). Antithymocyte globulin (ATG) was used in 44 patients (40%) and total body irradiation (TBI) in 56 (51%). Median follow up time was 14 months (range 4-35).
Results: Eighty-six patients presented sustained allogeneic recovery (no differences among sources). Of these, median time to neutrophil engraftment was 18 days (range: 8-52). The cumulative incidence (CI) of non-relapse-related mortality (NRM) was significantly higher in those with lower counts of NK56++16– during first 3 weeks after HSCT (34% at 1 year for patients with less than 30 cells/uL at day 21 vs 11% for patients with higher counts, *P*=0.03). Overall survival was significantly worse in patients with lower counts of NK56++16-subpopulations in the day 21 after engraftment (86% at 1 year vs 54% for patients with less than

30cells/uL – *P*=0.003). CI of grade II-IV acute GVHD and relapse were not significantly affected by NK counts. The number of NK56+16+ cells did not affect any endpoint studied. Cell source, age and conditioning regimen did not affect any of the NK subpopulations counts. In multivariate analysis, NK56++16- counts lower than 30 cells/uL at 21 and 60 days after engraftment remain an independent risk factor for non relapse mortality [HR: 4.8, CI (95%): 1.2-18.8].
Discussion: Low NK56++16- counts in the first weeks after HSCT are associated with increased non relapse related mortality, but not acute GVHD or relapse. The mechanisms that rules the NK56++16- role on immunity deserve further investigations.
Disclosure of Interest: None Declared.

Autoimmune Diseases

PH-P304
AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR TAKAYASU'S ARTERITIS: REPORT OF THREE CASES

D. Aparecida Moraes[1,], J. Bernardes Elias Dias[1], A. B. Lima Stracieri[1], F. Pieroni[1], R. Guerino Cunha[1], R. Malta[1], A. Ferreira Zombrilli[1], L. Nilsen[2], V. Leopoldo[1], B. Pinto Simões[1], M. C. Oliveira[1]*
[1]Bone Marrow Transplantation, Internal Medicine, Ribeirão Preto Medical School, University of São Paulo, [2]Ribeirão Preto College of Nursing, University of São Paulo, Ribeirão Preto, Brazil

Introduction: Takayasu's arteritis is a rare autoimmune vasculitis that affects medium and large vessels, especially the aorta and its main branches. Disease pathogenesis includes inflammation of artery walls and subsequent narrowing of vessels with progressive impairment of blood flow.
Materials (or patients) and Methods: We report outcomes of three patients with refractory Takayasu's arteritis treated with autologous hematopoietic stem cell transplantation (AHSCT), followed for 127, 43 and 15 months, respectively. **Case 1:** 41 year-old female, with a history of dizziness, claudication of upper and lower limbs, non-palpable right radial pulse and transient visual impairment for over 13 years. Arteriography showed irregularity and stenosis of abdominal aorta, right and left iliac arteries, left subclavian and carotid arteries. She had been unsuccessfully treated with steroids, methotrexate, cyclophosphamide, chlorambucil and mycophenolate mofetil. **Case 2:** 30 year-old female, with chronic history of intermittent visual deficits, upper limb claudication, subclavian steal syndrome and low blood pressure measurements. Within the last year before enrolment, she had presented three episodes of transient ischemic strokes. Similarly to the first patient, she had been previously treated with several immunosuppressive drugs, including steroids, azathioprine, cyclophosphamide and mycophenolate mofetil, besides a stent placed in the right carotid artery. **Case 3:** 39 year-old male with intestinal claudication, renovascular hypertension, upper limb claudication and dizziness. Magnetic angioresonance images evidenced right renal and mesenteric artery stenosis, and bilateral critical narrowing of carotid, subclavian, brachial and axilary arteries, as well as irregularities along the aorta. Patients were enrolled for AHSCT, mobilized with 2g/m^2 cyclophosphamide plus G-CSF, and had their hematopoietic stem cells harvested by leukoapheresis and cryopreserved, non-manipulated. In sequence, each patient received 200mg/kg IV cyclophosphamide and 4.5mg/kg rabbit anti-thymocyte globulin (ATG) over five consecutive days, followed by infusion of the previously cryopreserved cells.
Results: The procedure was well tolerated by all three patients. Side effects included fever, rash, nausea and vomiting and neutropenic fever. None of the patients presented severe side effects during or after AHSCT. One patient presented positive CMV antigenemia after transplant and was preemptively treated, without any clinical manifestations of disease. On long-term follow-up, patients #1 and #3 presented remission of disease activity, with improvement of symptoms, normalization of acute phase reactant (C-reactive protein and ESR) levels and partial reversal of vessel stenosis. Patient #2 remained symptomatic and maintained high levels of acute phase reactants, indicating refractoriness to AHSCT. After transplant, she also failed etanercept injections, but disease activity was further controlled with tocilizumab infusions, started at approximately 2 years post-AHSCT and maintained until last follow-up.
Discussion: Very few cases of vasculitides treated with AHSCT have been reported in the literature. To the best of our knowledge, this is the largest reported series of transplanted Takayasu's patients. Although response to transplant was not universal, beneficial effects were verified in two out of three patients, who remained in remission for long periods.
Disclosure of Interest: None Declared.

PH-P305
AUTOLOGUS HEMATOPOIETIC STEM CELLS TRANSPLANTATION IN PATIENTS WITH SYSTEMIC SCLEROSIS- LONG TERM FOLLOW-UP

S. Kyrcz-Krzemień[1], M. Krawczyk-Kuliś[1,], M. Widuchowska[2], A. Koclęga[1], A. Kopińska[1], I. Grygoruk-Wiśniowska [1], E. Kucharz[2]*
[1]Department of Hematology and Bone Marrow Transplantation, Medical University of Silesia, Department of Hematology and Bone Marrow Transplantation, Medical Univesity of Silesia, [2]Department of Internal Medicine and Rheumatology Medical University of Silesia, Department of Internal Medicine and Rheumatology Medical University of Silesia, KATOWICE, Poland

Introduction: Systemic sclerosis (SS) is one of autoimmune diseases in which autologus hematopoietic stem cells transplantation (ASCT) may be potential curative treatment, which may reverse of skin and organ fibrosis, and what is more can stop progression of the disease.
Materials (or patients) and Methods: 16 patients- median age 48 (range 24-68 years) with a mean predominance (9 male and 7 female) with the rapidly progressive SS were treated with AHSCT between 2003 and 2013. All patients received previously treatment. Mobilisation of PBSC was achieved with Ctx with granulocyte-colony stimulating factor in 9pts, or G-CSF alone in 7pts. Conditioning regimen used combinations Ctx with Gemtuzumab ozogamycin in 15pts or Melphalan with Thymoglobulin in 2pts. Medium 4.0x10^6 CD34 positive cells/kg was transplanted and all patients received G-CSF since +1 days after transplantation until regeneration white blood cells (WBC>1G/L).
Results: Mobilization of stem cells was successful in all cases. Engraftment was successful in 12 cases (2 patients died because of acute circulatory failure, one of them during conditioning regimen. 2 patients died before 30-days after transplant procedure, during aplasia period because of pneumonitis and respiratory failure). One patient died 6 months after transplantation because of circulatory failure. In one case disease progression occurred 1.5 years after transplantation requiring systemic treatment. The patient died 3.5 years after transplantation. One patient is lost to follow up. Nine patients are alive. All of them achieved disease improvement after autoHSCT. Major improvement was seen in skin (mRSS) and disability index. Organ function remained stable. Autoantibodies are still detectable. Median observation time is 4.7 years (range: 3–10 years).
Discussion: Our preliminary results indicate that Auto-HSCT has favourable influence on course severe SS. The extension of the study by both increasing the number of included patients and prolonging follow-up time is required to confirm current preliminary results.
Disclosure of Interest: None Declared.

PH-P306

QUALITY OF LIFE EVALUATIONS IN MULTIPLE SCLEROSIS PATIENTS TWO YEARS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

E. Arantes Oliveira-Cardoso[1], F. Bronzi Guimarães[2], J. Tomé Garcia[2], J. Bernardes Elias Dias[3], D. Aparecida Moraes[4], B. Pinto Simões[3], M. C. Oliveira[3,], M. Antonio Santos[5]*

[1]Bone Marrow Transplantation Unit, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, [2]Bone Marrow Transplantation Unit, Hospital das Clínicas de Ribeirão Preto, Universidade de São Paulo, [3]Bone Marrow Transplantation, Internal Medicine, [4]Bone Marrow Transplantation Unit, Ribeirão Preto Medical School, University of São Paulo, [5]Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

Introduction: In addition to physical complications, psychological aspects should also be considered in multiple sclerosis (MS) patients. The low quality of life and life expectancy of these patients justify the application of very aggressive therapies such as high dose chemotherapy, immunotherapy and/or radiotherapy, with or without hematopoietic stem cell support. Because hematopoietic stem cell transplantation (HSCT) is an innovative approach in the treatment of autoimmune diseases (AID), there is an urgent need for studies that can assess not only the effectiveness of the technique, but also its impact on patients' lives. The goal of our study was to evaluate the impact of HSCT in the Health-Related Quality of Life (HRQoL) of MS patients, two years after the procedure.

Materials (or patients) and Methods: The sample consisted of 34 patients, treated at a University Hospital in the state of São Paulo, Brazil. Selection criteria included diagnosis of definite MS; minimum age of 18 years; having medical and psychological conditions to tolerate the treatment; willing to cooperate voluntarily with the research. For data collection MOS SF-36 scales were applied 2 years post-transplantation. The MOS SF-36 scale consists of 36 items that assess two main components: the physical health component (PHC) and the mental health component (MHC). The PHC encompasses the following domains: physical functioning (PF), role limitations due to physical problems (RP), bodily pain (BP) and general health perceptions (GH). The MHC comprises: vitality (VT), social functioning (SF), role limitations due to emotional problems (RE) and general mental health (MH). The results of each domain range from 0 to 100, in which zero represents the worst and 100 the best state of health.

Results: The physical health component: a) PF: mean (x)=50.15, SD=29.81, median (Md)=60; b) RP: x=39.71, SD=33.78, Md: 25; c) BP: x=83.97, SD=24.13, Md=100; GH: x=85.97, SD=16.78, Md=97 and the mental health component: a) VT: x=71.91, SD=17.97, Md=75; b) SF: x=71.7, SD= 24.1, Md=75 c) RE: x=65.7, SD: 40.61, Md= 83 d) MH: x=79.65, SD: 19.1, Md=84.

Discussion: Our results indicate that only the physical aspects were abnormal in this studied population and that both General Health and Mental Health were well preserved. The positive perception of HRQoL in the late post-transplantation period may be related to the fact that the patients may not face the constant possibility of disease progression, translated by stabilization or improvement of neurologic function 2 year after the procedure. These results may be interpreted as positive outcomes of the HSCT for MS.

Disclosure of Interest: None Declared.

PH-P307

ANALYSIS OF LONG TERM HEMATOPOIETIC AND IMMUNE RECONSTITUTION AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT) FOR SEVERE SYSTEMIC SCLEROSIS

D. Farge[1,], F. BRIGANT[2], C. Douay[3], E. Clave[4], Z. Marjanovic[5], A. Hij[5], D. Kheav[4], L. Homyrda[2], C. Wu[1], A. Toubert[5], H. Moins-Teisserenc[6]*

[1]Internal Medicine Unit-GFRS, Saint Louis Hospital, [2]INSERM UMRS1160, IUH-Saint Louis Hospital, [3]Université Paris Diderot, [4]Inserm UMRS1160, IUH- Saint Louis Hospital, [5]Saint Louis Hospital, Paris, France, [6]INSERM UMRS1160, Saint Louis Hospital, Paris, France

Introduction: Autologous hematopoietic stem cell transplantation (AHSCT) for severe Systemic Sclerosis (SSc) has been developed in the past fifteen years and shown as an effective therapy, based on its capacity of resetting the immune response (IR) and inducing de novo tolerance after transplant. However, no analysis of the very long term hematopoietic and immune reconstitution after AHSCT for SSc or for other autoimmune diseases has yet been performed. We therefore designed the present study to analyse long term IR after AHSCT for severe SSc.

Materials (or patients) and Methods: Twelve SSc patients, who had been treated by AHSCT in a single transplant center hospital using the same mobilization procedure with cyclophosphamide (CYCLO) 4 g/m^2 and filgrastim 10 µg/kg/day and then conditioning, 6 weeks to 3 months later, with CYCLO (200 mg/kg total dose) without (*n*=5) or with (*n*=7) rabbit antithymocyte globulin (7·5 mg/kg total dose) followed by reinfusion of CD34+ selected AHSCT, were prospectively analysed. All patients were routinely followed using repeated measure of Fonctionnal Status, Rodnan modified skin score, heart, kidney and lung functions for at least 2 years up to 11 years after AHSCT. Two groups were retrospectively constituted according to whether patients had a favorable clinical response (group A, responders; *n* = 5) or no response or relapse (group B, non responders; *n*=7) as previously published (Farge D *et al*, Arthritis and Rheumatism 2005; 52:1555-63). IR was studied before and at least yearly after AHSCT, using repeated clinical examination plus simultaneous extensive lymphocyte immunophenotyping, T cell receptor (TCR) diversity and thymic function analyses by spectratyping and T cell excision circles (TREC) quantification.

Results: Patients had similar characteristics at study entry. Before AHSCT, absolute numbers of CD4+ T cells and B cells were lower in group A comparing to Normal Controls (NC) (median TCD4+=450 microL^{-1} versus 680 microL^{-1} in NC; median BCD19+=70 microL^{-1} versus 143 microL^{-1} in NC), whereas an important increase of B-cells in group B was detected (median BCD19+=350 microL^{-1}). A normalization of B-cell counts was observed from the second year after HSCT in group A (median BCD19+=170 microL^{-1}), whereas a trend of B hyperlymphocytosis remained in group B (*P*=0.04) at 96 months after AHSCT. After 5 years, group A patients had shown higher T cell reconstitution compared to group B. The analysis of the T-cell repertoire showed the reconstitution of an overall broader clonal diversity in 4/8 SSc patients with sustained oligoclonal abnormalities in the others, irrespectively of clinical outcome. In our hands, the study of the thymic function was not informative to evaluate the immune reconstitution in this context.

Discussion: To our knowledge, this is the first study analyzing the long-term immune reconstitution of patients with SSc after AHSCT. We showed substantial differences between responders and non-responders according to the distribution of lymphocyte subsets, which may have further clinical implications for improving the conditioning regimen for SSC patients. However, the persistence of abnormalities even in good responders requires further investigations such as the analysis of lymphocyte subsets including B-cell differentiation and maturation.

Disclosure of Interest: None Declared.

PH-P308

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN NEUROMYELITIS OPTICA: A RETROSPECTIVE STUDY OF THE EBMT AUTOIMMUNE DISEASES WORKING PARTY IN COLLABORATION WITH THE UNIVERSITY OF SAO PAOLO RIBEIRÃO PRETO BRAZIL

R. Greco[1,], A. Bondanza[1], M. C. Oliveira[2], M. Badoglio[3], J. Burman[4], F. Piehl[5], H. Hagglund[6], E. Krasulova[7], B. Pinto Simoes[2], K. Carlson[8], D. Pohlreich[9], M. Labopin[10], R. Saccardi[11], G. Comi[12], G. L. Mancardi[13], A. Bacigalupo[14], F. Ciceri[1], D. Farge[15], on behalf of EBMT Autoimmune Diseases Working Party*

[1]Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milano, Italy, [2]Department of Clinical Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil, [3]EBMT Study Office, ADWP-EBMT, Hopital Saint-Antoine, Paris, France, [4]Department of Neuroscience, Uppsala University, Uppsala, [5]Department of Clinical Neuroscience, Neuroimmunology Unit, Karolinska Institute Solna, Center for Molecular Medicine, [6]Hematology Center, Karolinska University Hospital Huddinge, Stockholm, Sweden-Division of Hematology, Karolinska Institutet, Stockholm, Sweden, [7]Department of Neurology, Charles University in Prague, 1st Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic, [8]University Hospital, Uppsala, Sweden, [9]1st Department of Medicine-Department of Haematology, Charles University in Prague, 1st Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic, [10]EBMT Study Office, Hopital Saint Antoine, Paris, France, [11]Department of Haematology, Careggi University Hospital, Florence, [12]Neurology Department, Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Milano, [13]Department of Neuroscience, Ophthalmology and Genetics, San Martino Hospital, University of Genoa, [14]Division of Hematology, San Martino Hospital, Genoa, Italy, [15]ADWP-EBMT Chair, Unité de Médecine interne et Pathologie Vasculaire, Saint Louis Hospital, Paris, France

Introduction: Neuromyelitis optica (NMO) is an inflammatory and demyelinating disorder of the central nervous system, recently recognized as a distinct disease hallmarked by pathogenic anti-aquaporin 4 antibodies (AQP4). Currently NMO carries a poorer prognosis than multiple sclerosis. Use of Autologous stem cell transplantation (ASCT) has been reported worldwide for refractory autoimmune diseases (AD). NMO treatment resistant cases were considered for ASCT on a 'Clinical Option' basis, according to EBMT guidelines. The EBMT Autoimmune Diseases Working Party (ADWP) conducted the first survey to address NMO disease response following ASCT.

Materials (or patients) and Methods: This retrospective study followed the EBMT study guidelines. All centers were invited to participate. Sixteen patients with aggressive forms of NMO refractory to standard treatments treated by ASCT between 2001 and 2011 had been reported to the EBMT registry. For each case, a specific questionnaire was sent to complete information by referring haematologist and neurologist about NMO, ASCT and outcome. Results are reported as median.

Results: Patients (13 females and 3 males) had a median age of 37 years at transplant. Previous treatments had included high-dose steroids (12/16), immunoglobulins (5/16), iv cyclophosphamide (Cy, in 8/16), rituximab (5/16), mitoxantrone (2/16), plasma exchanges (8/16), azathioprine (5/16) and methotrexate (1/16). Median time between NMO diagnosis and transplant was 20 months. Before ASCT, the median EDSS (the Kurtzke Expanded Disability Status Scale) was of 6.5, 10/16 patients were positive for AQP4 antibodies and 11/16 had active lesions on magnetic resonance imaging (MRI). Fifteen patients were successfully mobilized with cyclophosphamide and G-CSF, one with G-CSF alone. The conditioning regimen consisted of BEAM plus anti-thymocyte globulin (9/16) or Thiotepa-Cy (3/16) or Cy and anti-thymocyte globulin (4/16). Hematopoietic recovery was documented in all patients within 10 days (range 3-25) after ASCT, both for neutrophils and platelets. Infectious complications required specific treatment in 9/16 patients (6 febrile neutropenia, 5 CMV and 2 VZV reactivations, 1 aspergillosis). All patients initially stabilized their disease. Disease progression was observed in 9/16 patients at a median of 10 months after ASCT. Relapse occurred in 13/16 patients at a median of 7 months after transplant, requiring further treatments in thirteen cases. Relapse, necessitating further treatments, occurred in 13/16 at a median of 7 months after ASCT. In the eight patients evaluable for AQP4 antibodies in the follow-up phase, the pathogenic autoantibodies remained positive after ASCT. Disease-free survival at 3 and 5 years was 31% and 10%, respectively, while progression-free survival at 3 and 5 years was 48%. No secondary malignancy was documented. All patients, but one patient who died from disease progression, are alive at a median follow up of 44 (14-128) months after ASCT.

Discussion: This first retrospective survey of the EBMT ADWP in 16 refractory NMO patients shows the potential of ASCT to reduce the highly inflammatory picture typical of NMO in the short term, despite a tendency to relapse in the long term, together with a low incidence of toxicities.

Disclosure of Interest: None Declared.

PH-P309

LONG-TERM DISEASE CONTROL OF REFRACTORY NEUROMYELITIS OPTICA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

R. Greco[1,], A. Bondanza[2], L. Vago[3], L. Moiola[4], P. Rossi[4], R. Furlan[4], G. Martino[4], M. Radaelli[4], V. Martinelli[4], M. R. Carbone[5], M. T. Lupo Stanghellini[1], A. Assanelli[1], M. Bernardi[1], C. Corti[1], J. Peccatori[1], C. Bonini[5], P. Vezzulli[6], A. Falini[7], F. Ciceri[1], G. Comi[8]*

[1]Hematology and Bone Marrow Transplantation Unit, [2]Hematology and Bone Marrow Transplantation Unit, Experimental Hematology Unit, [3]Hematology and Bone Marrow Transplantation Unit, Unit of Molecular and Functional Immunogenetics, [4]Neurology Department, [5]Experimental Hematology Unit, [6]Neuroradiology Unit, University "Vita-Salute San Raffaele", [7]Neuroradiology Unit, University "Vita-Salute San Raffaele", [8]Neurology Department, University "Vita-Salute San Raffaele", San Raffaele Scientific Institute, Milano, Italy

Introduction: Neuromyelitis Optica (NMO) is a rare neurological autoimmune disorder characterized by recurrent attacks of optic neuritis (ON) and longitudinally extensive trasverse myelitis (LETM), associated with peculiar pathogenic auto-antibodies to aquaporin-4 (AQP4). Immunosuppression can halt disease progression, but some patients are refractory to multiple treatments. Thus, new therapeutic strategies for NMO are warranted. Here we update the successful follow-up of two patients with refractory NMO after allogeneic hematopoietic stem cell transplantation (HSCT).

Materials (or patients) and Methods: Both treated patients had aggressive forms of NMO and serum positivity for AQP4 antibodies. Upn#1 is a 30 yrs old male affected by severe relapsing LETM, with paraparesis and thoracic spinal cord lesion on magnetic resonance imaging (MRI). Upn#2 is a 28 yrs female with recurrent attacks of ON and LETM. Both had received several lines of treatment without benefit, including high-dose corticosteroids, cyclophosphamide, rituximab, natalizumab, alemtuzumab, plasma exchange, high-dose thiotepa/cyclophosphamide and/or BEAM with Anti-Thymocyte Globulin (ATG) and cyclosporine followed by autologous HSCT rescue. Before HSCT the two patients showed a Kurtzke Expanded Disability Status Score (EDSS) of 6 and 8.5 respectively, while both of them presented active contrast enhancing lesions on MRI and positivity for the pathogenic AQP4 auto-antibodies.

Upn#1 underwent allogeneic HSCT from his HLA-identical sibling, while Upn#2 from a matched unrelated donor. Conditioning regimen consisted of full-dose treosulfan and fludarabine. Graft versus host disease (GvHD) prophylaxis combined ATG-Fresenius with cyclosporine and a short course of methotrexate (Upn#1) or mycophenolate and rapamycin (Upn#2); B cell depletion of both patients and graft was obtained by rituximab.

Results: Hematopoietic recovery occurred in both patients within day 30, and was accompanied by rapid achievement of full donor chimerism. Each patient experienced an episode of febrile neutropenia and one of them a CMV reactivation, both responsive to medical therapy; no serious adverse events were reported. None

of the patients experienced neither acute nor chronic GvHD. MRI demonstrated a sustained disappearance of inflammatory lesions, without any sign of disease progression. The immunological data obtained from the follow-up of these two patients suggest that allogeneic HSCT can alter the course of NMO through several concurring mechanisms: the eradication of autoreactive cell clones by high-dose chemotherapy and by the *in vivo* T and B cell depletion used for conditioning, elimination of long-lived plasma cells producing anti-AQP4 antibodies by putative donor T cell-mediated alloreactivity, the re-establishment of thymic central tolerance and renewal of the immune repertoire. Strikingly, these results correlated with a marked and sustained improvement of neurological functions in both treated patients. In UPN#1 EDSS dropped from 6 to 3.5, while in UPN#2 EDSS decreased from 8.5 to 7.5. At last follow-up (55 and 42 months after HSCT, respectively), both patients are alive, well and relapse-free.
Discussion: Allogeneic HSCT can provide long-term disease control in NMO and should be considered as a treatment option for patients with aggressive and refractory forms of disease.
Disclosure of Interest: None Declared.

PH-P310
EXCELLENT LONG-TERM SURVIVAL AFTER AUTOLOGOUS STEM-CELL TRANSPLANTATION FOR RCDII

P. Nijeboer[1,], O. Visser[2], R. L. J. van Wanrooij[1], T. van Gils[1], N. J. Wierdsma[3], H. J. Bontkes[4], C. J. J. Mulder[1], G. Bouma[1]*
[1]Gastroenterology, [2]Haematology, [3]Nutrition and Dietetics, [4]Pathology, VU Medical Center Amsterdam, Amsterdam, Netherlands

Introduction: RCDII can be defined as a low-grade intraepithelial lymphoma. This entity frequently transforms into an aggressive enteropathy-type-associated T cell lymphoma (EATL) with dismal prognosis. Current treatment strategies include cladribine (2-CDA) and autologous stem cell transplantation (AST) but their effectiveness in prevention of EATL is not well documented. Here we evaluated long-term follow-up and survival of 2-CdA monotherapy and 2-CdA-AST combination therapy.
Materials (or patients) and Methods: Diagnosis of RCDII was based on persisting or recurring clinical symptoms and small intestinal villous atrophy, in patients with celiac disease, despite a strict GFD for over 12 months and a clinically validated cut-off value of more than 20% aberrant intraepithelial lymphocytes (IELs) detected by flow cytometric analysis. Patients received either 2-CDA monotherapy (*n*=25) or combination therapy (*n*=11) 2-CDA followed by AST. AST was performed in patients <70 years. Overall survival, EATL development and change in clinical, histological and immunological parameters were monitored.
Results: Median age of the diagnosis RCD II was 63 years (range 42-78). Overall, 16 out of 36 patients (44%) died during a median follow-up of 59 months. In the monotherapy group, 14 out of 25 patients died (56%). Eight out of 14 (57%) died due to an EATL and 2 due to progressive refractory state. Progression into EATL developed after a median follow-up of 22 months after RCDII diagnosis (range 4-72 months). In the 2-CdA-AST combination therapy group, only 2 out of 11 patients died (18%), one as the consequence of an EATL. Overall survival in the monotherapy group was 47 months (range 3-106 months) compared to 87 months (range 18-190 months) in the combination-therapy group ($P < 0.05$). One, three- and five-years OS in the combination therapy group was 100%, 91% and 81% respectively, compared to 81%, 66% and 56% in the monotherapy group. No significant differences could be identified in clinical characteristics, histological or immunological response in those who developed EATL and those who did not.
Discussion: Both monotherapy and combination therapy induce improvement in the majority of patients, however progression to EATL was found and dismal outcome was found more often in the monotherapy group. These observations argue for an aggressive therapeutic approach for those RCDII patients eligible for AST.
Disclosure of Interest: None Declared.

PH-P311
CMV AND EBV REACTIVATION AFTER AUTOLOGOUS HAEMOPOIETIC STEM CELL TRANSPLANTATION WITH BEAM CONDITIONING REGIMEN FOR MULTIPLE SCLEROSIS AND LYMPHOMA: A RETROSPECTIVE SINGLE CENTER CASE-CONTROL STUDY

M. Di Gioia[1,], C. Innocenti[1], I. Donnini[1], C. Nozzoli[1], A. Bosi[1], S. Guidi[1], L. Rigacci[1], A. Repice[2], L. Massacesi[2], E. Portaccio[2], M. P. Amato[2], R. Saccardi[1]*
[1]Department of Hematology, [2]Department of Neurology, Careggi University Hospital, Florence, Italy

Introduction: High dose chemotherapy followed by autologous haemopoietic stem cell transplantation (aHSCT) was proposed in the last 10 years as a treatment for rapidly progressive Multiple Sclerosis (MS). Toxicity of aHSCT can be heterogeneous and depends by disease phase and conditioning regimen intensity. BEAM, an intermediate-intensity conditioning regimen plus Anti-T Lymphocyte Globulin (ATG) currently represents the standard regimen in Europe. A better selection of patients and the use of lower intensity conditioning regimens resulted in a significant drop of Transplant-Related Mortality (TRM). However CMV and EBV tend to reactivate in MS patients, possibly due to the inclusion of ATG in the conditioning regimen. We carried out a retrospective case-control study to investigate the impact of ATG administration in post-transplant viral reactivation.
Materials (or patients) and Methods: 55 patients underwent auto-HSCT for severe MS from 1999 to 2013. All MS were mobilized by Cyclophosphamide 4 g/sqm + G-CSF and conditioned with BEAM plus rabbit ATG (Thymoglobulin©) at a dose ranging between 7.5 and 10 mg/Kg. Monitoring of CMV/EBV DNA by quantitative PCR on either circulating Mononuclear Cells (MNC) or Whole Blood (WB) was performed in 33/55. In order to assess the incidence of viral reactivation in non-MS patients conditioned with BEAM we selected consecutive patients transplanted from 2009 to 2013 for HL and NHL and conditioned with BEAM alone. Controls were propensity-score matched, with nearest neighbor method, for sex and age. Pre-emptive treatment was administered in case of viral DNA load $>1x10^3$ copies/10^5 MNC or $>1x10^4$ copies/ml WB in two subsequent determinations. Antiviral treatment was established in case of symptomatic disease.
Results: Overall 144 total determination for CMV and EBV reactivation were carried out in both groups, 68 in the experimental group and 76 in controls. Positive PCR for CMV were detected at median of 34 days from aHSCT (28-44) in 9/33 patients (27.5%) of MS and at 26 days (12-39) in 7/33 (21.2%); the difference was not statistically significant. Among those who showed CMV reactivation, 3 (10%) in MS group and 3 (10%) in controls underwent pre-emptive antiviral treatment (P=0.8; CI 0.15–1.13). Positive PCR for EBV were detected at median of +33 days (22–98) in 8/33 patients (24.2%) of MS and at 21 days in 1/33 (3.1%) of controls, showing a statistically significant difference between the two group (P<0.001; CI 1.2–8.7). Among those who showed EBV reactivation 4/33 (12.1%) in MS group and 1 (10%) in controls underwent pre-emptive antiviral treatment with Rituximab (P=0.9; CI 0.02-2.23) without any statistically significant difference, also due to the small number of cases. Two patient in the MS group developed a CMV disease at

+47 and +27 days respectively, successfully treated the iv administration of Ganciclovir and Foscarnet
Discussion: Our data suggest that ATG may have an impact on viral reactivation. Monitoring of viral load and, whenever necessary, pre-emptive and anti-viral treatment should be administered in order to improve the safety profile of HSCT in this subset of non-neoplastic patients.
Disclosure of Interest: None Declared.

PH-P312
LONG-TERM FOLLOW-UP RESULTS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN SYSTEMIC SCLEROSIS

N. Del Papa[1,], C. Annaloro[2], G. Saporiti[2], S. Cavalieri[2], E. Zaccara[1], E. Tagliaferri[2], F. Grifoni[2], R. Giordano[3], G. Mometto[2], W. Maglione[1], B. Motta[2], A. Cortelezzi[2], F. Onida[2]*
[1]Day Hospital Rheumatology Unit, Istituto Ortopedico Gaetano Pini, [2]Hematology-BMT Unit, [3]Franco Calori Cell Factory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico-University of Milan, Milan, Italy

Introduction: Although various immunosuppressive treatments have been used to treat Systemic Sclerosis (SSc), currently there is no known therapy that can change the natural history of the disease. In the recent years, autologous haematopoietic stem cell transplantation (AHSCT) has been shown to represent an effective therapeutic option for patients suffering from severe SSc, although the number of available clinical trials at present is relatively scant.
Materials (or patients) and Methods: Since 2003, 17 patients affected by rapidly progressive diffuse cutaneous SSc (male 3, female 14; median age 41 years, range 21–62), underwent AHSCT using positively selected CD34+ cells. Patients with advanced pulmonary hypertension (>50 mmHg), and/or with renal (clearance <40 mL/m), lung (TLCO <40%) or heart (EF <45%) impairment were ruled out. Mobilisation was performed with CTX 4000 mg/m^2 given over two days and G-CSF 10 μg/m^2/day. Conditioning regimen included CTX 50 mg/kg/day on days-5 to -2 and rabbit ATG 2.5 mg/kg/day on days-3 to -1. The major outcome variables were treatment safety and clinical response (evaluated by means of the modified Rodnan skin score - mRSS, TLCO and the clinical activity score according to the European Scleroderma Study Group – EscSG).
Results: After a median follow-up of 86 months (range 13–128), 82% (14/17) of the patients demonstrated a beneficial clinical response, with sustained significant improvements of median mRSS and EScSG clinical score associated to stability of TLCO 2 years and 4 years after transplantation.

Outcome	Baseline	+ 2 years	+ 4 years
mRSS	22 (11-28)	3 (2-6)	3 (2-6)
TLCO	67% (38-95)	67% (31-87.5)	66% (37.5-73%)
EScSG clinical score	5 (4-6)	2 (1-3.5)	1.75 (1-3.5)

Two patients died during follow-up of SSc from pulmonary and cardiac complications of the disease. One patient died from interstitial pneumonia at day + 65, leading to a TRM of 5.8% (1/17).
Discussion: This study confirms that AHSCT in selected patients with severe diffuse cutaneous SSc results in sustained improvement of skin thickening and stabilisations of organ function up to 10 years after transplantation, so leading to a global clinical improvement, as showed by the persistent reduction in the EScSG clinical activity score. According to other experiences, TRM resulted reasonable, as a possible result of patients selection. These finding are in keeping with the view that AHSCT is effective in improving the inflammatory and active phase of SSc, while letting unchanged and stable the fibrosclerotic one. Further studies are needed to evaluate the importance of CD34 selection, the need of immunosuppressive therapy post-AHSCT and the best timing of HSCT in the treatment of SSc patients.
Disclosure of Interest: None Declared.

PH-P313
AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN SEVERE AUTOIMMUNE DISEASES : ANALYSIS OF 97 PATIENTS FROM THE FRENCH REGISTRY. SFGM-TC AND EBMT

L. Terriou[1,] on Behalf Of ADWP, Z. Marjanovic[2], S. Hadj-khelifa[3], T. Martin[4], B. Lioure[4], R. Saccardi[5], M. Badoglio[2], A. Cras[6], D. Launay[7], I. Yakoub-agha[7], D. Farge[6] and ADWP*
[1]unite d'immunologie clinique, service de médecine interne, hôpital CI HURIEZ, CHRU LILLE, LILLE, [2]hôpital St Antoine, Paris, [3]CHRU Toulouse, toulouse, [4]CHRU Strasbourg, Strasbourg, [5]St antoine, [6]hôpital St Louis, Paris, [7]CHRU LILLE, Lille, France

Introduction: Autoimmune diseases are relatively common, affecting 5-8% of the population. The majority of the patient are treated successfully by disease specialists with an evolving array of therapeutics and supportive care measures. However, exceptional patients have refractory, life threatening states. In this context, autologous haematopoietic stem cell transplantation (AHSCT) has been applied for severe autoimmune diseases (SADs) for the last 15 years. Retrospective analyses and prospective studies subsequently demonstrated the feasibility and safety of autologous HSCT, and efficacity and biological changes in various specific SADs.
Materials (or patients) and Methods: Patients who underwent an AHSCT for a SAD from 1996 and August 2013 were included in this analysis. Core Data were derived from the SFGM-TC and EBMT data base. Initial data submitted included basic demographics, indication and AHSCT types.
Results: Between 1996 and august 2013 there were 97 adult patients with SADs treated with AHSCT (Table 1). Median age at transplant was 45 years old (Range 20-69). Indications were Systemic Sclerosis (*n*=53), multiple slerosis (*n*=14), Connective Tissue disease (*n*=12), Immune cytopenia (*n*=5), Crohn's disease (*n*=3), inflammatory demyelinating polyneuropathy (*n*=2), vasculatis (*n*=2), lupus (*n*=2), and rhumatoid arthritis (*n*=1). By the date of august 2013, median follow up was 52 months (range 1-142). At last follow up, 70 patients were Alive, 3 were lost of follow up and 24 were dead. Death was related to disease activity for 13 patients and related to AHSCT for 7 patients (2 of them had total body irradiation conditioning regimen). Thirteen of the 24 deaths occurred before the year 2001 (for a total of 34 AHSCT) whereas 11 death occurred after 2001 for a total of 63 AHSCT.
Discussion: This retrospective national analysis provides useful informations regarding frequency, indications and broad outcomes in the context of translational and development phases of this treatment in poor prognosis and refractory SADs. There is therefore a case for a french national network (MATHEC) linking AHSCT centres with relevant regional level autoimmune disease specialists who are able to identify poor prognosis patients with refractory disease, before. Advanced, irréversible organ damage and chronic immunosuppression potentially compromise outcomes of AHSCT. National guidelines, written beyond the french society of bone marrow transplantation (SFGM-TC), will help centres dealing with general status for AHSCT, infection prophylaxis, supportive care and follow up after AHSCT.
Disclosure of Interest: None Declared.

PH-P314
LONG TERM EFFICACY AND SAFETY OF UNSELECTED HAEMATOPOIETIC STEM CELLS AUTOTRANSPLANTATION FOR REFRACTORY CROHN'S DISEASE

F. Onida[1,], A. Cassinotti[2], S. Cavalieri[1], G. Saporiti[1], E. Tagliaferri[1], F. Grifoni[1], G. Mometto[1], P. Fociani[3], P. Usardi[4], M. Fichera[2], B. Motta[1], M. Nebuloni[3], R. De Franchis[2], A. Cortelezzi[1], C. Annaloro[1], S. Ardizzone[2]*
[1]Hematology-BMT Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico-University of Milan, [2]Gastroenterology Unit, [3]Pathology Unit, "L. Sacco" University Hospital, [4]Clinical Apheresis Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico-University of Milan, Milan, Italy

Introduction: Autologous haematopoietic stem cell transplantation (HSCT) has recently been used to treat refractory Crohn's

disease (CD). We analyze the long term effects of a protocol using unselected stem cells autotransplantation in refractory CD.
Materials (or patients) and Methods: Eleven patients (4 male, age 22-46 years) with moderate-severe CD (median CDAI 336, range 236-395; four with perianal disease), refractory to multiple drugs including immunomodulators, were enrolled. Unselected PBSCs were collected after mobilisation with CTX 1.5 g/m^2 and G-CSF 10 mg/kg. The conditioning regimen included CTX 50 mg/kg on days –5 to –2 and rabbit ATG 2.5 mg/kg on days –4 to –2. Toxicity, clinical remission (CDAI < 150), endoscopic remission (SES-CD), extramucosal response (ultrasound sonography) and quality of life (IBD-Q) were assessed after mobilisation and at 3, 12 months and then every year after HSCT. Blood samples for immunologycal analysis were collected at baseline, after mobilisation, and 3, 6 and 12 months after transplantation. Immunologycal analyses evaluated: 1) CD4+/CD25high+/FoxP3+ regulatory T cells (Tregs); 2) Toll-like receptor 2- (TLR2) and TRL4- expressing monocytes (CD14+ cells); 3) IL-12, IL-10, TNF-alpha-production by mitogen-stimulated CD14+ cells and IFN-gamma production by CD4+ T cells. Immunological results were compared with healthy donors and associated with clinical and endoscopic response during 12 months of follow-up.
Results: No improvement was observed after mobilisation (median CDAI 363, range 201-404). At the third month, all patients were in clinical remission (median CDAI 84, range 56-132). After a median follow-up of 80 (range 32-95) months, clinical remission was maintained in 64%, 55%, 45%, 36% and 36% at the 1st, 2nd, 3rd, 5th and 7th year after HSCT, respectively. Complete mucosal healing was observed in 40% and 70% after 3 and 12 months, respectively, but maintained in only 30% at their last visit. Perianal fistulas closure was observed in 3/4 patients. No deaths or life-threatening infections occurred. Adverse events included mild-moderate infections at different sites and de novo autoimmune disorders. Satisfactory quality of life (IBD-Q >170) was reached in all patients after HSCT, and maintained up to their last follow-up or relapse. Overall, T-regs increased, while TLR4-expressing cells, as well as TNF-alpha and IL-10, all higher than healthy donors at baseline, significantly decreased after transplantation. Full responders at T3 had higher T-regs and lower IFN-gamma and IL12. Tregs decreased and IL12 and TLR2 increased in the only relapsed patient.
Discussion: Unselected CD34+ cells transplantation can induce and maintain clinical and endoscopic remission up to 7 years in refractory CD patients, which is associated with immunomodulation. The imperfect long term remission rate should be balanced against toxicity.
Disclosure of Interest: None Declared.

Early Complications / Late Effects & Quality of Life

PH-P315
IMPACT OF ABO INCOMPATIBILITY ON OUTCOME AFTER UNRELATED ALLOGENEIC STEM CELL TRANSPLANTATION
E. Bojtarova[1,], B. Ziakova[1], L. Sopko[1], V. Rimajova[1], D. Pavlikova[1], A. Batorova[1], M. Mistrik[1]*
[1]Hematology and Transfusiology, University Hospital, Bratislava, Slovakia

Introduction: AB0 incompatibility is not considered as an obstacle in hematopoietic stem cell transplantation (HSCT). However, might be associated with specific complications: delayed erythropoesis recovery, higher level of transfusion support required and pure red cell aplasia (PRCA) in major incompatibility, acute haemolytic reactions during first three months after HSCT in minor incompatibility. The aim of this analysis was to assess if AB0-incompatibility leads to delayed engraftment, higher incidence of graft versus host disease (GvHD), disease-free survival and overall survival after unrelated allogeneic HSCT.
Materials (or patients) and Methods: We retrospectively evaluated results of unrelated allogeneic HSCT in 101 consecutive patients (pts) between February 1999 and May 2013 at University Hospital in Bratislava (Slovakia). The results were evaluated to 31st August 2013. 41 pts had AB0-compatible donor, 60 pts AB0-incompatible donor (33 minor, 20 major, 7 bidirectional). AB0-compatible and incompatible patient groups were comparative in age, primary diagnosis and peripheral blood use as the source of stem cells in majority of pts. The median age of pts at the time of HSCT was 35 years (range, 18-68). AML/ALL was indication for HSCT in 58 pts, CML/MPN in 15 pts, MDS in 17 pts, SAA/PNH in 8 pts, MM/CLL/lymphoma in 3 pts. Median follow-up was 12 months after AB0-compatible and 10 months after AB0- incompatible HSCT.
Results: We didn't observe any acute hemolysis or pure red cell aplasia (PRCA) after unrelated HSCT transfusion. Median time to neutrophile engrafment (i.e. neutrophil count exceeding 0.5x 10^9/l) was 18 (range, 13-41) days in AB0-compatible and 18 (range, 11-51) days in AB0-incompatible HSCT, 5 pts after compatible and 4 pts after incompatible HSCT had graft failure. Acute GvHD grades II to IV was observed in 45% vs. 43% of pts in AB0-compatibility vs. incompatibility, chronic GvHD in 10% vs.12%. Relaps incidence was observed in 14/40 evaluable pts in AB0-compatible (35%) and in 10/59 evaluable pts (17%) in AB0-incompatible HSCT.
21/41 pts (51%) with AB0-compatible and 30/60 pts (50%) with AB0-incompatible donor are alive to date 31.8.2013. Survival probability in 5 years (according to Kaplan-Meier) was 45% vs. 42%, with median survival of 31 vs. 15 months (P=0.56) in AB0-compatible vs. AB0 incompatible HSCT.
Discussion: AB0 mismatch does not appear to impact time needed to engraftment, incidence of acute and chronic GvHD, disease-free survival and overall survival after unrelated allogeneic HSCT.
Disclosure of Interest: None Declared.

PH-P316
LONG-TERM SURVIVORS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN EARLY LIFE. A DANISH NATIONAL COHORT STUDY
J. Nathan Jensen[1,], F. Gøtzsche[1], C. Heilmann[2], H. Sengeløv[3], H. Baekgaard Larsen[2]*
[1]Faculty of health science, University of Copenhagen, [2]Department of Pediatric Oncology, [3]Department of Hematology, University Hospital of Copenhagen, Copenhagen, Denmark

Introduction: Allogeneic stem cell transplantation (SCT) is an important treatment option for certain severe, life threatening malignant and non-malignant diseases. The conditioning regimen prior to SCT may along with the immunological effect of the SCT result in severe side effects and complications, the most important being graft versus host disease (GvHD).
The aim of this study is to examine the influence of demographic factors, transplantation procedures and chronic GvHD on health related quality of life (HRQoL) and the emotional state (anxiety and depression), in long-term survivors of SCT during childhood and adolescence.
Materials (or patients) and Methods: The patients had to be younger than 25 years at the time of SCT and older than 15 years at the time of the study. This national cohort consists of 151 patients of whom 132 patients responded (89%) to a mailed questionnaire. The patients were treated between 1972 and 2008, and the mean age at SCT was 14.9 years and the mean age at time of the study was 28.2 years. Data was collected using a demographical information questionnaire. For HRQoL the Short Form 36 (SF-36) was used and for the evaluation of the emotional state the Hospital Anxiety and Depression Scale (HADS) was used. For comparison we used normal data from Danish, Swedish and British population samples.
Results: This study revealed no major impact of prior SCT on HRQoL and the emotional state in young SCT survivors. Regarding

the SF-36 the only subscales that were different from those of the general population were the General Health and Vitality scales. With respect to the HADS there was no significant difference between the SCT patients and the norm data for the general population.
Factors found to influence one or more of the SF-36 subscales were: gender on Physical Function (*P*=0.015) with men scoring higher that women, age on General Health (*P*=0.015) with older survivors scoring lower, cohabitant status on General Health (*P*=0.0015) with those living with parents scoring the highest, and on Mental Health (*P*=0.0091) with those living alone scoring the lowest, total body irradiation (TBI) on Physical Functioning (*P*=0.021), with those receiving TBI scoring lower, and graft source on Role-Physical (*P*=0.0056) with bone marrow recipients scoring higher than recipients of peripheral blood. Factors influencing emotional state were BMI (*P*=0.045) with underweight patients showing more signs of depression, and graft source (*P*=0.026) with bone marrow recipients showing fewer signs of depression than recipients of peripheral blood. Chronic GvHD was not found to have any significant influence on HRQoL or emotional state.
Discussion: In this Danish national cohort study, the majority of long-term survivors of paediatric and adolescent SCT reported a normal level of HRQoL and emotional state compared to a normal population. This includes patients treated with SCT when this was still a new treatment with severe side effects and a high mortality rate. For a more contemporary picture and to follow the progress of HRQoL in SCT survivors more studies need to be conducted.
Disclosure of Interest: None Declared.

PH-P317
PREDICTING FACTORS FOR INPATIENT MORTALITY AND LONG TERM SURVIVAL IN ALLOGENEIC STEM CELL TRANSPLANTATION RECIPIENTS ADMITTED TO INTENSIVE CARE UNIT

L. Floro[1,], H. de Lavallade[1], R. Szydlo[2], J. Clay[1], D. McLornan[1], K. Raj[1], M. Kenyon[1], V. Potter[1], A. Kulasekararaj[1], J. Marsh[1], G. Mufti[1], A. Pagliuca[1]*
[1]Haematology Department, Kings College Hospital, [2]Imperial College, London, United Kingdom

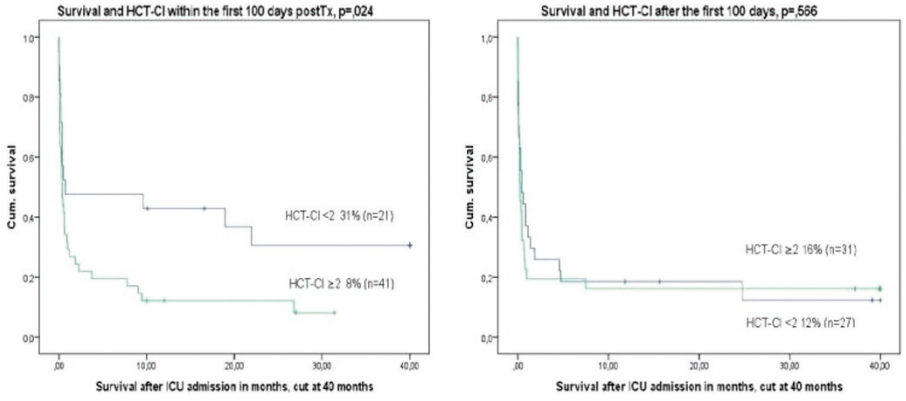
Introduction: Complications associated with allogeneic hematopoietic stem-cell transplantation (allo-SCT) can lead to critical illness with multiple-organ failure and allo-SCT recipients are considered as poor candidates for intensive care unit (ICU) admission. The decision to admit patients to ICU following allo-SCT remains a matter of debate and prognostic markers are lacking to help in the decision making process. Moreover the impact of pre-ICU admission factors on long-term survival have been rarely analysed.
Materials (or patients) and Methods: We retrospectively analysed the factors affecting short and long term survival in a single centre cohort of allo-SCT patients admitted to ICU.
Results: Between December 1999 and February 2012, 860 consecutive patients received an allo-SCT at King's College Hospital, of whom 124 (14%) were admitted to ICU for full organ support (reduced intensity conditioning *n*=83, myeloablative conditioning *n*=41).
The median time from transplant to ICU was 94 days (1-2292) of whom 62 patients were admitted within 100 days following allo-SCT, 39 between 100 and 365 days and 23 after one year.
At admission 73 patients presented with one organ failure (respiratory failure 56), 18 with two and 18 with 3 organ failure (others *n*=15).
The overall ICU discharge rate, hospital discharge rate, one year and 2 year survival rates were 35%, 29%, 19% and 14% respectively. 90 (73%) patients required invasive ventilation during ICU care with an ICU discharge and 2 years survival significantly lower (13% vs 91%, *P*<,001 and 4.4% vs 39%, *P*<,001 respectively).
Hematopoietic Cell Transplantation–Specific Comorbidity Index (HCT-CI) significantly predicted ICU discharge in patients admitted to ICU within 100 days following allo-SCT while there was no impact of HCT-CI in patients admitted after 100 days (63% vs 37%, *P*=0.02 and 46% vs 54%, *P*= 0.6 respectively; see figure).
Patients with steroid-treated GvHD at ICU admission had a worse outcome, with none of the 33 patients on steroids at ICU admission being alive after 2.1 years. Similarly none of the 15 patients admitted to ICU with active disease were discharged alive from ICU. Time from allo-SCT to ICU admission also significantly affected outcome between patients admitted before 100 days, from 100 to 365 days and after 365 days (two year survival of 18%, 8% and 30% respectively, *P*=0.02). Of note neutrophil count before ICU admission did not significantly impact the percentage of patients discharged alive from ICU (neutrophils ≥1.0 vs <1.0, 35% vs 34% *P*=0,53).
Discussion: Extensive unlimited intensive care support is justified for allo-SCT recipients without active GvHD or active disease. HCT-CI score predicts only for patients admitted within 100 days following allo-SCT. In line with others, allo-SCT recipients requiring invasive ventilation have poorer prognosis. Further studies are needed to define a scoring system to better predict outcome of allo-SCT recipients at the time of ICU referral.
Disclosure of Interest: None Declared.

PH-P318
MECHANISMS AND PREVENTION OF FATAL CARDIOTOXICITY FOLLOWING HIGH-DOSE CYCLOPHOSPHAMIDE THERAPY

T. Nishikawa[1,], K. Kurauchi[1], E. Miyahara[1], Y. Okamoto[1], Y. Kawano[1]*
[1]Pediatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Introduction: Acute fatal cardiotoxic effects due to the use of high-dose cyclophosphamide (CY) in HSCT have been recognized. The

[PH-P317]



inclusion of high-dose CY in transplant regimens may sometimes incidentally lead to immediate death. However, the mechanism underlying this phenomenon has not yet been elucidated. In addition, the occurrence of acute heart failure following treatment with high-dose CY is unpredictable.
In this study, we investigated the cardiotoxic mechanisms of CY and evaluated the protective effects of antioxidants in order to determine the preliminary mechanisms that prevent the occurrence of CY-induced cytotoxicity in H9C2 cells.
Materials (or patients) and Methods: A rat cardiac myocardial cell line, H9C2, was exposed to CY or CY metabolites, 4-hydroxycyclophosphamide (HCY) or acrolein (Acr), with or without various antioxidants (N-acetylcysteine (NAC), dexrazoxane, silymarin, isorhamnetin (ISO)). The degree of cytotoxicity was then evaluated using a MTT assay, lactate dehydrogenase (LDH) release measurements and reactive oxygen species (ROS) production measurements. In addition, we observed the cytotoxicity of CY and CY metabolites and the protective effects of various antioxidants using a real-time, live-cell imaging system (Incucyte™). Using the leukemia cell lines HL-60 and U-937, we confirmed whether each of the above antioxidants inhibit an antileukemic effect.
We also investigated how the myocardial cellular effects of CY was changed by S9 mix. S9 mix is rat liver homogenate (S9) with co-factors. We thereafter developed and validated an assay using liquid chromatography coupled with electrospray tandem mass spectrometry (LC/MS/MS) for the quantification of CY and CY metabolites, as well as HCY and *o*-carboxyethylphosphoramide mustard (CEPM). We then assayed the culture supernatant of CY plus S9 mix.
Results: The MTT and LDH release assays showed that treatment with CY (125–500 μM) and Acr (10–100 μM) did not cause cytotoxicity. In addition, neither of these compounds increased the ROS production. However, HCY (1–10 μM) exhibited myocardial cytotoxicity in a concentration-dependent manner and increased the ROS levels, including superoxide, hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and the hydroxyl radical (·OH). Both NAC and ISO inhibited HCY-induced cytotoxicity; however, the other antioxidants did not. A real-time live-cell imaging system also confirmed acute cytotoxicity induced by HCY and the inhibition of this cytotoxicity. Neither NAC nor ISO inhibited the HCY-induced antileukemic effect. CY (250 μM) plus S9 mix induced cytotoxicity more severely than HCY (10 μM). In addition, the concentration of HCY was approximately 10 μM when 250 μM of CY was metabolized for two hours using the S9 mix. Both NAC and ISO inhibited CY plus S9 mix induced cytotoxicity.
Discussion: CY itself did not exert a cardiotoxic effect. The cardiac toxicity of CY may thus be primarily caused by HCY, not Acr. Furthermore, HCY induced cardiotoxicity via variable ROS generation. However, treatment with CY plus S9 mix induced cytotoxicity more severely than treatment with HCY alone. Therefore, various metabolites of CY additively, rather than HCY alone, induce cardiotoxicity. Since both NAC and ISO inhibited CY plus S9 mix induced cytotoxicity, these compounds potentially serve as novel cardioprotective agents that can prevent the occurrence of high-dose CY-induced cardiotoxicity.
Disclosure of Interest: None Declared.

PH-P319
SLEEP DISORDERS AND PSYCHOLOGICAL DISTURBANCES DURING HOSPITALIZATION FOR HEMATOPOIETIC CELL TRANSPLANT

A. Barata[1,], R. Martino[1], P. Barba[2], G. Artaza[2], G. Ballarin[1], N. Castillo[2], S. Cortés[1], A. Esquirol[1], I. Garcia-Cadenas[1], J. Romans[2], I. Mensa[2], P. Giraldez[2], D. Valcárcel[2], S. Brunet[1], R. Laborda[1], I. Gich[3], J. Sierra[1]*
[1]Hematology, Hospital de la Santa Creu i Sant Pau, [2]Hematology, Hospital Vall d'Hebrón, [3]Epidemiology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

Introduction: Hospitalization during hematopoietic cell transplantation (HCT) is a highly stressful period for recipients. Few studies have focused specifically on the emotional disturbances during inpatient stage. Thus, the aim of this study is to longitudinally assess sleep disorders, anxiety, depression and quality of life of HCT patients during this phase.
Materials (or patients) and Methods: As part of a larger ongoing prospective study, sleep disorders were assessed by the Pittsburgh Sleep Quality Index (PSQI) which was administered previous to the HCT admission (pre-HCT) and at hospital discharge, and by sleep diaries which were completed daily by patients. Self-reported anxiety and depression were assessed with the Hospital Anxiety and Depression Scale on the pre-HCT, weekly during hospitalization, and at hospital discharge. Quality of life was examined by the Functional Assessment Cancer Therapy-BMT on the pre-HCT and at hospital discharge.
Results: We present data of 140 patients at pre-HCT of whom 106 were currently scored at the time of hospital discharge. Of the initially included patients, 71 (50%) were scheduled to receive an autologous HCT and 70 (50%) an allogeneic HCT. Fifty eight percent were men and median age was 49 years (range 18-71). At baseline, 49% of the total sample referred sleep disorders according to the PSQI, which increased to 67% at hospital discharge (P<0.001). Specifically, a subsample of patients (n=100) completed sleep diaries daily and showed that worse sleep disturbances occurred during the conditioning regimen (P<.001) when compared with other phases of the HCT hospitalization. Symptoms of anxiety were reported by 32% of the patients on the pre-HCT, and decreased each week to 20% at the time of hospital discharge (P<0.001). Symptoms of depression were present on 8% of the patients on the pre-HCT and increased significantly each week to 17% at hospital discharge (P<0.001). Quality of life remained stable when comparing both study points (P> 0.05). No differences were observed between autologous and allogeneic groups in any of the assessed disorders during this study period. Multivariate analysis showed that women were at a higher risk for presenting sleep disorders (P= 0.007), anxiety (P< 0.001) and depression (P= 0.044) at hospital discharge, while older patients were at a higher risk for developing depressive symptoms at the end of the hospitalization (P= 0.022).
Discussion: Our results point out the high psychological morbidity observed at baseline and maintained during the HCT hospitalization, highlighting that women and older patients are at a higher risk to develop complications. The incidence of psychological disorders observed emphasizes the need for more clinical attention to the HCT population in order to help patients better coping with the transplant procedure.
Disclosure of Interest: None Declared.

PH-P320
DUODENAL TUBE FEEDING: A SAFE METHOD OF NUTRITIONAL SUPPORT IN STEM CELL TRANSPLANTATION PATIENTS

L. Hoes[1,], H. Koene[1]*
[1]Department of Internal Medicine, St. Antonius Hospital, Nieuwegein (the Netherlands), Nieuwegein, Netherlands

Introduction: Almost all patients undergoing hematopoietic stem cell transplantation (SCT) develop some form of mucositis due to conditioning chemotherapy. This results in decreased food intake and often requires invasive nutritional support in the form of tube feeding or parenteral feeding. Most centers in The Netherlands prefer parenteral feeding in this patient category due to the perceived risk of gastrointestinal bleeding and/or perforation during tube placement. Due to lack of evidence concerning this issue and extensive experience in duodenal tube feeding in patients with mucositis at St. Antonius Hospital Nieuwegein (The Netherlands), we performed a retrospective study to evaluate the effectiveness of duodenal tube feeding patients with mucositis.
Materials (or patients) and Methods: A retrospective database study was conducted in which all patients who underwent autologous SCT and received a duodenal tube during hospitalization between January 2010 and March 2013 were included. Primary endpoint was the safety of duodenal tube feeding as measured by the number complications that occurred. Secondary endpoint was the effectiveness of duodenal tube feeding expressed

in weight loss and failure. All required data were retrieved from computerized medical records.
Results: There were 63 patients included in this study. No gastrointestinal bleeding or perforation occurred. Complications were reported in 39.7% of all patients and were mainly of mechanical nature, notably dislocation. Mean weight loss at discharge was 3.0 kg (SD ± 5.5) and in 42 patients (68.9%), weight loss at discharge was no more than 5% of initial weight at admission. A switch to parenteral feeding (failure) was required in 14 patients (22.2%).
Discussion: Our study shows that duodenal tube feeding is a safe method for providing nutritional support in SCT patients with mucositis. It may also have an acceptable effectiveness in the prevention of weight loss and is relatively well tolerated by patients.
Disclosure of Interest: None Declared.

PH-P321
AUTOIMMUNE HEMOLYTIC ANEMIA AFTER DUAL CORD BLOOD TRANSPLANTATION

D. Morillo[1,], R. Fores[1], A. De Laiglesia[1], G. Bautista[1], C. Regidor[1], J. L. Bueno[1], M. Rojas[1], N. Dorado[1], C. Fernandez[1], A. Morales[1], J. R. Cabrera[1]*
[1]Hematology, Hospital Universitario Puerta De Hierro, Madrid, Spain

Introduction: Autoimmune hemolytic anemia (AIHA), defined as hemolysis owing to antibodies produced by the donor´s immune system against donor red cell antigens, is a rare complication observed after allogeneic Hematopoietic Stem Cell Transplantation (HSCT). It has been associated with a variety of circumstances, such as chronic graft-versus-host disease (cGVHD), T cell depletion, unrelated donor, and the indication of HSCT for non malignant disorders. The incidence and risk factors for the development of AIHA, as well as its prognosis and response to treatment are still not well defined.
We describe our experience in the management of this complication after dual Cord Blood Transplant (CBT).*
Materials (or patients) and Methods: Between October/2008 and September/2013 we have performanced 109 allogeneic transplants at our centre, of these 38 cases were dual CBT. In this group, the median age was 32 years old (16-61). All of them received Antithymocyte globulin as part of conditioning treatment. Four patients developed AIHA after dual CBT (10.5%) with a median of 292 days post-transplant (range 186-485). None of the patients presented ABO mismatch. The direct antiglobulin test (DAT) was strongly positive in all of them (2 with anti-IgG, 2 with anti-IgG and anti-C3d), detecting a warm-reactanting panspecific IgG in serum and eluate.
Results: Table 1 shows patients characteristics.
Patient 1: developed aGVHD grade II (skin) which responded to steroids and mesenchymal stem cells; and mild cutaneous cGVHD controlled with MMF. He was diagnosed of AIHA at day +485, in situation of complete chimerism, and treated with corticosteroids, Rituximab (375 mg/m^2), iv Immunoglobulins; cyclophosphamide (750 mg/m^2) + rituximab + dexametasone; cyclophosphamide pulses (1g/m^2) and splenectomy, with partial responses. The AML relapsed at day +910, maintaining the patient a compensated hemolytic anemia. Finally, he died at day +1294.
Patient 2: was diagnosed of AIHA and autoimmune thrombocytopenia at day +186. She was treated with corticosteroids, Rituximab (375 mg/m^2), iv Immunoglobulins and Romiplostim (500 mcg/week), with slow recovery of both hematopoietic series in next weeks. She also presented CMV reactivation. Currently she remains in complete chimerism without signs of hemolysis, with normal levels of hemoglobin and platelets.
Patient 3: developed mild cutaneous cGVHD that responded to topic steroids. At day +210 he was diagnosed of AIHA, treated with systemic steroids, acquiring a complete recovery in few days. Currently (day +900), he remains in complete chimerism with persistence of DAT positive, without clinic signs of hemolysis.
Patient 4: presented aGVHD grade II (skin) controlled with steroids and etanercept. Later, she was diagnosed of AIHA at day +288, in situation of mixed chimerism. She was refractory to several treatment strategies: corticosteroids, iv Immunoglobulins, Rituximab (375 mg/m^2); cyclophosphamide (1g/m^2), splenectomy and bortezomib (1.3 mg/m^2). She did not respond to the treatment, and finally she died at day +328 because of pneumonia.
Discussion: In our experience, AIHA after dual CBT is a frequent and serious complication which develops as a late onset. It is refractory to several therapeutic strategies. It does not have relationship with the severity of GVHD, ABO mismatch (included third party donor) or CMV reactivation.
*Magro E, Regidor C, Cabrera R, *et al. Haematologica.* 2006 May;91(5):640-8.
Disclosure of Interest: None Declared.

PH-P322
IN ACUTE LEUKEMIA A PROPHYLAXIS USING DEFIBROTIDE DURING ALLOGENEIC HSC TRANSPLANTATION REDUCES TRM INDEPENDENTLY FROM ANY IMPROVEMENT OF LIVER TOXICITY

G. Milone[1,], S. Leotta[2], A. La Fauci[2], D. Berritta[2], A. Cupri[2], A. Di Marco[2], P. Spina[2], M. Parisi[2]*
[1]Bone Marrow Transplantation Program, Azienda Policlinico Vittorio Emanuele, [2]Hematology, BMT UNIT, Catania, Italy

Introduction: Aim of our study was to determine clinical results that can be achieved using a prophylaxis with Defibrotide in adult acute leukemia patients receiving allogeneic HSC transplantation.
Materials (or patients) and Methods: 107 patients affected by Acute Leukemia were studied, all received a fully myeloablative conditioning. 51 patients received prophylaxis with Defibrotide from start of conditioning to discharge. Controls were 56 patients who did not receive this prophylaxis. All patients transplanted from 2000 to 2007 received Defibrotide while since 2008 no patient received Defibrotide. The two groups were comparable for

[PH-P321]

Patient	Sex/ Age	Diagnosis	Blood group Patient / Cord / 3rd party	Conditioning	GVHD (acute / chronic)	CMV reactivation	Time to AIHA
1	M/ 56	AML	0 pos 0 pos 0 pos	Flu-Bu-Cy-ATG	II / mild skin	Yes	485
2	F/ 61	Ph+ ALL	A pos A pos A pos	Flu-Bu-Cy-ATG	0 / 0	Yes	186
3	M/ 49	AML	0 pos 0 neg 0 neg	Flu-Bu-Cy-ATG	I / mild skin	No	210
4	F/ 30	MDS	0 neg 0 pos 0 pos	Flu-Bu-Thy-ATG	II / 0	No	288

	Age	Active disease	1 CR	MUD transplants	Cum. Inc. for TRM	Cum. Inc. for Relapse	OS (3y)	TRM risk After adjustment for MUD effect (RR)
Yes Defibrotide	37 y	19%	60%	20%	10%	30%	55%	1.000
No defibrotide	40 y	19%	62%	42%	30%	25%	45%	2.822
	NS	NS	NS	0.01	0.02	NS	NS	0.05

age (*P*=0.18), diagnosis of AML vs ALL (*P*=0.9), percentage having active disease at transplant (*P*=0.9), previous transplant (*P*=0.4) and bilirubin level at admission (*P*=0.20), BU-FLU was administered in 53% in "No Defibrotide" group versus 25% in "Defibrotide treated", (*P*=0.02). MUD transplants were more frequent in "No Defibrotide " group (42%) in comparison to group treated by Defibrotide (20%), (*P*=0.01).
Results: Overall, TRM at 3 years was 20%. It was not influenced by Diagnosis (*P*=0.7), Type of conditioning (*P*=0.6), Age of patients (*P*=0.18) and Disease activity at time of transplantation (*P*=0.7). TRM at 3 y was higher in MUD transplants (30%) compared to family donor transplants (10%), *P*=0.05. Cumulative Incidence of TRM, taking into account competing risks, at 3 y was 30% in "no Defibrotide" group, compared to 10% in "Defibrotide treated" group (log-rank *P*=0.02). Univariate significant factors (MUD transplant and Defibrotide use) were entered multivariate analysis using Cox's regression. "No use of Defibrotide" remained significant for an increased risk of TRM at 3y (RR: 2.822, *P*=0.05) also after adjustment for MUD transplant effect. TRM at 100 days was 2% in "Defibrotide treated" group and 12% in "No Defibrotide" group (*P*=0.05). TRM at 100 days was not influenced by Diagnosis (*P*=0.3), Unrelated donor (*P*=0.9), Stage of Disease at Transplant (*P*=0.5), and Type of Conditioning (*P*=0.2), TRM at 100 days was, however, influenced by Recipient Age (*P*=0.004). OS was 50% at 3 years, it was 55% in "Defibrotide Treated" and 45% in "No Defibrotide" groups (*P*=0.6). No patient suffered of severe Liver toxicity, clinically mild SOS was diagnosed in 3.9% of patients in "Defibrotide treated" group and in 5.3% in "No Defibrotide" group (*P*=0.7); Bilirubin above 2.0 mg/dl was registered during the first 30 days in 21% of patients in "No Defibrotide" group and in 15% of "Defibrotide treated" group (*P*=0.4).
Discussion: Prophylaxis using Defibrotide after allogeneic hematopoietic transplantation using a myeloablative conditioning determines, in adults patients, a significant reduction of TRM. Reduction of Liver toxicity did not reach statistical significance in the sample we have studied. Thus, beneficial effect of Defibrotide on TRM is independent from any lessening of liver toxicity and may depend from modulation of allo-reactivity.
Disclosure of Interest: None Declared.

PH-P323
CARDIAC TROPONIN LEVEL MONITORING IN PATIENTS WITH MALIGNANT LYMPHOMA DURING HIGH-DOSE CHEMOTHERAPY IN COMBINATION WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

D. Kolesnikova[1,], V. Sarzhevskiy[1], V. Melnichenko[1], M. Vakhromeeva[2]*
[1]Hematology and cell therapy, [2]Diagnostic Radiology, National Medical Surgical centre named after Pirogov, Moscow, Russian Federation

Introduction: It is known that the levels of cardiac troponins can be raised as a consequence of cardiotoxic action of chemotherapeutic agent used in oncology. In the last few years high-sensitive assay for cardiac troponin measurement became widely used in cardiological practice. However, there is no data to support the predictive value of cardiac troponin levels in patients with malignant lymphomas (ML) during the high-dose chemotherapy (HDC) combined with autologous hematopoietic stem cells transplantation (auto-HSCT).
It is known that the levels of cardiac troponins can be raised as a consequence of cardiotoxic action of chemotherapeutic agent used in oncology. In the last few years high-sensitive assay for cardiac troponin measurement became widely used in cardiological practice. However, there is no data to support the predictive value of cardiac troponin levels in patients with malignant lymphomas (ML) during the high-dose chemotherapy (HDC) combined with autologous hematopoietic stem cells transplantation (auto-HSCT).
The goal of the study was to compare the changes in the troponin T («conventional troponin») and troponin I levels measured by the high-sensitive assay (hs-cTnI) at different time points before and after the HDC and auto-HSCT in ML patients.
Materials (or patients) and Methods: 73 patients with ML were enrolled in the study. Troponin T was measured in 52 patients (group 1), hs-cTnI was measured in 21 patients (group 2). The groups were matched for age and sex. Serum troponin levels in both groups of ML patients were evaluated before the HDC, directly after HDC (D0), on day 7 and 12 after auto-HSCT (D+7 and D+12).
Results: Increasing troponin T level was observed in 2 of 52 patients (3.8%), increasing troponin Hs-cTnI level – in 6 of 21 patients (28.6%) (*P* <0.01). No increase in troponin levels were detected at D0. Troponin T level (group 1) was raised only on D+7 in both two patients, hs-cTnI level (group 2) was increased in 5 patients on D+7 and in 1 patient on D+12. No cases of myocardial infarction were observed.
Discussion: Hs-cTnI is more sensitive marker of cardiotoxicity of HDC and auto-HSCT in comparison with «conventional» troponin T. Further studies and observation of the patients during the post-transplantation period are needed to clarify the predictive value of determining the level of hs-cTnI in the studied group of patients.
Disclosure of Interest: None Declared.

PH-P324
INTRAPULMONARY RECOMBINANT FACTOR VII FOR DIFFUSE ALVEOLAR HEMORRHAGE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN

J. A. Park[1,], B. J. Kim[1]*
[1]Pediatrics, Inje University Haeundae-Paik Hospital, Busan, Korea, Republic Of

Introduction: Diffuse alveolar hemorrhage (DAH) after allogeneic HSCT is a life threatening pulmonary complication. It is associated with a high mortality rate; however, current treatment options using steroids, transfusions, immunosuppressants, mechanical ventilation, and ECMO are very limited and mostly unsuccessful, as well as accompanying multiple complications. Intrapulmonary administration of activated recombinant factor VII (rFVIIa) via bronchoscopy has been reported, but there is scarce data on the use in transplantation setting and especially in children. We report 2 children treated with intrapulmonary rFVIIa for DAH after HSCT.
Materials (or patients) and Methods: During 2011-2012, 2 pediatric patients with acute, bronchoscopically confirmed DAH were included. One patient received PBSCT from matched unrelated donor for familial hemophagocytic lymphohistiocytosis (HLH), and the other received umbilical cord blood transplantation for myelodysplastic syndrome. Their ages were 11 months and 11 years, and DAH developed at day 30 and day 31 post-transplant,

respectively. They were treated with intrapulmonary administration of 50 µg/kg rFVIIa by bronchoalveolar lavage (BAL), concurrently with methylprednisolone pulse thereapy, fresh frozen plasma and maintenance of platelet count >50,000/mm^3.
Results: Complete and sustained hemostasis after a single dose of rFVIIa was observed in both patients. No toxicity or adverse events were observed with rFVIIa treatment. The oxygen capacity (PaO_2/FiO_2 ratio) increased significantly, and rapid clinical and radiological improvements were observed. However, they suffered from subsequent infection, one with MDS died from respiratory syncytial virus pneumonia, and the other infant with HLH died of multidrug resistant Acinetorbactor baumanni infection at day 51 and 70 post-transplantation.
Discussion: Our experience indicates that intrapulmonary administration of rFVIIa is an effective and safe treatment option for DAH after HSCT in children; Although high-dose corticosteroids has been the mainstay of treatment of DAH, high-dose corticosteroids deteriorate immunity and increases the risk of opportunistic infections. Therefore, new treatment modalities for DAH are desperately needed, not only to aid in the cessation of the acute bleeding episode, but also to spare the use of steroid. Although this study is limited by small numbers of patients, and further clinical studies are needed, intrapulmonary rFVIIa could be successfully used in pediatric patients with DAH after HSCT and it might spare the use of steroid.
Disclosure of Interest: None Declared.

PH-P325
PROGNOSIS OF ALLOGENEIC HEMATOPOIETIC STEM CELL RECIPIENTS ADMITTED TO INTENSIVE CARE UNIT. A RETROSPECTIVE SINGLE-CENTER STUDY.
S. Christy Lindgaard[1,], J. Nielsen[2], A. Lindmark[1], H. Sengeløv[1]*
[1]Department of Hematology, [2]Intensive Care Unit, National University Hospital Rigshospitalet, Copenhagen, Denmark

Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) is a procedure with inherent complications such as graft versus host disease (GVH) and severe infections. Intensive care may be necessary in some HSCT patients, and previous studies have reported a dismal prognosis in these patients. An important clinical dilemma is when to stop therapy in these patients, which often has several organ failures. Clinical features, which can aid decision-making at the intensive care unit (ICU), are therefore much warranted.
Materials (or patients) and Methods: We performed a retrospective study examining the outcome of the adult hematological patients receiving allogeneic HSCT and requiring critical care at the ICU at our institution. Patients who were admitted to the ICU between the 1st of January 2007 and the 31st of March 2012 were included. In total 54 patients were included. There were 32 male (59.3%) and 22 female (40.7%). The top three underlying diseases that necessitated HSCT were acute myeloid leukemia (20 patients, 37.0%), myelodysplastic syndrome (12 patients, 22.2%) and acute lymphoblastic leukemia (7 patients, 13.0%). The underlying disease for the remaining patients was one of the following: chronic lymphoblastic leukemia, chronic myeloid leukemia, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, severe aplastic anaemia and myelomatosis. The median age was 48 years (min-max 18-71). 36 patients (66.7%) received hematopoietic stem cells from a matched unrelated donor (MUD), 11 patients (20.4%) from a sibling donor and 7 patients (13.0%) from umbilical cord blood (UCB). 55.6% of the HSCT were myeloablative and 44.4% were nonmyeloablative. The median time from HSCT to ICU admission was 60.5 days (min-max 0-958).
Results: Hematological engraftment was present in 79.6% of the patients at the time of ICU admission. The overall in-ICU, in-hospital, 6-month, and 1-year mortality rates were 46.3%, 75.9%, 79.6% and 86.5% respectively. Mechanical ventilation had a statistically significant negative effect on the in-ICU (P=0.02), 6-month (P=0.049) and 1-year (P=0.014) mortality. Renal replacement therapy also had a statistically significant negative effect on in-hospital (P=0.038) and 6-months (P=0.026) mortality. Treatment with vasopressor drugs did not show any effect on mortality, neither did age nor underlying hematological disease.
Short ICU admissions had a positive effect on patient outcome. Our data showed a distinct difference between patients admitted <10 days and ≥10 days to the ICU in regards to in-hospital, 6-month and 1-year mortality (all with P<0.001).
The predicted mortality using SAPS II, APACHE II and SOFA score was 52.2%, 56.8% and 40-50% respectively. These scoring systems grossly underestimated the actual in-hospital mortality observed for this group of patients (75.9%).
Discussion: The poor prognosis of critically ill HSCT recipients admitted to the ICU was confirmed in the present study. Mechanical ventilation, renal replacement therapy and an ICU-admission of 10 days or more, were each risk factors for mortality in the first year following ICU-admission. Standard critical care scoring systems grossly underestimated the mortality for these patients.
Disclosure of Interest: None Declared.

PH-P326
SIGNIFICANT IMPACT OF IRON CHELATION AFTER ALLOGENEIC HEMATOPOETIC STEM CELL TRANSPLANTATION ON DISEASE RECURRENCE: POTENTIAL ANTI-LEUKEMIC ACTIVITY
M. Michallet[1,], M. Sobh[1], H. Labussière[1], S. Ducastelle[1], F. Barraco[1], L. Gilis[1], X. Thomas[1], F. E. N. Franck E. Nicolini [1], C. Chapuis-Cellier[2]*
[1]Hematology, [2]Biochemistry, Centre Hospitalier Lyon Sud, PIERRE BENITE CEDEX, France

Introduction: Iron overload (IO), primarily related to multiple red blood cell transfusions, is a relatively common complication in allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients. Iron chelators have been widely used leading to normalisation for ferritine level and lower IO-related complications. The objective of this study was to determine at a first time the impact of serum ferritin level measured at time of allogeneic HSCT in adult patients with hematological disorders on the different outcomes and to investigate at a second time the role of iron chelation on relapse incidence.
Materials (or patients) and Methods: We included 158 patients, 100 males and 58 females with a median age of 45 years (18-67) who underwent allo-HSCT between 2002 and 2010. There were 83 acute myeloid leukemias, 10 chronic myeloid leukemias, 11 myelodysplastic syndromes, 7 myeloproliferative disorders, 19 myelomas, 9 non-Hodgkin lymphomas, 6 Hodgkin diseases, 5 aplastic anemias and 3 hemoglobinopathies. Sixty-seven (42%) patients were sex mismatched (F→M:37; M→F:30); for ABO compatibility, 61% were compatible, 18% had minor incompatibility and 21% had major incompatibility. Concerning the HSCT procedures, 60 patients (38%) received peripheral blood stem cell and 98 (62%) received bone marrow from 97 (61%) HLA related donors [matched, n=76; mismatched, n=21], and 61 (39%) HLA unrelated donors [matched, n=36; mismatched, n=25] after myeloablative [n=64, (41%)] or reduced intensity conditioning [n=94, (59%)]. At transplantation, 91 (58%) were in complete remission (CR) or chronic phase [CR1: n=61 (67%); ≥CR2: n=30 (33%)]. The median serum ferritin level at HSCT was 1327 microg./l (26-14136); 31(20%) patients had a level 26-500, 33 (21%) had a level 500-2500, and 94 (59%) >2500. There was no significant correlation between the different ferritin levels, disease kind and status at HSCT.
Results: After transplantation, 23 patients received iron chelating agents after a serum ferritine level of 1000 microg/l and stopped when the level decreased below 1000. The cumulative incidence of acute GVHD ≥ II at 3 months was 14% (11-16.5) with 10.5% (8-13) for grade III and 7% (5-9) for grade IV; the 1 year cumulative incidence of limited and extensive chronic GVHD were 4% (2-6) and 12.4% (9-16) respectively. After a median follow-up of 18 months (1-106), the 5 years OS probability was 65% for patients with ferritin level below 500 microg./l, 39% for level between 500 and 2500 microg./l and 28% for level >2500 micog./l, [Hazard ratio= 3.5 (1.5-8.1), P=0.002]; this was explained by a significant higher TRM in patients with level >2500 [Hazard ratio= 4.3 (1.02-18), P=0.04]. Interestingly, we found in multivariate analysis that

patients receiving iron chelators had significantly better OS [5 years OS= 59% vs. 34% for non-chelated patients, Hazard ratio= 0.34 (0.15-0.76), *P*=0.008], and experienced less disease relapse [5 years relapse incidence= 18% vs. 41% for non-chelated patients, Hazard ratio= 0.22 (0.07-0.73), *P*=0.012].
Discussion: We confirmed the negative impact of iron overload on the outcomes allo-HSCT recipients. More importantly, we demonstrated that iron chelators have a positive impact in reducing disease relapse by the possible mechanism of iron deprivation in leukemic cells. This clinical observation needs to be confirmed by prospective randomized trials.
Disclosure of Interest: None Declared.

PH-P327
RISK FACTORS AND OUTCOME OF PULMONARY COMPLICATION OF PEDIATRIC PATIENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

J. Ohshima[1,], M. Sugiyama[1], Y. Terashita[1], T. Sato[1], Y. Cho[1], A. Iguchi[1], T. Ariga[1]*
[1]Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo-Shi, Japan

Introduction: Pulmonary complication of hematopoietic stem cell transplantation (HSCT) is one of the most important issues for pediatric recipient. Although various infectious or non-infectious etiologies of this complication have been showed, a substantial number of patients have remained undiagnosed and subsequently died without effective treatment.
Materials (or patients) and Methods: We reviewed pulmonary complications developed in allogeneic- or autologous- HSCT for 259 children with malignant or non-malignant disease in Hokkaido University Hospital from February 1988 to November 2013.
Results: Of total 259 patients, 25 (9.7%) developed pulmonary complications at median time of 97 days (9-597) after HSCT: age, 1.5-20.8 (median 13.5) years old; gender, 14 male and 11 female; primary disorder, 22 with malignant disorder and 3 with non-malignant. In 12 cases, infectious causes were identified or strongly suspected; fungus in seven, tuberculosis complex in three, virus in three. The remaining 13 patients developed respiratory failure without overt source of infection. Eighteen (72%) of 25 patients with pulmonary complication died: caused by respiratory failure in 10, by tumor-related in three, by treatment-related in three, and by unknown reason in one. At a median follow-up period of 45.2 months, the overall survival rate of patients with pulmonary complication was significantly lower than others (28.0 % vs. 63.2 %, *P*<0.001). The significant risk factors for the development of pulmonary complication were over 10-years of age (*P*<0.001), allogeneic source of stem cell (*P*=0.037), conditioning with TBI regimen (*P*=0.001), and acute GVHD above Grade 2 (*P*=0.022). We then stratified the patients, according to time after HSCT, in 4 groups: less than 30 days (*n*=5), all 5 patients were non-infectious and dead; 30-200 days with infection (*n*=8), all of 5 patients with fungus infection died and those with viral or tuberculosis complex infection were rescued; 30-200 days of non-infectious (*n*=9), most patients were strongly associated with chronic GVHD; over 200 days (*n*=3), all patient were infectious and dead.
Discussion: In present study, we showed that pulmonary complication in pediatric recipients increases mortality significantly and that risk factors of which are older age, allogeneic source of stem cell, presence of acute GVHD and TBI-based conditioning. Furthermore, we found that mortality, type of infection and association with chronic GVHD vary by time after HSCT. It is an issue in the future to provide diagnosis and treatment for childhood "non-infectious" recipients with pulmonary complication, those who would be potentially infected with fungus, tuberculosis complex, and some viruses.
Disclosure of Interest: None Declared.

PH-P328
PREDICTORS OF ADVERSE REACTIONS DURING STEM CELL INFUSION IN CHILDREN RECEIVING AUTOLOGOUS AND ALLOGENEIC STEM CELL TRANSPLANTATION

T. H. Truong[1,2,], R. Moorjani[1], D. Dewey[3,4], G. M. T. Guilcher[1,2], V. Lewis[1,2], N. L. Prokopishyn[5]*
[1]University of Calgary, [2]Oncology, Alberta Children's Hospital, [3]Pediatrics and Community Health Sciences, University of Calgary, [4]Pediatrics, Alberta Children's Hospital, [5]Cellular Therapy Lab, Foothills Medical Center, Calgary, Canada

Introduction: Adverse reactions during hematopoietic stem cell infusion are common in patients undergoing stem cell transplantation (SCT), mainly due to high white blood cell (WBC) content as shown in adult studies and red blood cell replete cord blood stem cells. We studied a large cohort of pediatric patients undergoing SCT to determine the incidence, grade and predictors of adverse reactions.
Materials (or patients) and Methods: All patients undergoing autologous and allogeneic SCT at our institution from 2004 to 2012 were included. We reviewed clinical data from medical records to collect adverse reactions during the infusion of stem cells on Day 0 of SCT, as well as for 24 hours following the end of stem cell infusion. Adverse reactions were graded according to the National Cancer Institute grading criteria (NCI CTCAE version 4.0). Covariates of interest included patient-related data, transplant type, stem cell source, and cellular content (total nucleated cell, CD34, CD45, CD3, granulocyte) of the infused product. Statistical analysis was performed using *t*-tests, chi-square and multivariate logistic regression.
Results: In the nine year period, 120 allogeneic and 93 autologous patients received SCT. The majority of allogeneic SCT were for leukemia while autologous SCT were mostly for brain tumors and solid tumors. Some patients received 2 or more transplants (15 allogeneic and 37 autologous patients) and others received multiple stem cell infusions in a single transplant (7 allogeneic and 30 autologous transplants), contributing to a total of 361 infusion episodes. Grade 2 reactions occurred in 109 (30%) infusion episodes and grade 3 reactions occurred in 28 (7.7%). There were no grade 4 or 5 reactions. Common grade 2 reactions included vomiting, headache, fever and hypertension. Common grade 3 reactions included oxygen desaturation and hypertension. Univariate predictors of any grade 2 or 3 reactions were: female gender (*P*=0.008), earlier transplant period before 2009 (*P*=0.004), allogeneic SCT vs. autologous SCT (*P*<0.0001), and stem cell source (*P*<0.0001). Stem cell product characteristics that were associated with any grade 2 or 3 reactions were WBC/kg (*P*=0.03), DMSO volume (*P*=0.02), total stem cell product volume (*P*=0.01), and if the product was washed (*P*=0.001). Manipulated products were negatively associated with reactions (*P*=0.003). Multivariate analysis identified three independent factors associated with any grade 2 or 3 reactions: stem cell source (Odds Ratio (OR) 14.6 for cord vs. marrow, *P*<0.0001), female gender (OR 1.9, *P*=0.025), and manipulated products which were protective against reactions (OR 0.4, *P*=0.002).
Discussion: Pediatric patients commonly experience mild adverse reactions on Day 0 of SCT with 30% of infusion episodes having a grade 2 reaction. However a small group of 8% experience a grade 3 reaction. Some reactions may have been present prior to Day 0 and therefore may not be related to stem cell infusion. In our dataset, Day 0 reactions were associated with cord blood stem cells and female gender. Manipulation of stem cell products, such as plasma or red cell depletion, was associated with a lower risk of having an adverse reaction. Unlike previous adult studies, an association with WBC content was not found to be a risk factor in the pediatric population.
Disclosure of Interest: None Declared.

PH-P329

RISK FACTORS FOR POSTERIOR REVERSIBLE ENCEPHALOPATHY SYNDROME IN PEDIATRIC ALLOGENEIC STEM CELLS TRANSPLANTATION: A TWO-CENTERS EXPERIENCE.

D. Zama[1,], F. Vendemini[1], R. Masetti[1], D. Maria Cordelli[2], L. Giordano[3], R. Rondelli[1], E. Massaccesi[1], W. Morello[1], F. Porta[4], E. Franzoni[2], A. Prete[1], A. Pession[1]*

[1]Department of Pediatrics, "Lalla Seràgnoli", Hematology-Oncology Unit, [2]Pediatric Neurology Unit, S.Orsola Malpighi Hospital, Bologna, [3]Pediatric Neurology Unit, Spedali Civili, [4]Pediatric Oncohaematology and BMT Unit, Children's Hospital, Brescia, Italy

Introduction: Risk factors predisposing to posterior reversible encephalopathy syndrome (PRES), which is a severe neurological complication occurring after hematopoietic stem cell transplantation (HSCT), are still under debate. Retrospective analysis of the risk factors potentially associated with PRES in pediatric allografted patients was carried out.

Materials (or patients) and Methods: Data of 287 patients receiving allogeneic HSCT between January 2000 and November 2012 at the Pediatric HSCT Unit of S. Orsola Malpighi Hospital in Bologna and of Spedali Civili in Brescia were collected. Univariate analysis was conducted on the following variables using Pearson's chi-square test: sex, age at HSCT, baseline disease (oncological vs non-oncological), type of HSCT (MFD, MMFD, MUD, MMUD), number of previous HSCT, disease status at HSCT, stem cells source (BM, PB, CB), donor graft T-depletion, TBI, busulfan or fludarabine, MAC or RIC regimen, G-CSF administration, acute and chronic GVHD, administration of calcineurin inhibitors, disease relapse after HSCT. Variable with $P<0.05$ according to univariate analysis were entered in a Cox regression model for multivariate analysis. Overall survival (OS) of patients who did or did not develop PRES were estimated using Kaplan-Meier method.

Results: One houndred-eightyeight and 125 HSCT were performed in oncologic and non oncologic patients, respectively, for a total of 313 consecutive procedures in 287 patients (162 male). Twenty-six out of 313 HSCT procedure (8,3 %) were complicated by the development of PRES. Univariate analysis identified the following risk factors for PRES: age at HSCT > 2 years, hemoglobinopathy, G-CSF administration, MMUD, CB stem cells source and fludarabine-based conditioning. Multivariate analysis confirmed the use of CB as stem cells source to be an independent risk factor for the development of PRES (Tab. 1). Eight-year OS of patients with PRES did not significantly differ from those of patients who did not experience this complication (35.1% compared to 56.1%, P: 0.27).

Discussion: Our result showed a strong association between CB HSCT and PRES. In this setting, steroid therapy used for GVHD prophylaxis may partially explain the association with PRES onset through exacerbation of hypertension which is a renowned risk factor for PRES. Interestingly, fludarabine based regimen seems to be associated with PRES development as previously published in adults[1]. **Reference:** 1. Beitinjaneh A. *et al*. Toxic leukoencephalopathy following fludarabine-associated hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2011;17(3):300-8.

Disclosure of Interest: None Declared.

PH-P330

POST-TRANSPLANT ALTERATIONS OF BODY-MASS-INDEX (BMI) AND THEIR POTENTIAL IMPACT ON THE OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (SCT)

T. Diederichs[1], T. Weber[1], L. P. Müller[1,]*

[1]Klinik für Innere Medizin IV, Universitätsklinikum Halle, Halle, Germany

Introduction: A small number of studies have shown varying results on the relevance of the pre-transplant BMI for various

[PH-P329]

		NO PRES *n=313*	PRES *n=26*	Univariate Analysis	Multivariate analysis
Sex	M/F	169/118	13/13		
Age at HSCT	< 2years/≥ 2years	98/189	3/23	P.018	ns
Diagnosis	Oncological Disease	171	17		
	Non oncological diseases	116	9	P.003	ns
	Hemoglobinopathies	*31*	*8*		
	Immunodeficiencies	*68*	*1*		
Centre	Bologna/Brescia	170/117	4/19	P.027	ns
N° ofHSCT	1/>1	238/49	22/4		
Disease status at HSCT	CR/AD	114/133	7/13		
Type of HSCT	MFD	71	4		
	MMFD	49	2		
	MUD	112	9		
	MMUD	55	11	P.006	ns
Source of stem cells	BM	206	16		
	CBSC	17	7	P.006	
	PBSC	64	3		
Conditioning regimen	MAC/RIC	180/89	14/12		
	Busulphan	*160*	*11*		
	Total Body Irradiation	*37*	*5*		
	Fludarabine	*85*	*13*	P.046	P.033
G-CSF	Yes/No	120/139	18/3	P.001	
GVHD prophylaxis	CNI	270	25		
	T-cell depletion	38	1		
aGvHD	Yes/No	104/146	9/12		
cGvHD	Yes/No	41/166	2/11		
Disease Relapse	Yes/No	53/154	5/15		

Tab. 1. AD: active disease; BM: Bone Marrow; CBSC: Cord Blood Stem Cells, G-CSF: Granulocyte colony stimulating factor; GvHD: Graft versus Host Disease; MAC: myeloablative conditioning regimen; MFD: match family donor; MMFD: mismatch family donor; MMUD: mismatch unrelated donor; MUD: match unrelated donor; PRES: posterior reversible encephalopathy syndrome, CR: complete remission; PBSC: Peripheral Blood Stem Cells, RIC: reduced intensity conditioning regimen.

outcomes including non-relapse mortality (NRM) after allogenic SCT. However, the post-transplant course of BMI and its effect on the outcome of allogeneic SCT has not been well studied. We hypothesized that the longitudinal course of BMI may provide helpful information regarding the risks of complications and NRM during the first three months and the first year after allogeneic SCT.
Materials (or patients) and Methods: Data from 98 adult patients, who underwent allogenic SCT in our centre between 2005 and 2013, were retrospectively analysed. Body weight and relevant laboratory parameters (total protein, albumin etc.) were recorded before conditioning as well as on day +30, day +120, day +180 and day +365 after SCT. Data were censored due to death, relapse or progress of disease or reached day +365 past SCT. BMI was calculated by standard formula and categorized as follows: underweight <18.5kg/m^2, normal weight 18.5-<25kg/m^2, overweight 25-<30kg/m^2, obesity >30kg/m^2. The longitudinal course of weight and BMI-category were evaluated in the following time intervals: start of conditioning until day +120 (IN1) and day +120 until day +365 past SCT (IN2). The association of weight and BMI-category as well as their longitudinal course with the occurrence of NRM during IN1 and IN2 were evaluated by statistical analysis.
Results: Median age of patients at the time of transplantation was 57 years (18–72 years). The analysed group comprised 60 men (61.2%) and 38 women (38.8%) with different haematological-oncologic diseases: acute myelogenous leukaemia (AML; 35%), lymphomas (22%), multiple myeloma (16%) and others (27%). In the longitudinal course a reduction of weight was prominent during IN1 with 81% of patients recording a weight loss and only 2% recording a weight gain. In contrast, no general trend in weight was observed during IN2 with 42% of patients losing and 34% gaining weight. This resulted in a significant change of BMI-categories during IN1 between start of conditioning (3% underweight, 42% normal weight) and day +120 (7% underweight, 58% normal weight; P<0.0001). In a preliminary analysis of 62 patients, a higher incidence of NRM was observed during IN2 for those patients who lost weight with a resulting lower BMI-category compared to patients with stable or increased BMI-category (19% vs. 3%; P=0.1). In contrast, patients suffering a weight loss with a resulting lower BMI-category during IN1 showed no higher incidence of NRM during IN2 compared to patients with stable or higher BMI-category (12% vs. 17%; P=0.6).
Discussion: After allogeneic SCT the majority of patients lose weight in the first post-transplant interval until day +120. In contrast, the second interval from day +120 until day +365 seems to be characterized by a differential course of patient weight. Our preliminary data suggest that this differential weight course may correlate with NRM in the second interval while weight loss during the first interval is not associated with NRM in the second interval. More detailed data on the association of weight changes as well as of changes in laboratory data with NRM for the entire patient group will be presented at the meeting.
Disclosure of Interest: None Declared.

PH-P331
PREVALENCE, RISK FACTORS AND OUTCOMES OF BRONCHIOLITIS OBLITERANS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

L. Bertelli[1,], A. Martoni[1], G. Bardasi[1], E. Massaccesi[1], D. Zama[1], W. Morello[1], S. Cazzato[1], R. Masetti[1], R. Rondelli[1], A. Prete[1], A. Pession[1]*
[1]Clinica Pediatrica Università degli studi di Bologna, Policlinico S. Orsola-Malpighi, Bologna, Italy

Introduction: Bronchiolitis obliterans (BO) after allogeneic hematopoietic SCT (HSCT) is a late-onset, lifethreatening respiratory complication with a reported prevalence of 2–10% [1,2].
Aim: Analyse the incidence of, risk factors and outcome of BO in a single institution cohort of pediatric patients who underwent allogeneic HSCT.
Materials (or patients) and Methods: Between January 2000 and November 2012, 89 transplanted pediatric patients were retrospectively evaluated. The diagnosis of BO was based on the fulfilment of all NIH criteria [3]. Differences in frequencies of discrete variables were tested using two-sided Fisher's exact. Univariate and multivariate analysis was conducted on the following risk factors: age at HSCT, sex, high risk, type of donor and type of diagnosis, conditioning regimen, total body irradiation, busulfan based regimen, acute and chronic GVHD, donor sources, ABO blood group incompatibility, Rh incompatibility, HLA, CMV reactivation. The median follow up time was of 60 months (range 4–153). Log-rank test was used to analyse the overall survival of the BO positive vs BO negative patients.
Results: Seven patients met the diagnostic criteria for BO. Prevalence rate of BO was 7,8% among all long-term surviving hematopoietic SCT recipients. Risk factor significantly associated with the development of BO was the presence of chronic hepatic and/or cutaneous GVHD (P<0.0001). All patient received a treatment consisted of inhaled beta agonists, steroids, increase in the dosage of systemic immunosuppressive therapy, repeated courses of oral antibiotics (azithromycin) and supportive antinfectious prophylaxis. Six patients showed improvement in BO and one patient (14%) died after developing BO of pulmonary insufficiency. The 5-year overall survival (OS) resulted not significantly different between the two groups (60%,SE 5.7 vs 80%,SE 17, P=0,38).
Discussion: We found a BO prevalence of 7,8% which was consistent with previous studies. Recipients with existent chronic GVHD have a greater risk of developing BO. Early diagnosis and intensive treatment may improve the prognosis of patients with BO resulting in a global OS not statistically different from the other transplanted patients.
References
1. Ditschkowski M *et al*. Bronchiolitis obliterans after allogeneic hematopoietic SCT: further insight new perspectives? *Bone Marrow Transplantation* 2013; 48: 1224–1229
2. Nakaseko C *et al*. Incidence, risk factors and outcomes of bronchiolitis obliterans after allogeneic stem cell transplantation. *Int J Hematol* 2011; 93: 375–382
3. Filipovich AH *et al*. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005; 11: 945–956
Disclosure of Interest: None Declared.

PH-P332
DATA FROM AN INDEPENDENT REGISTRY CORROBORATES RESULTS OF A PREVIOUS STUDY CONFIRMING THE EFFECTIVENESS OF DEFIBROTIDE IN THE TREATMENT OF SEVERE VENO-OCCLUSIVE DISEASE

M. Eapen[1,], P. Richardson[2], G. Prentice[3], M. Ehrhardt[1], S. Korman[1], M. M. Horowitz[1]*
[1]Medical College of Wisconsin, Milwaukee, [2]Dana Farber Cancer Institute, Boston, United States, [3]Pharmion Corporation, London, United Kingdom

Introduction: Severe VOD is known to have a high mortality rate, ranging between 75 and 85%[1]. In a pivotal study[2,3] involving 102 patients with sVOD i.e. with associated Multi Organ Failure (MOF) following hematopoietic stem cell transplantations (HSCT), defibrotide (DF) was compared with an historical control group of patients who had received standard supportive care. DF was shown to improve the survival rate at day+100 post HSCT to 38.2% versus 25.0% in the historical control group (P=0.034). The VOD complete response (CR) rate was 23.5% with DF versus 9.4% in the historical control group (P=0.013).
Materials (or patients) and Methods: Using data reported to the Center for International Blood and Marrow Transplant Research, patients with severe VOD with MOF who received HSCT between 2008 and 2011 were studied. These patients were not included in the pivotal trial.[2] Most of the CIBMTR cohort (96%) received allogeneic HCT and 4%, autologous HSCT. Day+100 survival was the primary endpoint. Severe VOD was defined as hepatic VOD with serum bilirubin >2mg/dL and MOF (renal failure requiring dialysis and/or pulmonary dysfunction that was not attributed to interstitial pneumonitis or adult respiratory distress syndrome). Of 101 eligible patients 96 were studied; 5 patients were excluded

for incomplete records. Patients were grouped as standard of care which includes no treatment / any treatment that was not DF (*n*=55) vs. standard of care plus DF or DF alone (*n*=41).
Results: Patients who did and did not receive DF were similar except that patients who received DF were more likely to be younger than 16 years of age and more likely to have renal and pulmonary failure. Day+100 survival rate was 39% for DF +/– standard of care compared to 31% for those with no DF treatment with percent improvement in outcome of 8% (95% confidence interval [CI] – 11.2 – 27.4). The corresponding rates for resolution of sVOD were 51% and 29%, respectively, with percent improvement in outcome of 22% (95% CI 2.6 – 41.6).
Discussion: The observed day+100 survival after sVOD/MOF and DF treatment, in the current analysis, are consistent with previous experience suggesting that DF is effective for the treatment of sVOD/MOF. DF has recently been approved in the European Union for the treatment of this life-threatening condition.
References:
1. Coppell JA *et al. Biol Blood Marrow Transplant*. 2010; 16: 157– 168
2. Richardson PG *et al*. *Blood*. 2009; 114: 654a
3. Defitelio Summary of Product Characteristics available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR - Product Information/human/002392/WC500153150.pdf. accessed November 2013
Disclosure of Interest: M. Eapen Conflict with: Study was funded by Gentium, P. Richardson Conflict with: Study was funded by Gentium, Conflict with: Advisory Board - Gentium, G. Prentice Conflict with: Study was funded by Gentium, Conflict with: Consultant - Gentium, M. Ehrhardt Conflict with: Study was funded by Gentium, S. Korman Conflict with: Study was funded by Gentium, M. Horowitz Conflict with: Study was funded by Gentium.

PH-P333
PLATELET CONSUMPTION AND PLATELET TRANSFUSION REFRACTORINESS IS A RELIABLE EARLY MARKER OF VENO-OCCLUSIVE LIVER DISEASE IN A SUBGROUP OF PAEDIATRIC HAEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

P. Svec[1,], S. Sufliarska[1], I. Bodova[1], A. Chocholova[1], O. Fabri[1], V. Cingel[2], J. Horakova[1]*
[1]Department of Paediatric Haematology and Oncology, [2]Department of Paediatric Surgery, Comenius University Children's Hospital, Bratislava, Slovakia

Introduction: The currently used clinical criteria for diagnosis of veno-occlusive liver disease (HVOD) carry substantial degree of uncertainty in some cases, often limiting specificity when febrile neutropenia/ sepsis is present. We analysed the applicability of increased platelet consumption and platelet transfusion refractoriness (IPC/PTR) as a diagnostic marker with respect to early recognition of HVOD.
Materials (or patients) and Methods: At the single centre level, we retrospectively reviewed medical records of 262 consecutive paediatric patients transplanted in the period 1996-2011 (157 allogeneic, 102 autologous). Modified Seattle criteria were used to verify the diagnosis and the presence of organ dysfunction to judge the severity of HVOD (Corbacioglu *et al*., Lancet 2012). 25 age-matched patients with febrile neutropenia (FN) before day +21 were compared with 27 HVOD patients in terms of platelet consumption, all given standardised apheresis platelet products. Mann-Whitney U test was used for comparison, $P<0.05$ was considered significant.
Results: The incidence of HVOD (10.3%, 27/262), severe HVOD (51.8% of HVOD, 14/27), overall survival of patients with HVOD at d+100 (78%, 21/27) and survival of patients with severe HVOD at d+100 (57%, 8/14) were estimated. 81.4% of patients (22/27) were treated with defibrotide. The corrected post-transfusion platelet count increment (CCI) early after SCT (calculated as average CCI of platelet transfusions given until day +7) and at time of event (onset of HVOD or FN) did not differ significantly between HVOD and the FN group (6.2 ± 4.7 x10^9/L vs. 6.2 ± 4.1 x10^9/L and 2.4 ± 2.1 x10^9/L vs. 3.8 ± 3.0 x10^9/L, respectively). Similarly, the time from the first to third platelet transfusion (reflecting the transfusion frequency) was not significantly shorter in HVOD compared to FN group (4.8 ± 2.0 days vs. 5.5 ± 2.3 days, respectively). 37% (10/27) of HVOD and 40% (10/25) FN patients fulfilled the criteria for having IPC /PTR early after SCT (CCI < 5x10^9/L) – here no further significant drop of CCI could be expected at the onset of VOD or FN. On the contrary, we observed a steep significant drop in CCI in HVOD patients with initially normal CCI: 11.6 ± 5.5 x10^9/L vs. 2.3 ± 2.2 x10^9/L, $P<0.0001$. Importantly, the CCI decrease occurred 6.6 ± 4.1 days before diagnosis of HVOD ($P<0.001$) and 4.2 ± 3.1 days before the threshold weight gain of 5% ($P<0.01$). The drop in CCI was also observed in FN patients with initially normal CCI: 10.3 ± 5.8 x10^9/L vs. 4.5 ± 4.8 x10^9/L, $P<0.005$. The CCI change difference between these HVOD and FN patients was not significant.
Discussion: The high incidence of increased platelet consumption /platelet transfusion refractoriness early after allogeneic and autologous SCT precluded to use this marker as indicator of HVOD in substantial part of the cohort. Febrile neutropenia /sepsis was also accompanied by significant IPC/PTR, thus the CCI drop or platelet transfusion frequency would not help identify HVOD in concurrent sepsis. However, in the setting of initially transfusion non-refractory children without febrile neutropenia, the sudden drop in CCI seems to be a reliable, objective and importantly, an extraordinary early marker of HVOD.
Disclosure of Interest: None Declared.

PH-P334
BRONCHOALVEOLAR LAVAGE FLUID PROTEIN PROFILE IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

M. Bhargava[1,], K. Viken[1], S. Dey, [2], M. S. Steinbach[2], B. Wu[3], P. D. Jagtap[4], L. Higgins[4], V. Kumar[2], P. B. Bitterman[1], M. Arora[1], D. H. Ingbar[1], C. H. Wendt[1]*
[1]Medicine, [2]Computer Science and Engineering, [3]Biostatistics, [4]Biochemistry, Molecular Blology and Biophysics, University of Minnesota, Minneapolis, United States

Introduction: Idiopathic Pneumonia Syndrome (IPS) is a devastating complication in Hematopoietic Stem Cell Transplant (HSCT) recipients. Bronchoalveolar Lavage Fluid (BALF) protein profiling has the potential to identify diagnostic biomarkers and provide novel insights into the mechanisms underpinning its pathogenesis. Our overall objective is to characterize the global protein expression of IPS to identify novel pathways that differentiate IPS from infectious lung injury in HSCT recipients.
Materials (or patients) and Methods: Patients with lung injury within 180 days of Allogeneic HSCT were classified as IPS based on criterion outlined in 2011 American Thoracic Society statement. For determining the global protein expression profile BALF was depleted of high abundance proteins, treated with trypsin and labeled with iTRAQ® 8-plex reagent for mass spectrometry (MS). The complex mixture of iTRAQ® labeled peptides was analyzed by 2D capillary LC-MS/MS on an Orbitrap Velos system in HCD mode for data dependent peptide tandem MS. Protein identification and relative quantification was performed using a target decoy strategy. Relative abundance of the proteins was determined with reference to pooled BALF from patients with no history of HSCT and respiratory failure (controls). The proteins that were differentially expressed were identified using false discovery rate of the t-test empirically using the random permutation testing. To determine the biological relevance of the proteins identified, Gene Ontology enrichment analysis was performed using the Functional Annotation Clustering algorithm in Database for Annotation, Visualization and Integrated Discovery (DAVID).
Results: BALF was available on 21 patients with infectious lung injury after HSCT and 11 patients with IPS. The mean duration from transplantation to BALF collection was not different in the two groups (56.9 ± 53.3 vs. 59.6 ± 39.4 in the two groups, respectively). No statistically significant difference was seen in age, serum creatinine, BALF leucocyte count, neutrophil and lymphocyte count. BALF protein profile is available from 4 patients with infectious

lung injury and 2 patients with IPS. We identified 542 proteins at a global FDR of 1%. These proteins represent diverse biological processes such as programmed cell death, proteasome-ubiquitin dependent protein catabolism, cellular ion homeostasis, response to oxygen radicals, carbohydrate catabolism, actin filament polymerization, plasma protein involved acute inflammation and protein remodeling. Of these, 16 proteins demonstrated differential expression in subjects with IPS when compared to infectious lung injury. These proteins will be explored as potential diagnostic biomarkers for IPS.
Discussion: High-resolution mass spectrometric platforms provide extended proteome coverage in BALF. A protein expression profile in BALF likely represents the pathophysiologic mechanisms involved in development of IPS. Early findings suggest presence of candidate biomarkers in the BALF for rapid diagnosis of IPS.
Disclosure of Interest: None Declared.

PH-P335
OCCURRENCE, CLINICAL PRESENTATIONS AND MANAGEMENT OF ISOLATED EXTRAMEDULLARY RELAPSES AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIAS: AN UPDATED SINGLE INSTITUTIONAL ANALYSIS OF 571 PATIENTS

D. Kata[1,], S. Kyrcz-Krzemien[1], M. Markiewicz[1], M. Kopera[1], A. Waclawik[1], K. Bialas[1], E. Dobrzynska[1]*
[1]Dept. of Hematology and BMT, Silesian Medical University, Katowice, Poland

Introduction: Extramedullary (EM) relapses of acute lymphoblastic leukemia (ALL) and acute myeloid 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).
Materials (or patients) and Methods: We retrospectively analyzed incidence, clinical chatracteristics, treatment options and long-term outcome of this mode of leukemia recurrence in a cohort of 571 consecutive patients (pts) (223 with ALL, 348 with AML) who underwent allo-HSCT in our center between June 1993 and June 2012. 94 pts (43 with ALL, 51 with AML) relapsed (any site).
Results: 17 (18 %) out of all pts who relapsed (5 with high-risk B-line ALL, 2 with T-line ALL, 10 with AML, F/M 9/8, median age 34 years, range 23 – 60 years) experienced histologically proven IEMR after a median time of 11 months (mts) (range, 5 – 80 mts) following allo-HSCT. 7 pts (5 with ALL, 2 with AML) developed skin and/or subcutaneous tissue infiltrates. Other sites of IEMR included (No. of cases/diagnosis): central nervous system (2/ ALL, 1/AML), paraspinal soft tissues (1/AML), small intestine (1/AML), lymph nodes (1/AML), paranasal sinuses (1/AML), pleura (1/ALL), breast (1/AML), retro-orbital region (1/ALL). Treatment plans for those isolated EM relapses included (No. of cases/diagnosis): 1/ involved-field radiotherapy (IF-RT) (2/ALL, 2/AML), 2/ IF-RT followed by chemotherapy (CHT) and interferon-alpha (2/ ALL), 3/imatinib + CHT + steroids and methotrexate intrathecally (1/ ALL), 4/ imatinib + CHT (1/ ALL), 5/ CHT (3/ AML, 3/ALL), 6/ dasatinib (1/ CD117+ AML), 7/ surgery (1/AML), 8/ CHT and secondary alloHSCT (1/ALL, 2/AML). 10/17 pts died after a median time of 9,5 mts (range, 1 – 30 mts) due to resistant systemic relapse and/or infectious complications, 7/17 pts are currently under IF-RT/CHT or after secondary allo-HSCT.
Discussion: Our data indicate that EM disease following allo-HSCT affects a significant proportion of pts with acute leukemias. Sites of relapses vary widely among the pts, however, in most of them leukemic infiltrates are localized outside the well-defined sanctuaries (central nervous system or testis), predominantly within the skin and/or subcutaneous tissue. IF-RT seems to be effective initial treatment option, but it does not prevent from systemic relapse. An aggressive approach combined of local and systemic therapy including secondary allo-HSCT can produce favorable response in a percentage of patients with isolated EM relapse.
Disclosure of Interest: None Declared.

PH-P336
OCCUPATIONAL STATUS AMONG ADULT SURVIVORS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDHOOD

J. Winterling[1,], E. Johansson[1,2], L. Wettergren[1], P. Ljungman[2], K. Alexanderson[3]*
[1]Department of Neurobiology, Care Sience and Society, [2]Department of Medicine, [3]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Introduction: Although being in work is recognized to be an important factor for well-being, little is known about occupational status among childhood survivors treated with allogeneic hematopoietic stem cell transplantation (allo-SCT) for haematological disorder. The aim was to elucidate occupational status and factors associated with sick-leave/disability pension among adult survivors treated with HSCT during childhood.
Materials (or patients) and Methods: All former child and adolescent patients (<18 years) who between January 1978 and June 2008 underwent allo-SCT, due to haematological disorderl, who at data collection in 2009 were alive, aged ≥18 years, had follow-up's at a clinic for adults, and lived in Sweden (n=96), were approached for participation. Fifty-nine (62%) survivors (46% women) with a median age of 26 (18-45) years consented participation and responded to a questionnaire regarding life situation and occupation. Clinical data were extracted from their medical charts. Predictors for being on sick leave or disability pension were analyzed using multivariate logistic regression.
Results: Overall, the majority of long-term survivors were working or studying at follow-up. Nevertheless, a substantial number was on sick leave/disability pension presumably associated to sequelae from their underlying disease or treatment. At a median of 17 (3-28) years following the HSCT, 55% were working (44% full-time, 11% part-time), 28% studying (24% full-time, 4% part-time), 7% unemployed (5% full-time, 2% part-time) and 10% on full-time sick leave (>3 months) or had disability pension. In total were 19% (10% full-time and 9% part-time in combination with work, studies or unemployment) on sick leave or disability pension. Decreased physical and mental/social work capacity due to the HSCT was reported by 18% and 19% of the participants', respectively, and 32% reported economic difficulties. The reduced physical and mental/social work capacity was significantly associated with being on sick leave/disability pension (P = 0.01 and P = 0.02, respectively), while there were no such associations with having economic difficulties. Being on part- or full-time sick leave/disability pension was associated with self-reported multi-morbidity (≥2 co-morbidities: OR 12.6, CI, 1.99–80.09, P = 0.01).
Discussion: Overall, the majority of long-term survivors were working or studying at follow-up. Nevertheless, a substantial number was on sick leave/disability pension presumably associated to sequelae from their underlying disease or treatment.
Disclosure of Interest: None Declared.

PH-P337

DISCONTINUING IMMUNOSUPPRESSIVE THERAPY FOLLOWING AN ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANT (HSCT) : A CROSS SECTIONAL ANALYSIS OF 799 PATIENTS, 1 YEAR POST TRANSPLANT

A. Dominietto[1,], F. Galaverna[1], C. Di Grazia[1], T. Lamparelli[1], F. Gualandi[1], S. Bregante[1], M. T. van Lint[1], R. Varaldo[1], A. Ghiso[1], A. Signori[2], A. Stasia[1], A. M. Raiola[1], A. Bacigalupo[1]*

[1]U.O. EMATOLOGIA TRAPIANTO di MIDOLLO, [2]Statistica Medica, IRCCS Azienda Ospedaliera Universitaria San Martino – IST, Genoa, Italy

Introduction: Background. Unlike solid organ transplant, HSCT induce permanent bidirectional tolerance in most patients, who therefore become free of immunosuppressive therapy (IST). However the number of reports on the actual probability of discontinuing IST, are scares.

Aim of the study: We therefore studied patients one year post HSCT, to assess the proportion who were off IST, and predictive factors.

Materials (or patients) and Methods: Patients. We identified in our relational data base, 799 patients grafted between 1990 and 2012, with hematologic malignancies, alive 1 year post-HSCT, without evidence of disease, both molecular or hematologic. All of them had received CyclosporinA (CyA) for GvHD prophylaxis. We divided patients in 3 transplant eras <2000, 2001–2005 and 2006–2012.

Results: The overall proportion of patients off CyA at 1 year, was 68%: it was 59%, 67%, 81% respectively, in patients grafted before year 2000 (n=394), between 2000 and 2005 (n=154) or 2006-2012 (n=251) (P<0.001). This result was seen despite the increased number of HSCT from alternative donors in the 3 transplant era (23%, 42%, 62%, P<0.001), increased proportion of patients over 50 years (6%, 22%, 33%, P<0.0001), and more patients with advanced disease (36%, 38%, 57%, P<0.001).

In univariate analysis, other factors predictive of discontinuing CyA were: donor gender (male 71% vs female 63%, P=0.03), stem cell source (84% for CB, 68% for BM 57% for PB, P<0.001), previous acute GvHD (71% for grade 0-I, 61% for grades II-IV) and chronic GvHD (74% for no-minimal cGvHD, 57% for moderate severe cGvHD, P<0.0001).

There was no effect of donor/patient age, patient gender, intensity of the conditioning regimen. In multivariate analysis the same predictors remained significant, with the exception of acute GvHD. We also looked at the proportion of patients off steroids at 1 year, and this was 68% (<2000), 81% (2001-2005) and 90% (>2005). The same variables identified for CyA also predicted discontinuation of steroids.

Discussion: Conclusion. The majority of allogeneic HSCT recipients, are off IST, 1 year post transplant. Negative predictors are the use of PB as a stem cell source, moderate/severe cGvHD, female donor, early disease and HSCT before 2005. The higher proportion of patients oof IST beyond 2005, may be due to a combination of better prevention of cGvHD and reduced use of PB cells.

Disclosure of Interest: None Declared.

PH-P338

Abstract Withdrawn

PH-P339

DEFERASIROX IMPROVES HEMATOPOIESIS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

G. Visani[1,], B. Guiducci[1], C. Giardini[1], F. Loscocco[1], T. Ricciardi[1], A. Isidori[1]*

[1]AORMN, Hematology and Stem Cell Transplant Center, Pesaro, Italy

Introduction: 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. Leucopenia and thrombocytopenia could be frequently observed. Pancytopenia obviously complicates the management of the post transplant period. Iron overload is frequently observed in hematological patients before and after hematopoietic stem cell transplantation (HSCT), as multiple red blood cell (RBC) transfusions are generally administered, owing to the presence of disease and marrow suppression after cytotoxic treatment. Moreover, iron overload is considered a significant contributor to treatment related mortality, in thalassemias as well as in leukemias and myelodysplastic syndromes (MDS). Whereas several reports have focused on iron overload before transplant, little is known, up to now, on the effects of iron overload on the recovery of hematopoiesis after transplant.

Materials (or patients) and Methods: we report on 10 patients, transplanted for hematological diseases (9 acute leukemia, 1 aplastic anemia) heavily transfused before transplant that, considering the iron overload, were treated after HSCT with deferasirox. Before starting deferasirox, the patients were fully engrafted and in complete remission (acute leukemia), 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 haematological recovery; 3) transfusion-dependence; 4) serum ferritin > 1800 ng/mL; 5) normal creatinine value. All patients received an initial dose of deferasirox 10 mg/kg/day, later adjusted according to side effects.

Results: All patients experienced an increase in haemoglobin levels, with a reduction in the frequency of RBC transfusions, followed by transfusion independence (median time: 23 days from the first dose of deferasirox). In addition, it was promptly (median time: 26 days) associated with haematological improvement, with sustained values and no further platelet support or growth factors administration. Moreover, ferritin values were progressively reduced with deferasirox treatment. The workup for other aetiologies resulted negative; no concomitant infection was documented (CMV: negative; HHV-6: negative; EBV: negative). No relevant modifications with immunosuppressive or myelosuppressive drugs were made during deferasirox treatment. Deferasirox was well tolerated.

Discussion: The role of iron overload post transplant is not completely understood. No reports, in our knowledge, have up to now focused on the possible effect of iron chelators after transplant on the restoration of normal hemopoiesis and, in particular, transfusion independence. Basing on our results, we think that deferasirox determined stimulatory, and/or derepressive effects on hematopoiesis after allo-HSCT. In conclusion, 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 design an efficient strategy to reduce iron loading after transplant.

Acknowledgements: supported in part by AIL Pesaro Onlus.

Disclosure of Interest: None Declared.

PH-P340

TREATMENT OF POST-HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) HAEMORRHAGIC CYSTITIS WITH INTRAVESCICAL HYALURONIC ACID

C. M. Dellacasa[1,], P. Bertapelle[2], A. Busca[1], S. D'Ardia[1], B. Allione[1], L. Giaccone[1], S. Aydin[1], C. Frairia[1], C. Pecoraro[1], M. Festuccia[1], L. Brunello[1], E. Maffini[1], F. Ferrando[1], B. Bruno[1], R. Cavallo[3]*

[1]Hematology, [2]Urology, [3]Microbiology, AO Città della Salute e della Scienza di Torino, TORINO, Italy

Introduction: Haemorrhagic cystitis (HC) is a relatively common complication of allogeneic HSCT, occurring in 10 to 70% of the patients, however the optimal treatment has not yet been established.

We present our preliminary experience with intravescical hyaluronic acid for the treatment of HC in patients receiving an allogeneic HSCT.
Materials (or patients) and Methods: Between december 2011 and november 2013, 12 patients with hematological malignancies presented with HC after receiving an allogeneic HSCT from a matched sibling (*n*=1), an haploidentical relative (*n*=1) or an unrelated donor (*n*=10). Stem cell source was peripheral blood in 11 patients and bone marrow in 1 patient. All patients received myeloablative conditioning regimens including cyclophosphamide (Cy) in 7 cases. Haploidentical HSCT recipient received post-transplant Cy as *in vivo* T-cell depletion. In all cases the administration of Cy was combined with 2-mercaptoethane sodium (MESNA) and hyperhydration.
Results: HC developed at a median of 44 days (range 27-119 days) post-HSCT. HC has been classified as grade II (macroscopic haematuria) in 4 patients, grade III (with the presence of clots) in 4 patients and grade IV (requiring instrumentation for clot evacuation or leading to urinary retention) in 4 patients. The median platelet count at the time of HC onset was 70 x 10^9/l (range 24-234). Ten evaluable patients had BK viruria (viral titres: 5 x 10^6 –1 x 10^8 copies/ml) and 3 of these were treated with intravescical (*n*=1) or i.v. cidofovir (*n*=2). Seven patients had concomitant CMV infection requiring antiviral treatment, and 6 patients had grade II-IV acute GVHD at the time of HC onset. Slow instillation over 10-20 minutes of 50 ml of hyaluronic acid (Cystistat) was performed through a single use hydrophilic transurethral catheter. Patients were invited not to void the bladder for at least one hour. In 5 cases propiverine 15 mg p.o. bid was associated with the instillations. Hyaluronic acid was administered at a median of 7 days (range 1-27 days) from the onset of HC: patients received instillations 1-2 times/week on the basis of the clinical status, for 2-4 weeks. Patients received a median number of 4 intravescical instillations (range 2-10). In 10 cases (83%) instillations were performed as outpatients. A complete response, with improvement of urinary symptoms and resolution of gross haematuria, has been reported in 8 patients (67%); two patients did not respond to the instillations; one patient experienced a relapse of HC following early discontinuation of the treatment and responded to hyperhydration with bladder irrigation a second course of intravescical instillations. One patient had initial response to the first three doses and is still receiving the treatment. One patient did not tolerate the intravescical treatment; no other adverse events were reported.
Discussion: In conclusion, our results suggest that intravescical instillations of hyaluronic acid may represent a safe and minimally invasive treatment for patients with post-HSCT HC. Interesting to note that the therapeutic procedure has been performed in the outpatient setting in a consistent proportion of patients. Further studies are mandatory to confirm our preliminary results.
Disclosure of Interest: None Declared.

PH-P341

THE INFLUENCE OF ANTI-HLA ANTIBODIES ON OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM HLA-MISMATCHED UNRELATED DONORS

A. Koclega[1], M. Markiewicz[1,], S. Mizia[2], U. Siekiera[3], A. Dobrowolska[3], M. Dzierzak-Mietla[1], P. Zielinska[1], K. Bialas[1], S. Kyrcz-Krzemien[1]*
[1]Hematology and Bone Marrow Transplantation, Medical University of Silesia, Katowice, [2]Clinical Immunology, Lower Silesian Center for Cellular Transplantation with NBMDR, Wroclaw, [3]Immunogenetics and HLA Laboratory, Regional Blood Center, Katowice, Poland

Introduction: Although anti-HLA Antibodies (Abs) are considered an important factor of graft failure in solid organ transplants and post transfusion complications, their role in allogeneic hematopoietic stem cell transplantation (allo-HSCT) is still undiscovered. Large polymorphism and immunogenicity of HLA-antigens and heterogeneity of anti-HLA Abs warrant the need of their investigation. The purpose of this study was to define the presence of anti-HLA Abs before allo-HSCT from HLA-mismatched unrelated donors and their impact on engraftment, post-transplant full donor's chimerism, acute and chronic graft versus host disease, relapse and overall survival.
Materials (or patients) and Methods: 70 HLA-mismatched donor/recipient pairs entered the study. Indication for allo-HSCT was: ALL, AML, CML, SAA, PNH, MDS and CLL. Preparative regimen was myeloablative in 68pts (97%) and reduced in 2pts (2.3%). Standard GVHD prophylaxis consisted of cyclosporine, methotrexate and pre-transplant anti-thymocyte globulin (69pts) or Alemtuzumab (1pt). HLA-A,B,C,DR,DQ alleles were PCR-typed. Single HLA-antigen was mismatched in 46pts, single HLA-allele in 16pts, double antigens or alleles in 2 pts and another 2 pts had combined antigenic/allelic HLA mismatch. Anti-HLA A,B,C,DR,DQ,DP Abs were identified in sera collected prior to the conditioning treatment with use of automated DynaChip assay utilizing microchips bearing purified class I and class II HLA antigens. Post-transplant chimerism was analyzed using STR-PCR method at 30, 100-days and 1-year after allo-HSCT.
Results: Anti-HLA Abs pre-formed before allo-HSCT were detected in 32pts: against class I, II or both in 13(18.6%), 7(10%) and 12(17.1%) pts, respectively. Anti-HLA Abs directed against the mismatched HLA antigens were observed in 4 pts before allo-HSCT. Although no Abs specific to mismatched HLA alleles were detected, Abs belonging to the same Cross-Reactive Groups (CREGs) were present in 5pts.
No graft failure has been observed (graft failure was defined as absence of neutrophil recovery by day 30 after allo-HSCT or loss of donor's chimerism). The detection of anti-HLA Abs before allo-HSCT was associated with decrease of post-transplant donor's chimerism (18/31 vs 11/35, *P*=0.03). Anti-HLA Abs had no significant impact on engraftment of platelets nor neutrophils. The median time to neutrophils engraftment was 16.9 days (range 7-31 days) in pts with and 18.9 days (range 13-30 days) in pts without anti-HLA Abs (*P*=0.188).
The median time to platelets engraftment was 16.9 days (range 9-31 days) in patients with and 18.3 days (range 10-32 days) in pts without anti-HLA Abs (*P*=0.274). There was no significant impact of anti-HLA antibodies present before transplantation on incidence of relapse (4/32 vs 6/38, *P*=0.961), aGvHD (23/32 vs 30/38, *P*=0.495), cGvHD (14/32 vs 19/38, *P*= 0.602) and overall survival (3-years overall survival: 42% vs 56%, long-rank test *P*=0.535).
Discussion: Our results indicate, that anti-HLA Abs are present before transplantation in mismatched allo-HSCT recipients. They influence post-transplant full donor's chimerism. The detection of anti-HLA antibodies before transplantation has no significant impact on engraftment, incidence of relapse, aGvHD, cGvHD nor overall survival.
Disclosure of Interest: None Declared.

PH-P342

SOCIAL SUPPORT AND PSYCHOLOGICAL ISSUES IN HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANT PATIENTS IN THE KINGDOM OF SAUDI ARABIA

S. Akhtar[1,], H. Soudy[1], M. Shahzad Rauf[1], T. Ahmed M. Elhassan[1], I. Maghfoor[1]*
[1]Oncology Center, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia

Introduction: Outcomes in patients undergoing high dose chemotherapy and autologous-SCT (HDC-ASCT) is also influenced by pschychosocial issues. These patients require significant support from their caregivers and community during and after HDC ASCT. Magnitude of this support in quantitative way is currently not available in our society (Middle East / Arab culture). Purpose of this study is to evaluate the impact of social support and

psychological issues in ASCT patients, especially the relationship with family members, educational institutional and work place support.
Materials (or patients) and Methods: Patients who underwent HDC ASCT for non Hodgkin's lymphoma and Hodgkin's lymphoma were identified. They participated in the project of "Functional Assessment of Cancer Therapy –Bone Marrow Transplant" (FACT-BMT) quality of life questionnaire study. An Arabic questionnaire along with FACT-BMT was also developed to explore specific social issues pertinent to this specific population, not captured in FACT-BMT.
Results: One hundred and eight, males 68 (63%), females 40 (37%) participated in the study. Hodgkin's lymphoma in 69%, diffuse large cell lymphoma in 31% patients. At the time of study, median age was 29 years (14–62 years). All patients easily understood all items of questioner. Close relatives were aware of patient's situation / condition in 83% of cases. More than 90% patients received appropriate support from family and friends. Relationship with parents (99%), spouse (93%) and children (91%) was not affected due to HDC ASCT. No patient (74 eligible patients) faced separation from spouse due this condition. Getting married was considered difficult in 27% of eligible patients. Young patients observed significant issues related to their education. Twenty-seven patients (37%) mentioned that their education was affected and 58% reported that their educational institution was not supportive during this time. Six (10%) patients were unable to get admission in any educational institution as a result of their illness / absences / failure to get scholarships despite their efforts. Employer either fired or stopped 11% of patients from working and another 22% stopped working electively due to their condition. Finding a new job was difficult due to this condition in 23%.
Discussion: In Middle East and Arab countries, this is the first study which objectively assessed family and social support issues. These patients had good family support from close relatives. There is a significant need to optimize appropriate social support in our community for these patients; especially from educational institutions and employers. Enough social support to highly vulnerable transplant patients may improve HDC ASCT outcome.
Disclosure of Interest: None Declared.

PH-P343
FLUDARABINE-BASED SECOND TRANSPLANTATION FOR SECONDARY GRAFT FAILURE AFTER FIRST ALLOGENEIC STEM CELL TRANSPLANTATION
Y. Sun[1,], L. Xu[1], D. Liu[1], K. Liu[1], X. Huang[2]*
[1]Peking University Institute of Hematology, [2]Peking Univerisity Institute of Hematology, Beijing, China

Introduction: Graft failure is a rare but life-threatening complication after allogeneic transplantation (SCT). Second allogeneic SCT are the main therapeutic options, and immunopressive agents rather than myeloablative agents were adopted in conditioning regimen. In this retrospective study, we report the results of second allogeneic SCT containing fludarabine-based regimen for secondary graft failure.
Materials (or patients) and Methods: Nine patients with secondary graft failure who received second transplantation in Peking University Institute of Hematology from 2002 to 2013 were retrospectively reviewed. Secondary graft failure was defined as persistent neutropenia and thrombocytopenia after initial reconstitution.
Results: Nine patients received second transplantation with fludarabine-based regimen, including 4 male and 5 female. The median age was 28 (3-39) years old. There are 5 malignant disease and 4 aplastic anemia. The onset of secondary graft failure was 6 (2-36) months after first transplantation. The conditioning regimen was fludarabine plus basiliximab or busulfan or cyclophosphamide or total body irradiation. As for the graft, 7 was peripheral blood, 2 was combination of bone marrow and peripheral blood. Only 2 patients received second transplantation from a different donor. Seven of 9 patients acquired fast engraftment, with the median neutrophil engraftment time was 14 (7-16) days and the median platelet engraftment time was 12 (7-14) days. One patients occurred grade IV acute GVHD, and 4 patients with extensive chronic GVHD. Finally, 5 patients survived with median survival of 45 (2-60) months. In the 4 patients died, 3 was due to infection and one attributed to recurrence of malignant disease.
Discussion: Fludarabine based second transplantation may be a effective way for secondary graft failure after first transplantation.
Disclosure of Interest: None Declared.

PH-P344
THE APPLICATION OF SECOND TIME ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: REASONS AND OUTCOME
P. Topcuoglu[1,], M. Gunduz[1], M. Ozen[1], E. Atilla[1], O. Arslan[1], M. Ozcan[1], H. Akan[1], M. Beksac[1], G. Gurman[1]*
[1]Hematology, Ankara University, Faculty of Medicine, Ankara, Turkey

Introduction: We aimed to share the experience on second time allogeneic hematopoietic stem cell transplantation (allo-HSCT) in 22 patients.
Materials (or patients) and Methods: Second allo-HSCT was performed in 22 patients from 1993 till now. Median age was 27.5 years (16-64 years). There were fourteen males and 8 females. Most of the patients underwent 2nd transplantation from the same donor of their 1st one (*n*=18). We retrospectively analyzed the transplantations' feature and outcome from the computer based data obtained from Adult Stem Cell Transplantation Unit of Ankara University.
Results: The reasons of 2nd transplantation were primary or second graft failure (*n*=11/1) and the relapse or refractory primary disease (*n*=11) (Table). Successful engraftment was obtained in the half of patients with primary graft failure. Median time from 1st to 2nd transplantation in the relapse or refractory patients was 8.2 months (5.7-92.4 months). Their primary diagnosis were 9 acute myeloid leukemia (*n*=3 MDS or CMML secondary leukemia), 1 acute lymphoblastic leukemia and 1 chronic myeloid leukemia. We made 2nd transplantation of this group in four patients from the different donor than 1st transplantation (haploidentical donor from their mothers in 2 patients; 1 identical other related donor and 1 other identical sibling). Complete remission was obtained in 59.1 percent of all the patients. Acute and chronic graft versus host disease (GvHD) developed in 43.8 % and 41.6 % of the patients applicable for the evaluation of GvHD, respectively. The possibility of two-year-overall survival (OS) was 32.3±11% from the 2nd transplantation. Although the OS was longer in graft failure group than relapse patients (45.5±15% vs 22.7±13.6%), statistical difference was not found (*P*=0.67).

Table: Results and outcome

Reasons	
1st&2nd graft failure	10/1
Relapse or refractory disease	11
Median time from 1st to 2nd tx (Range), months	5.8 (0.9-92.4)
Stem Cell Source	
Peripheral Blood/Bone Marrow	17/5
Conditioning regimen	
Ablative/Reduced intensive	9/13
Complete remission	13 (59.1%)
Early transplantation related mortality	11 (50.0 %)
Nonresponse including death during aplasia or relapse	7 (31.8 %)
Acute GvHD, % (*n*)	43.8 % (7/16)
Gr I/II/III, *n*	3/2/2
Chronic GvHD	41.6 % (5/12)
Limited/extensive	3/2
2-year-OS	32.3%±11%

Discussion: In conclusion, second allo-HSCT should be successfully performed in graft failure and relapse patients after 1st allotransplantation from same or different donor. Moreover, that success of second transplantation in relapse or refractory patients is limited. Therefore, new approaches and treatment modalities to prevent the relapse or increase the rate of response should have been developed during or posttransplantation period after 1st transplantation instead of 2nd one.
Disclosure of Interest: None Declared.

PH-P345
TACROLIMUS EXTENDED RELEASE FORMULATION GIVEN AS AN ALTERNATIVE TO CICLOSPORINE A IN CASE OF RENAL IMPAIRMENT AFTER ALLOGENEIC HSCT: RESULTS FROM A PROSPECTIVE PILOT STUDY

M. Y. Detrait[1,], S. Morisset[1], S. Cohen[2], M. Manchon[2], V. Schwiertz[3], F. Ranchon[3], N. Vantard[3], C. Serrier[1], A. Quintela[1], C. Cazac[1], C. Hospital[1], V. Chapel[1], S. Sar[1], C. Rioufol[3], M. Michallet[1], F. Barraco[1]*
[1]Hematology 1G, [2]Biochemistry, [3]Pharmaceutical department, Centre Hospitalier Lyon Sud (CHU Lyon), Pierre Benite, France

Introduction: Calcineurin inhibitor toxicity remains among the most common causes of acute renal impairment after alloHSCT. Neverthless Tacrolimus and CsA are a part of the most validated drugs used in alloHCST for prophylaxis and treatment of GvHD. Recently tacrolimus is available in both standard-release (twice-daily) and extended-release (once-daily) formulations. In kidney recipients, the extended-release formulation of tacrolimus was followed by a clinically significant improvement in kidney graft function. Based on this observation, we have conducted a prospective study in wich CsA is switched for tacrolimus ER in case of renal impairment after alloHSCT.
Materials (or patients) and Methods: We enrolled 16 consecutive patients with renal impairment (serum creatinine >110 µMol/L) from May 2012 to May 2013 during the follow-up at outpatient clinic after exclusion of other cause of renal impairment as thrombotic microangiopathy syndromes or obstructive cause, and after correction of dehydration, dose adjustment of CsA, discontinuation of other nephrotoxic drugs and exclusion of disease relapse. A satisfaction and adherence survey for tacrolimus ER treatment was performed 3 months after the switch.
Results: All patients in this cohort had hematological malignancies, the median age was 52 years (range, 28-66). Eight patients (50%) had a matched related donor, 7 patients (43.75%) had a HLA-10/10 matched unrelated donor and 1 patient underwent alloHSCT with two cord blood units. Seven patients (43.75%) received a myeloablative regimen with 12 Gy TBI and 9 patients (56.25%) a reduced intensity regimen. The stem cell source was bone marrow for 7 patients (43.75%), PBSC for 8 patients (50%) and cord blood for one patient. The status at transplantation was CR1 for 6 patients (37.5%), CR2 or more for 6 patients (37.5%) and partial response for 4 patients (25%). The median follow-up of the cohort was 7 months (range,2-14). Non-parametric tests such as exact Wilcoxon Mann-Whitney test or Kruskal Wallis were performed for the analysis of the physiological parameters. The median of serum creatinine was 112 µmol/L (range, 51-262) with CsA and 87 µmol/L (range, 50-125) with tacrolimus ER (P <.0001). The reduction of serum creatinine level was correlate with an improvement of GFR for all patients in the study. The median of potassium level was 4.2 (range, 3.5-5.2) with CsA and 4.0 (3.4-4.5) with tacrolimus ER (P =.001). The median time of switch was 45 days (10-162) and the median of serum creatinine in which the switch was realized was 138 µmol/L (range,110-262). The median blood trough level was 300 µg/L (range,100-438) for CsA 14 days after starting and 7.2 (range,3-15) for tacrolimus ER 20 days after starting. The cumulative incidence of aGvHD grade >1 at 3 months was 25% (95%CI,14-36). After the switch for tacrolimus ER, no patient developped aGvHD. Two patients in this study developed severe cGvHD after the discontinuation of prophylaxis (5 months and 10 months). The survey demonstrated that patients were satisfied with this formulation of tacrolimus and the adherence was reinforced.
Discussion: The conversion from CsA to tacrolimus ER was followed by a significant improvement in kidney function and in adherence to immunosuppressive treatment. We have now enlarged the study to several centers to confirm these encouraging observations.
Disclosure of Interest: None Declared.

PH-P346
SIGNIFICANTLY REDUCED RISK OF SINUSOIDAL OBSTRUCTION SYNDROME OF THE LIVER IN RECENT YEARS IN PATIENTS RECEIVING BUCY PRIOR TO ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

J. Mattsson[1,], O. Ringden[1], M. Engstrom[1], S. Berglund[1], P. Ljungman[2], J. Winiarski[3], M. Uhlin[1], B.-M. Svahn[1], M. Remberger[1], M. Hassan[4]*
[1]Karolinska University Hospital, Centre for allogeneic stem cell transplantation, [2]Karolinska University Hospital, Dept of Hematology, [3]Karolinska University Hospital, Dept of Pediatrics, [4]Karolinska University Hospital, Dept of Laboratory Medicine, Stockholm, Sweden

Introduction: Sinusoidal obstruction syndrome (SOS) has been a common and potentially life-threatening complication after allogeneic hematopoietic stem cell transplantation (HSCT). Incidence of SOS has decreased in recent years according to several reports, in particular severe and fatal cases. This improvement has been thought to be related to increased use of less toxic and reduced-intensity conditioning regimens.
Materials (or patients) and Methods: In the present study we specifically focused on patients undergoing myeloablative preparatory treatment with busulfan (16 mg/kg) and cyclophosphamide (120 mg/kg) (BuCy) before allogeneic HSCT during the period of 1990-2013. In total 397 patients were given BuCy. All patients received oral busulfan. Since the end of 1990's dose adjustment based on pharmacokinetic analysis has been performed for busulfan. Before 1995 at our centre, females were treated with norethisterone to delay menstruation and had an incidence of SOS of 27% as opposed to 3% in females without this treatment (P=0.007). Therefore norethisterone therapy was discontinued in 1998.The most common diagnosis was AML (n=181) followed by MDS (n=65) and chronic leukemias (n=56). Seventy-two patients with non-malignant disorders received BuCy. Median age was 29 (<1-61) years and 154 (39%) of all patients were children. Donor age was median 31 (0-66) years. A matched related donor was used in 172 patients and matched unrelated donor in 173. A mismatched transplant was used in 52 patients. Stem cell source was bone marrow (BM) (n=182), peripheral blood stem cells (PBSC) (n=192) and cord blood (CB) (n=24). Median $CD34^+$ cell dose (x10^6/kg) was 6.6 (0.01-46.0). The majority of patients received ATG during conditioning therapy (n=236, 59%) and cyclosporine and MTX as graft-versus-host disease (GVHD) prophylaxis (n=343, 86%). Sinusoidal obstruction syndrome was diagnosed according to Jones criteria.
Results: Sinusoidal obstruction syndrome of the liver was diagnosed in 31 (8%) of the patients. Patients receiving PBSC had a SOS incidence of 2.6% (5/191) compared to 12.6% (23/182) and 12.5% (3/24) in patients given BM and CB, respectively (P<0.01). Patients given BuCy + VP16 had a high SOS incidence of 29.4% vs. 7% in patients given BuCy only (P=0.003). The incidence of SOS decreased over time with the highest incidence 1995-99 of 18.6% (8/43) compared to 1.37% (1/73) during 2010-2013.
In multivariate analysis, year of transplant (OR 0.89, CI 0.79-1.00, P=0.05) and use of PBSC (OR 0.30, CI 0.10-0.88, P=0.027) was protective against SOS, but addition of VP16 increased the risk (OR 3.66, CI 1.15-11.6, P=0.027). Overall survival at one year was 55% vs. 76% in patients with or without SOS. The corresponding numbers at five years were 35% vs. 65% (P<0.001).

Discussion: We believe this dramatic reduction of SOS in patients receiving BuCy is mainly due to the introduction of busulfan dose adjustment based on pharmacokinetic analysis in the end of 1990, stop of norethisterone in female patients and the use of acetylcysteine during conditioning therapy since 2010 in order to increase glutathione levels in the liver. The study also indicates clearly that oral busulfan is an excellent option without significant toxicity when administered with the measures mentioned above.
Disclosure of Interest: None Declared.

PH-P347
ASSESSING QUALITY OF LIFE BEFORE HSCT: TAKING CARE OF CAREGIVERS TO TAKE CARE OF PATIENTS

S. Giuliani[2,], F. Giglio[1], D. Alacqua[2], M. Morelli[1], R. Greco[1], G. Levati[1], M. T. Lupo Stanghellini[1], M. Carrabba[1], M. Marcatti[1], C. Corti[1], M. Bernardi[1], J. Peccatori[1], L. Sarno[2], F. Ciceri[1]*
[1]Hematology and bone marrow transplantation unit, [2]Health Clinical Psychology Unit, San Raffaele Scientific Institute, Milan, Italy

Introduction: Caregivers of hematopoietic stem cell transplantation (HSCT) patients play an important role in providing care and improving patients' Quality of Life (QoL) and adjustment process to the disease. However, a systematic assessment of family caregiver's distress is rarely practiced.
The aim of this study was to compare patients' and caregivers' QoL before HSCT and to identify caregivers at higher risk of poor outcomes, investigating which socio-demographic, clinical and psychological variables are significantly associated with worst QoL in order to plan early interventions that may reduce burden of caregivers themselves, while enhancing patients' QoL and well-being.
Materials (or patients) and Methods: From January to October 2012, 56 patients and their caregivers were studied.
Self-administered questionnaires were used to investigate patients and caregivers' QoL [*Medical Outcomes Study SF-36* (JE Ware and AL Stewart, 1992)], caregivers' perception of family environment [*Family Environment Scale* (R Moos and B Moos, 1994)] and burden of care [*Caregiver Burden Inventory* (M Novak and Guest C, 1989)].
The study compared patients' and caregivers' QoL, and analyzed the association between caregivers' QoL with their own socio-demographic and psychological factors and with patient's clinical variables. SPSS was used to test statistical significance of associations.
Results: Caregivers' psychological QoL, measured by SF-36 Mental Component Summary (MCS), was significantly lower ($P = 0.001$) than the patients', while patients' physical QoL, measured by SF-36 Physical Component Summary (PCS), was significantly lower ($P = 0.0001$) than the caregivers'.
Caregivers' psychological QoL (MCS) was negatively influenced by the need to move away from home for patient's hospitalization ($P= 0.04$); the difficulties to share with the family emotions and worries about the patient ($P = 0.4$); the feeling of being unfairly secluded from normal life compared to peers ($P = 0.0001$); the lack of energy ($P = 0.0001$); the difficulty to integrate the caring role with normal life roles ($P = 0.0001$).
No correlation between patient's medical condition and caregivers' QoL was found.
Discussion: These data highlight the need to plan systematic assessment of caregivers, in order to plan appropriate support and early psychological interventions to improve their QoL and therefore patients' QoL.
The need, real or perceived, to revolutionize their own life (geographically, emotionally or socially) stands out as the most stressing factor for caregivers. Interestingly caregivers level of distress does not relate to the severity of patient prognosis. This suggests the outstanding importance of supporting caregivers to build a continuum between previous life and new necessities, listening to their necessities, independently from patient's status.
Disclosure of Interest: None Declared.

PH-P348
VENO-OCCLUSIVE DISEASE IN CHILDREN FOLLOWING HAEMATOPOIETIC STEM CELL TRANSPLANTATION. A SEVENTEEN YEARS EXPERIENCE IN ONE PEDIATRIC BONE MARROW TRANSPLANTATION CENTRE

N. Mandel-Shorer[1,], M. Ben Arush[1], R. Elhasid[2], N. Arad Cohen[1], S. Postovsky[1], A. Ben Barak[1], I. Zaidman[1]*
[1]Pediatric Hemato-oncology, Rambam Medical Center, Haifa, [2]Pediatric Hemato-oncology, Dana Medical Center, Tel Aviv, Israel

Introduction: Veno-occlusive disease of the liver (VOD) is a complication of haematopoietic stem cell transplantation (HSCT) with high rate morbidity and mortality. Incidence of VOD after HSCT is about 14%. The diagnosis of VOD is based on clinical criteria, including elevated bilirubin levels, tender hepatomegaly, ascites and weight gain. A number of agents, including defibrotide, have been developed and investigated for use in both prophylaxis and treatment of VOD.
Materials (or patients) and Methods: Our objective herein was to review the incidence, risk factors, severity, treatment and outcome of VOD post HSCT.
Patients with VOD were identified by reviewing electronic charts of all children who underwent HSCT in 1996-2013. Diagnosis of VOD was based on the widely accepted Modified Seattle criteria and Baltimore criteria.
Results: VOD was diagnosed in 16/340 (4.7%) children who underwent HSCT in onco-hematological department during the study period. The median age of patients with VOD was 24 months. Underlying diagnoses were diverse, and included both malignant and non-malignant conditions. 15/16 patients developed VOD after first transplantation. One patient developed VOD following second transplantation. The source of stem cells included autologous SCT (n=3), matched sibling donor (MSD, n=5), matched family donor (n=1), family haploidentical donor (n=3), matched unrelated donor (MUD, n=2), and unrelated cord blood (UCB, n=2). Eight patients (50%) had evidence of liver disease/dysfunction prior to HSCT. Thirteen patients (80%) received Busulfan based conditioning regimen. Two patients were conditioned with TBI-VP16. Thirteen patients were treated with ursodeoxycholic acid as VOD prophylaxis, 8 of them (treated prior to 2007) received heparin as well. Fourteen patients developed signs and symptoms of VOD within 3 weeks of transplantation (average 12.6 days post transplantation). Two patients with AML were diagnosed with VOD several months post transplantation with Busulfan based Conditioning after salvage therapy for relapse.
All patients fulfilled the diagnostic criteria for VOD, including hepatomegaly and weight gain. All but two patients had elevated bilirubin levels (>2 mg/dL). 8/16 (50%) patients had evidence of reversal of portal vein blood flow on Doppler ultrasonography. All patients were treated with corticosteroids and thirteen patients (81%) were treated with Defibrotide, with treatment commencing on average 3 days (range 2-7 days) post appearance of VOD signs and symptoms. Defibrotide treatment was continued for an average of 11 days (range 4-21 days). Ten patients (62.5%) had severe VOD, all were treated with Defibrotide and corticosteroids, six (60%) died of VOD-related multiorgan failure. We have recently used defibrotide prophylaxis in two HSCT patients with several risk factors for development of VOD.
Discussion: VOD is a severe complication of HSCT, with high rates of morbidity and mortality despite early initiation of defibrotide and corticosteroid treatment. The use of defibrotide for prophylaxis of VOD in identified high risk patients has been recommended, and seems promising. Large scale studies are needed to evaluate the efficacy of VOD prophylaxis with defibrotide.
Disclosure of Interest: None Declared.

PH-P349

REHABILITATION NEEDS IN HEMATOLOGY AND BONE MARROW TRANSPLANTATION UNIT: ONE YEAR EXPERIENCE AT SAN RAFFAELE INSTITUTE

P. Cervi[2,], F. Giglio[1], G. Levati[1], M. Morelli[1], R. Greco[1], C. Messina[1], M. Carrabba[1], M. Bernardi[1], C. Soliman[1], J. Peccatori[1], R. Gatti[2], F. Ciceri[1]*
[1]Hematology and bone marrow transplantation unit, [2]Rehabilitation Service, Lab. of Analysis and Rehabilitation of Motor Function, San Raffaele Scientific Institute, Milan, Italy

Introduction: Hematologic treatments and allogeneic hematopoietic stem cell transplantation (HSCT) are associated with a high treatment-related burden for patients (pts) but little is known about specific motor impairments. Aim of the study was to assess feasibility of physiotherapy (PKT) and to analyse specific rehabilitation needs for these pts.
Materials (or patients) and Methods: 105 patients were included in a PKT program from Oct 2011 to Oct 2012. They were admitted to receive intensive chemotherapy (34 pts), HSCT (38 pts) or treatments complications (33 pts) for aggressive hemopathies (AML 53, ALL 11, lymphomas 18, MM 10, other 13). The inclusion of each patient in the PKT program was agreed by physicians and physiotherapists (PT), representing 23% of pts admitted in the same period. Each patient was trained for one or more issues depending on specific needs, 167 treatments were performed (TR).
Results: Exercise interventions, tuned on specific clinical conditions, were feasible in all patients, also in therapy-related neutropenic phases.
STRENGTH AND ENDURANCE RECOVERY: 52 pts (31% of TR) reported loss of strength and fatigue without losing autonomy in activities of daily living (ADL). To improve exercise tolerance, pts performed supervised or independent muscle strengthening or endurance exercise.
IMMOBILIZATION SYNDROME MANAGEMENT: 23 pts (14% of TR) were forced to stay in bed for longtime. Treatment consisted in gradual adaptation to the sitting position. Mobilization exercises and stretching were carried out to maintain joint mobility and preventing muscle retractions.
ADL AND WALKING RESTORATION: in 26 pts (16% of TR), reduction in muscle strength decreased autonomy in the ADL (sitting up, moving to chair, walking). Muscle strengthening, optimized transfer techniques and assistive devices for transfers and walking could restore autonomy.
BALANCE RESTORATION: in 9 pts (5% of TR) chemotherapy-induced neuropathy resulted in sensitivity and/or strength loss in limbs and in balance deficits. Goal of treatment was balance recovery and choice of aids (canes, crutches, types of ankle orthosis).
PAIN RELIEF: 8 pts (5% of TR) suffered from drug induced tendonitis, joint pain, limbs oedema and benefitted from passive mobilization for the maintenance of joint mobility and manual treatments of myofascial system.
END OF LIFE CARE: for pts confined to bed, especially in the end of life (12 pts, 7% of TR), interventions such as passive joint mobilization, change of posture in bed with the support of the nursing staff, were adopted to give pain relief and a comfortable position to pts.
RESPIRATORY PHYSIOTHERAPY: PT had a role in prevention or resolution of respiratory problems. Positioning the pts in sitting position, restoring upright position and walking increased lung volumes, facilitated gas exchange and bronchial clearance. PT performed and educated pts to bronchial clearance techniques (13 pts, 8% of TR). For 24 pts (14% of TR), treated with continuous positive airway pressure, PT helped to choose comfortable interface, provided instructions about operating principles of the device, placed pts in the most suitable posture to lung re-expansion.
Discussion: PKT is feasible in the delicate setting of intensive Hematology and HSCT. Many rehabilitation needs and motor impairments were detected and treated. At least 1/4 of all pts can benefit from PKT and the crucial role of the PT is confirmed.
Disclosure of Interest: None Declared.

PH-P350

THE QUALITY OF LIFE FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION – THE RETROSPECTIVE STUDY OF CZECH TRANSPLANT CENTRES

V. Valkova[1,], M. Trnkova[2], J. Jircikova[3], M. Lanska[4], K. Steinerova[5], P. Keslova[6], Z. Koristek[7], L. Raida[8], T. Dolezal[9], K. Benesova[10], P. Cetkovsky[11], M. Trneny[12]*
[1]Bone Marrow Transplantation, Institute of Haematolgy and Blood Transfusion, Prague, [2]Czech National Stem Cell Transplantation Registry, [3]VALUE OUTCOMES, Prague 2, [4]Department Hemato-oncology, Faculty Hospital, Hradec Kralove, [5]Department of Hemato-oncology, University Hospital, Plzen, [6]Pediatric Hematology and Oncology, 2nd Faculty of Medicine and University Hospital Motol, Prague, [7]2nd Department Hematooncology, Faculty Hospital, Brno, [8]Department of Hemato-Oncology, Faculty of Medicine Palacky University, Olomouc, [9]VALUE OUTCOMES, [10]Czech National Stem Cell Transplantation Registry, 1st Dept od Medicine, Charles University General Hospital, [11]Institute of Haematology and Blood Transfusion, [12]1st Dept od Medicine, Charles University General Hospital, Prague, Czech Republic

Introduction: Although allogeneic haematopoietic stem cell transplantation (allo-HSCT) offers a unique curative potential, it may be connected with high treatment-related morbidity and mortality. Besides many organ complications, allo-HSCT may significantly affect quality of life (QOL).
Materials (or patients) and Methods: Five hundred and ninety patients (pts) from 6 transplant centers in the Czech Republic filled questionnaire for the quantitative measurement of QOL using Functional Assessment of Cancer Therapy-General (FACT-G) version 4 between January 2011 and December 2012. Study cohort characteristics were as follows: 325 males, myeloablative conditioning received 340 pts, PBPC received 383 pts, diagnoses representation; acute leukemia (*n*=270), bone marrow failure (*n*=36), chronic myeloid leukemia (*n*=74), myelodysplastic/ myeloproliferative syndrom (*n*=110), lymphoproliferative disease (*n*=93). The median age at allo-HSCT was 43 years (range: 1.7 – 71), the median follow-up was 3 years (range: 0.1 – 22.7), median time from allo-HSCT to questionnaire compeling was 3,8 years (range: - 0.2 to 21.6). The earliest allo-HSCT was performed in November 1989, the last in September 2012. In this retrospective study, we investigated the impact of various factors on the QOL after allo-HSCT: age, gender, diagnosis, type of conditioning, time from diagnosis to allo-HSCT, disease stage, graft type, donor type, time from allo-HSCT to questionnaire compeling, GVHD, relapse. Only data from patients who were more than 3 months after allo-HSCT were used for the final multivariate analysis. This multivariate model accounted for 12,4 % of the actual variation of QOL.
Results: The overall results of the total FACT-G score (mean=83.4; SD 14.4, range: 29-108) as well as the results of each specific dimension - PWB (mean=21.9; SD 5.2, range: 5-28), SWB (mean=23; SD 4.1, range: 7-28), EWB (mean= 18; SD 4.1, range: 4-24), FWB (mean=20.5; SD 5.2, range: 2-28) showed a value in the upper 30% of the possible evaluation. In multivariate analysis, the inferior QOL score was reported for patients with aGVHD (*P*=0.0016), cGVHD (*P*=0.0006), QOL decreased with increasing age (*P*=0.048) and increased with time elapsed since allo-HSCT (*P*=0.0002).
Discussion: Allogeneic HSCT represents an important intervention into the overall integrity of the organism. In particular, the development of GVHD can cause very serious organ, but also mental problems which can significantly reduce QOL. All patients should be well informed, not only about the risks of treatment failure, organ complications or possible death, but also about chronic psychological consequences and the expected deterioration in the QOL, which can be finally perceived worse than the actual disease.
Supported by IGA of the Czech Ministery of Health – grant No. NT 11299-6/2010.
Disclosure of Interest: None Declared.

PH-P351

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IS FEASIBLE AFTER SOLID ORGAN TRANSPLANTATION: FINAL ANALYSIS BY THE EARLY COMPLICATIONS SUBCOMMITTEE OF THE COMPLICATIONS AND QUALITY OF LIFE WORKING PARTY, EBMT

G. Wladyslaw Basak[1,] on behalf of CQWP EBMT, W. Wiktor-Jedrzejczak[1], H. Schoemans[2], P. Ljungman[3], G. Kobbe[4], Y. Beguin[5], P. Lang[6], K. Walter Sykora[7], L. te Boome[8], A. van Biezen[9] on behalf of CQWP EBMT, S. van der Werf[9] on behalf of CQWP EBMT, M. Mohty[10] on behalf of ALWP EBMT, T. de Witte[11] on behalf of CMWP EBMT, J. Marsh[12] on behalf of AAWP EBMT, P. Dreger[13] on behalf of LWP EBMT, N. Kroeger[14] on behalf of CMWP EBMT, R. Duarte[15] on behalf of CQWP EBMT, T. Ruutu[16]on behalf of CQWP EBMT*

[1]Hematology, Oncology and Internal Diseases, Medical University of Warsaw, Warsaw, Poland, [2]University Hospital Gasthuisberg, Leuven, Belgium, [3]Karolinska University Hospital, Stockholm, Sweden, [4]Heinrich Heine Universität, Dusseldorf, Germany, [5]University of Liege, Liege, Belgium, [6]University Hospital, Tuebingen, [7]Hannover Medical University, Hannover, Germany, [8]University Medical Centre, Utrecht, [9]EBMT Data Office Leiden, Leiden, Netherlands, [10]Hospital Saint Antoine, Paris, France, [11]Radboud University - Nijmegen Medical Centre, Nijmegen, Netherlands, [12]GKT School of Medicine, London, United Kingdom, [13]University of Heidelberg, Heidelberg, [14]University Hospital Eppendorf, Hamburg, Germany, [15]Institut Català d'Oncologia, Hospital Duran i Reynals, Barcelona, Spain, [16]Helsinki University Central Hospital, Helsinki, Finland

Introduction: The recipients of solid organ transplants are infrequent candidates for allogeneic hematopoietic stem cell transplantation (alloSCT). Since the reported clinical experiences have been anecdotal, outcomes of alloSCT after SOT are not well known. In order to document the feasibility of alloSCT after SOT, we decided to perform a retrospective analysis of patients reported to the EBMT registry.

Materials (or patients) and Methods: In 18/101 centers, which responded to the survey, we identified 28 patients who fulfilled the criterion: alloSCT performed after SOT. This group included 12 patients with kidney, 13 with liver and 3 with heart transplant. The indications for alloSCT were: acute leukemia (*N*=10), chronic leukemia (*N*=4), lymphoma (*N*=3), MDS/MPN (*N*=4), bone marrow failure (*N*=4) and inherited disorders (*N*=2). The median time from SOT to diagnosis was 23 months (-206 to 294), in 8 patients the diagnosis was known before SOT. Patients underwent 31 alloSCT (3 double alloSCT after SOT). The median year of alloSCT was 2006 (1990-2012) and the median time between SOT and alloSCT was 35 months (1-315). The conditioning was standard (*N*=16) or reduced (*N*=14) and included TBI in 12 cases, but the regimens were heterogeneous. The SCT was performed with either PBSCs (*N*=20), BM (*N*=9) or both (*N*=1) obtained from MRD (*N*=12), MUD (*N*=11), mMUD (*N*=3) or haploidentical (*N*=5) donors. T cell depletion was performed in 20/29 cases (*in vivo N*=15, *ex vivo N*=2, *in vivo* and *ex vivo N*=3). The most common GvHD /SOT rejection prophylaxis was CsA and methotrexate (*N*=12) followed by CsA and MMF (*N*=5), tacrolimus and MMF (*N*=5) and tacrolimus with methotrexate (*N*=1), but frequently included other immunosuppressive drugs, especially steroids.

Results: Hematopoietic engraftment was achieved after 25/30 transplantations including 2 cases where the graft was lost. In evaluable patients, time to neutrophil recovery (>0.5 G/L) was 13.5 days (9-37, *N*=22), in 3 patients the platelet count never fell below 20 G/L and the rest recovered after median of 17 days (10-103). Acute GvHD was observed in 17/31 cases including 10 with grade II-IV disease. Terminal solid organ transplant failure was observed in 10 of 30 alloSCTs (1/3 heart, 3/13 liver and 6/11 kidney transplants) after median of 6 weeks (0-569) after HSCT (8/10 in early post-HSCT period). Six of 10 failure cases have been described as SOT rejections (4 kidneys, 1 liver, 1 heart). After median of 14 months from alloSCT (0,3-244) 13/28 patients were alive (46%, 8/13 patients after liver, 5/12 after kidney and none after heart transplantation). The estimated probability of survival after HSCT was 75% at 3 months, 60% at 12, 45% at 36, and 40% at 60 months. In one third of patients (5/15), death was directly related to transplanted solid organ failure.

Discussion: In summary, a significant proportion of patients enjoy long term survival without graft loss, especially after liver transplantation. Therefore our results suggest that alloSCT is a feasible treatment option for patients after SOT. However, the risk of transplanted solid organ failure is high, including the possibility of solid organ rejection.

Disclosure of Interest: None Declared.

PH-P352

EBMT SCORE OR SORROR'S COMORBIDITY INDEX : WHICH ONE IS THE BEST FOR PREDICTING TRANSPLANT OUTCOME?

Z. A. Yegin[1,], Z. Aki[1], G. Sucak[1]*

[1]Hematology, Gazi University, Ankara, Turkey

Introduction: Allogeneic hematopoietic stem cell transplantation (AHSCT) is a curative treatment modality for a variety of hematological malignancies. Risk adapted treatment decisions based on various scoring systems including EBMT and Sorror's models have been used to reduce transplant related morbidity and mortality.

Materials (or patients) and Methods: A total of 157 patients [median age: 35(15-64) years; M/F:108/49] who underwent AHSCT in Gazi University Stem Cell Transplantation Unit were retrospectively reviewed to evaluate the predictor role of pre-HSCT EBMT score, Sorror's comorbidity index (CI) and serum ferritin levels on transplant outcome and survival.

Results: Fourty five patients (28.7%) had acute myeloblastic leukemia, 41 patients (26.1%) acute lymphoblastic leukemia, 15 patients (9.6%) multiple myeloma, 11 patients (7.1%) myelodysplastic syndrome, 9 patients (5.7%) severe aplastic anemia, 9 patients (5.7%) non Hodgkin's lymphoma, 7 patients (4.5%) chronic myeloid leukemia, 5 patients (3.2%) primary myelofibrosis, 4 patients (2.5%) Hodgkin's lymphoma, 4 patients (2.5%) paroxysmal nocturnal hemoglobinuria, 2 patients (1.3%) plasma cell leukemia, 2 patients (1.3%) chronic lymphocytic leukemia, 1 patient (0.6%) chronic myelomonocytic leukemia, 1 patient (0.6%) Fanconi aplastic anemia and 1 patient (0.6%) thalassemia major. Conditioning regimens were Cyclophosphamide(Cy)–Busulfex(Bu) in 61 patients (38.9%), Fludarabine(Flu)–Melphalan(Mel) in 43 patients (27.4%), total body irradiation(TBI)–Cy in 30 patients (19.1%), Flu–Cy–antithymocyte globuline(ATG) in 10 patients (6.4%), TBI–Cy–Thiotepa in 5 patients (3.2%) Flu–Bu–ATG in 4 patients (2.6%) Flu–Bu–Cy–ATG in 1 patient (0.6%), Flu in 1 patient (0.6%), TBI–Flu in 1 patient (0.6%) and Bortezomib–Mel in 1 patient (0.6%). Cyclosporine A (CSA) and methotreaxate were used in 125 patients (79.6%) and CSA and mycophenolate mofetil in 32 patients (20.4%) for graft versus host disease prophylaxis. Pre-HSCT disease status was demonstrated in 126 patients. A total of 84 patients (66.7%) were in complete remission, 10 patients (7.9%) was in partial remission, 7 patients (5.6%) had stable disease and 25 patients (19.8%) had progressive disease. Median Karnofsky performance score (PS) was found to be 90 (40-100). Pre-HSCT serum ferritin level was 948,85(9,8-8660,44) ng/ml. Median time from diagnosis to AHSCT was 356(53-5961) days. Pre-HSCT EBMT score and Sorror's CI were 3(0-6) and 2(0-7) respectively. A total of 80 patients (51%) died at 324 (7-2077) days of follow-up. Pre-transplant disease status was correlated with both Sorror's CI (P=0.036; r=0.188) and EBMT score (P<0.001; r=0.424). An inverse correlation was demonstrated between Sorror CI and Karnofsky PS (P<0.001; r=–0.318). Pre-HSCT serum ferritin level was correlated with Sorror's CI (P=0.005; r=0.225) and Karnofsky PS (P<0.001; r=–0.310). In univariate analysis, Karnofsky PS (P=0.001), Sorror's CI (P=0.052) and EBMT score (P=0.002) were found to have significant impact on overall survival (OS). The impact of EBMT score (P=0.003) and Karnofsky PS (P=0.003) on OS remained significant on multivariate analysis.

Discussion: This preliminary report shows that EBMT score and pre-HSCT PS predicts transplant outcome. The significant

impact of EBMT score on survival underlines the importance of primary disease and transplant related factors rather than comorbidities.
Disclosure of Interest: None Declared.

PH-P353
LATE MORTALITY AND CAUSE OF DEATH AMONG LONG SURVIVORS AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

M. Iida[1,], H. Nakasone[2], Y. Inamoto[3], M. Inoue[4], Y. Ishida[5], H. Kaneko[6], K. Iwato[7], T. Miyamoto[8], H. Hashimoto[9], T. Mori[10], M. Sawa[11], M. Hirokawa[12], T. Yamashita[13], Y. Atsuta[14] and Late Effect and Quality of Life Working Group of the Japan Society for Hematopoietic Cell Transplantation*
[1]Department of Promotion for Blood and Marrow Transplantation, Aichi Medical University School of Medicine, Nagakute, Japan, [2]Division of Blood and Marrow Transplantation, Stanford University School of Medicine, Stanford, [3]Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, United States, [4]Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child, Osaka, [5]Department of Pediatrics, Ehime Prefectural Central Hospital, Matsuyama, [6]Department of Hematology, Japanese Red Cross Kyoto Daiichi Hospital, Kyoto, [7]Department of Hematology, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Hiroshima, [8]Department of Hematology and Oncology, Kyushu University Hospital, Fukuoka, [9]Department of Hematology /Division of Stem Cell Transplantation, Kobe General Hospital/Institute of Biomedical Research and Innovation, Kobe, [10]Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, [11]Department of Hematology and Oncology, Anjo Kosei Hospital, Anjo, [12]Oncology Center, Akita University Hospital, Akita, [13]Division of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, [14]Department of HSCT Data Management / Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan

Introduction: We evaluated risks of late mortality and causes of death among long survivors after autologous hematopoietic stem cell transplantation (HCT).
Materials (or patients) and Methods: Patients: 6,222 patients who underwent first autologous HCT for hematological diseases were analyzed. All of them were relapse-free and survived over two years after transplantation. Median age at transplant was 49 (0-78) and 94.6% of their stem cell source was peripheral blood. Median follow-up period of the survivors was 5.7 years (2 to 26).
Results: The overall survival rates at 5 and 10 years were 91.9% and 82.4%, respectively. Among 6,222 patients who survived with relapse-free at 2 years after autologous HCT, the number of any cause of death after the first two years was 743 including 412 recurrent diseases. As for the numbers of other cause-specific death, there were 118 new malignancies including 68 non-hematological and 49 hematological ones, 52 infections, 40 respiratory diseases, 18 cardiovascular diseases, 15 liver diseases, 10 neurological diseases, 8 kidney-genitourinary diseases. When we compared these figures to those from the Japanese general population, risk of overall mortality was significantly higher (observed / expected ratio [O/E] = 5.8, 95%CI, 5.4-6.2). The risk of mortality was significantly higher with infection (O/E=7.9, 95%CI, 5.9-10.3), new malignancy (O/E=2.1, 95%CI, 1.8-2.6) ;hematological (O/E=14.7, 95%CI, 10.9-19.4) and non-hematological (O/E=1.3, 95%CI, 1.0-1.7), kidney-genitourinary (O/E=4.7, 95%CI, 2.1-9.4), respiratory (O/E=10.8, 95%CI, 7.7-14.7) and liver (O/E=3.0, 95%CI, 1.7-5.0) compared to general population.
Discussion: Long-term survivors after autologous HCT are at higher risk of mortality due to recurrent diseases and various causes. Careful screening for relapse and early identification of disease outbreaks are essential for long-term follow-up of autologous HCT recipients.
Disclosure of Interest: None Declared.

PH-P354
PALIFERMIN DECREASES ORAL MUCOSITIS AND IMPROVES SURVIVAL IN CHILDREN UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

K. Czyzewski[1], J. Styczynski[1,], R. Debski[1], A. Krenska[1], M. Wysocki[1]*
[1]Department of Pediatric Hematology and Oncology, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland

Introduction: Palifermin, recombinant human keratinocyte growth factor, is used for mucositis prevention in adults following autologous and allogeneic hematopoietic stem cell transplantation (HSCT). It is known that palifermin decreases length of initial hospital stay, mean number of days of total parenteral nutrition and the use of opioids for pain control in oral mucositis in adults. There are no data evaluating palifermin use in children following autologous HSCT. The aim of this study was analysis of efficacy and safety of palifermin in children and adolescents following autologous HSCT.
Materials (or patients) and Methods: Ninety-nine consecutive patients were included into the study. Results of efficacy of palifermin in 18 patients were compared to data of 81 patients not treated with palifermin. Palifermin was administered on compassionate-use basis.
Results: Palifermin decreased the incidence of severe oral mucositis (grade 3-4) by 21% (44% vs 65%), however it did not contribute to the length of oral mucositis and total parenteral nutrition. There were no differences between palifermin and non-palifermin groups in opioid use, incidence of neutropenic fever, severe infection, hematological recovery and gastrointestinal hemorrhage. 5-year overall survival was better in patients treated with palifermin (0.94±0.05 vs 0.62±0.05, P=0.068). In univariate analysis, the use of palifermin was a positive risk factor for overall survival (P=0.032, HR=5.2, 95%CI=1.7-38). No side effects were observed after palifermin administration in all 18 patients.
Discussion: Palifermin decreases oral mucositis and improves overall survival in children undergoing autologous HSCT. However, the use of this drug is limited by high cost, and no direct reduction of treatment expenses is proved.
Disclosure of Interest: None Declared.

PH-P355
INCREASED RISK FOR NON-RELAPSE MORTALITY IN PATIENTS WITH IRON OVERLOAD UNDERGOING STEM CELL TRANSPLANTATION

L. Anna Gil[1,], M. Kozlowska-Skrzypczak[1], K. Mackowiak[1], A. Czyz[1], D. Dytfeld[1], A. Lojko-Dankowska[1], M. Matuszak[1], A. Nowicki[1], M. Komarnicki[1]*
[1]Department of Hematology, Poznan University of Medical Sciences, Poznan, Poland

Introduction: Iron overload is associated with increased morbidity and mortality among stem cell recipients. It has been reported that high pre-transplant ferritin level adversely impacts clinical outcome in transplant setting and is associated with early complication after HSCT. Growing body of evidence however suggest, that serum level of hepcidin, the main regulator of iron homeostasis, correlates better with tissue and plasma iron than serum ferritin. We evaluated the risk of NRM, infections and organ toxicity and acute GVHD within 100 days after HSCT with respect to pretransplant serum ferritin and hepcidin levels and transferrin saturation.
Materials (or patients) and Methods: A total of 51 patients (median age 40; range 20-65) were analyzed. Twenty four pts were treated for myeloid malignancies (AML, MDS, CML, MPD) and 27 for lymphoid neoplasia (ALL, NHL, HL, MM). Twenty six pts had advanced disease. Forty pts underwent allogeneic PBSCT (28) or BMT (12) from sibling (17) or unrelated (23) donors. In this group reduced-intensity conditioning was applied to 15 pts. Autologous transplant was performed in 11 pts with use of PBSC. Elevated serum

ferritin level >1000 ng/mL was found in 30 (59%) pts and >2000 ng/mL in 14 (27%) pts. Hepcidin level <50 ng/mL was seen in 42 (82%) pts. High transferring saturation (>50%) was detected in 25 (49%) pts.
Results: Neutrophil recovery occurred in 48 pts at 14 (11-34) days; 3 pts died before hematologic reconstitution due to infection (2) or multiorgan failure (1). Infection complications up to 100 days occurred in 49 (96%) pts including: FUO (36), bacterial (12), fungal (9); CMV (13), influenza (2) and toxoplasmosis (1) infections. Any organ dysfunction was seen in 22 (43%) pts, however severe renal, hepatic, or lung toxicity was diagnosed in 5 (10%) pts. GVHD>2° occurred in 8 (20%) pts from allogeneic group. Seven (14%) pts died up to 100 days due to bacterial infection (1), viral infection (2), toxoplasmosis (1), GVHD (2) or multiorgan failure (1). In univariate analysis we did not find statistically significant influence of ferritin level, hepcidin level or transferin saturation on infection risk (including fungal infection), organ toxicity, GVHD and NRM. However, among pts with hepcidin level >50 ng/mL there were no early deaths and the risk for infection was the lowest (ns). A simple model based on ferritin level >1000 ng/mL, hepcidin level <50 ng/mL and transferring saturation >50% was created, which identifies pts with iron overload and correlates significantly with higher non-relapse mortality (*P*=0.037, HR=6.0, 95%CI=1.1-32).
Discussion: Iron overload is common complication in HSCT recipients, especially when assessed with serum hepcidin measurement. A simple model based on ferritin, hepcidin levels and transferrin saturation indentifies patients with iron overload and higher risk for early non-relapse mortality both in autologous and allogeneic settings.
Disclosure of Interest: None Declared.

PH-P356
INCIDENCE AND RISK FACTORS OF HEPATIC VENO-OCCLUSIVE DISEASE AFTER HEMATOPOETIC STEM CELL TRANSPLANTATION IN CHILDREN

G. Karasu[1,], V. Uygun[2], S. Cakı Kılıç[1], Z. Berber[2], H. Daloglu[2], F. Pekun[1], Z. Dincer[2], V. Hazar[3], A. Yesilipek[1]*
[1]Pediatric Stem Cell Transplantation Unit, Bahcesehir University Faculty of Medicine Goztepe Medicalpark Hospital, Istanbul, [2]Pediatric Stem Cell Transplantation Unit, Antalya Medicalpark Hospital, [3]Pediatric Hematology and Oncology, Akdeniz University Faculty of Medicine, Antalya, Turkey

Introduction: Hepatic veno-occlusive disease (VOD) is an early complication of hematopoetic stem cell transplantation (HSCT). In this study, we evaluate the incidence and clinical

[PH-P356]

Table 1. Characteristics of VOD patients

	n (vod patients/totally transplanted patients)	%
VOD patients	67/441	15
Gender (M-F)	41/258 – 26/183	15.9 / 14.2
Age (Median)	7.4 y (3 m-17y)	
< 5 years	23 / 179	12.8
5-10 years	16 / 123	13.0
> 10 years	28 / 139	20.1
Findings		
Weight gain	45	67.1
Hiperbilirubinemia	51	76.1
Painful hepatomegaly	61	91.0
Ascites	41	61.2
Resisitant thrombocytopenia	46	68.6
Diagnosis		
Malign diseases	14 / 122	11.5
Non-malign (except thalassemia major)	26 / 194	13.4
Thalassemia major	27 / 125	21.6
Donors		
MSD	25 / 200	12.5
MFD	15 / 91	16.4
MUD	27 / 150	18.0
Stem cell source		
Bone marrow	48 / 294	16.3 (48/294)
Peripheral blood stem cell	13 / 103	12.6 (13/103)
Cord blood	6 / 44	13.6 (6/44)

characteristics of hepatic VOD in children after HSCT in our center.
Materials (or patients) and Methods: The data of allogeneic HSCT performed at Goztepe and Antalya Medicalpark Hospitals Pediatric Stem Cell Units between January 2011 and November 2013 were retrospectively evaluated with respect to hepatic VOD that was defined by the Seattle criteria.
Results: We had a total of 441 allogeneic transplantation performed in defined period. Hepatic VOD occurred in 67 of all transplants(15%). The characteristics of the patients with VOD out of totally transplanted patients are shown in Table 1. Any significant difference with respect to sex was not identified. When patients were stratified according to age groups, patients who were older than 10 years old at the time of transplant had a tendency for increased risk of VOD compared to younger than10 (P=0.05). VOD incidence was similar in malign and non-malign diseases (11.5% and 13.4%, respectively), however it was higher in thalassemia major patients (20.6%)(P<0.01). Although there was a decreased tendency for VOD in patients receiving graft from MSD compared to other donor types, it did not reach to statistical significance (P=0.057). With respect to stem cell source, VOD frequency was found 16.3% in BM, 12.6% in PBSC and 13.6% in CB recipients (P=0.637). We lost 12 of 67 VOD patients (17.91%) but only in two cases the causes of deaths were solely attributed to VOD.
Discussion: Risk factors identified in our study group are; transplant age older than 10 years old, a diagnosis of thalassemia major and a transplant from non-sibling donors. We also suggest that refractory thrombocytopenia may be another predictive factor for hepatic VOD.
Disclosure of Interest: None Declared.

PH-P357
ENDOCRINE DISORDERS AFTER AUTOLOGOUS AND ALLOGENEIC STEM CELL TRANSPLANTATION

B. Serio[1,], V. Giudice[1], M. Sessa[1], L. Pezzullo[1], I. Ferrara[1], M. Rocco[1], R. Fontana[1], S. Annunziata[1], C. Selleri[1]*
[1]Hematology and Hematopoietic Stem Cell Transplant Center, Department of Medicine and Surgery, University of Salerno, Salerno, Italy

Introduction: The endocrine system is one of the most frequent target of complications after autologous (auto) and allogeneic (allo) hematopoietic stem cell transplantation (HSCT).
Materials (or patients) and Methods: We evaluated for endocrine abnormalities a retrospective cohort of 100 consecutive patients (median age, 32; range, 20-52) who underwent auto- (n=50) and allo- (n=50) HSCT with a median follow-up of 6 years (range, 1-15) and a disease-free survival of at least 1 year post-HSCT. Primary diseases were acute (n=44) or chronic (n=13) myeloid leukemia, Hodgkin disease (n=17), non-Hodgkin lymphoma (n=12) and multiple myeloma (n=14).
Results: All women experienced ovarian insufficiency, manifested as secondary amenorrhea associated with hypergonadotrope hypogonadism and reduced volumes of ovaries and uterus. In allo-HSCT patients, serum 17beta-estradiol, delta-4-androstenedione, circulating androgens and dehydroepiandrosterone levels were significantly decreased, especially in women developing cGVHD. In auto-HSCT patients, only serum 17beta-estradiol levels were decreased. Impaired spermatogenesis damage was observed in all transplanted patients. Lower sperm counts were observed in patients affected by cGVHD when compared to unaffected patients. Testosterone was reduced in about 30% of patients up to 1 year after HSCT, particularly during acute and chronic GVHD. The onset of adrenal insufficiency (a total of about 20% of cases in the auto- and allo-setting) was always related to the duration (more than 100 days) and cumulative dose (greater than 10 gr/m^2) of corticosteroid treatment. Cortico-adrenal failure recovered in all patients after 3-12 months of short acting steroid substitution therapy.
All allo-HSCT recipients conditioned with BU/CY regimen showed growth hormone (GH) levels within the normal range, insulin-like growth factor (IGF)-I levels were lower in 38% of recipients affected by cGVHD, whereas IGF-1 resulted in the normal range in only 7% of subjects cGVHD-free.
Sub-clinical hypothyroidism was found up to 5 years after allo-HSCT and the "low T3 syndrome" after 12-48 months, especially in patients with extensive cGVHD. In the auto-HSCT setting, we detected subclinical hypothyroidism in 12% of cases at 12 months and the "low T3 syndrome" in about 30% at 3 months, but none at 12 months. The incidence of hypothyroidism was higher in patients previously treated with neck/thoracic radiotherapy than in untreated patients (50% vs 1.3%, respectively).
Bone mass density (BMD) at lumbar spine, femoral neck and phalanges were significantly reduced (Z score mean values: -0.4 vs -0.9, -0.6 vs -1.4 and -1 vs -1.5 in auto- and allo-HSCT recipients, respectively) and GVHD development was associated with a more severe reduction in all bone sites. Eight patients (6 allo- and 2 auto-HSCT) developed avascular necrosis, 1 to 15 years (median, 28) following HSCT.
Discussion: The underlying diseases, pre-transplant therapies, the age at HSCT, total body irradiation (TBI)- and high-dose chemotherapy-based conditioning regimens were the main risk factors of endocrine disorders after auto- and allo-HSCT. Our analysis further provide evidence that auto and allo-HSCT recipients show higher incidence of endocrine disorders suggesting that their early identification may greatly improve the quality of life of long-term survivors after HSCT.
Disclosure of Interest: None Declared.

PH-P358
ROLE OF PARENTERAL NUTRITION IN PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

F. Almagro Torres[1,], M. J. Martínez Quesada[1], P. González Sierra[1], E. de León Marcano[1], J. A. López López[1], S. Durán Nieto[1]*
[1]Hematology, University Hospital of Jaen, Jaen, Spain

Introduction: Nutrition plays an essential role in the processes of maintaining health. And the malnutrition is common in patients waiting for Hematopoietic precursor cell transplantation and represents a risk factor for post-transplant morbidity.
Patients at any stage of the transplantation process are at high nutritional risk and should undergo careful nutritional assessment for the early identification of nutritional support requirements. The parenteral nutrition (PN) is treatment modality remains an artificial feeding technique that can give rise to numerous complications of varying severity. Therefore, appropriate selection of those patients who will derive a true benefit from PN is essential.
Materials (or patients) and Methods: Prior to November of 2011, in our centre, we have administered through stipulated protocols Parenteral Nutrition from day +1 regardless of the individual characteristics of each patient. Since then, we have not used this universal strategy, we have administered only enteral nutritional supplements.
108 consecutive patients under ASCT have been performed in our center, 64 patients have received NP (from Jan 2008 to Oct 2011), and 46 belong to Non-NP group (from Nov 2011 to Nov 2013). The characteristics of both groups are shown in Table 1. There are no significant differences between both groups according to gender, age, diagnosis and mucositis incidence. We used baseline Serum Albumin and Total Proteins (TP) as parameters to assess the nutritional status of patients. Subsequently we have studied these values 5 and 10 days post-transplant to evaluate the impact of use parenteral nutrition.
Results: For statistical analysis we used the test of T student. We found no significant differences in baseline values of both determinations, so both groups are comparable. There are no differences (P>0.05) between the values of albumin and TP on day +5 and day +10. The values are listed in Table 1.

Table 1. Patient characteristics

	NP Group (*n*: 62)	Non-NP Group (*n*: 46)
Gender		
- · Male	37 (60%)	31 (67%)
- · Female	25 (40%)	15 (33%)
Age at ASCT (average)	45.43 years	45.65 years
Diagnosis		
- · Acute Leukemia	7 (11.3%)	5 (10.9%)
- · NHL	19 (37.1)	20 (43.48%)
- · Hodgkin disease	13 (20.9%)	7 (15.21%)
- · MM	19 (30.7%)	14 (30.41%)
Mucositis incidence	40/55 (72%)	40/47 (85%)
- · Grade I-II	20 (36%)	17 (36%)
- · Grade III-IV	20 (36%)	23 (49%)
Total Protein (g/dL)		
- · Day 0	6.37	6.4
- · Day 5	5.87	5.85
- · Day 10	5.77	5.64
Serum albumin (g/dL)		
- · Day 0	3.76	3.67
- · Day 5	3.39	3.25
- · Day 10	3.13	3.11

Discussion: 1.There are only a few prospective, randomized, controlled trials that have investigated the role of nutritional support in organ transplantation.
2.Total proteins and Albumin is a very good index of the status of the hepatic synthesis, but has a half life as very long (21 days) it takes to change with disorder and nutritional therapy recovered, hence, prealbumin determining to have a life shorter half (2 days) is much more effective to assess acute malnutrition and response to treatment.
3.The individualized prescription of parenteral nutrition, according to current recommendations established by scientific societies, helps to improve the safety and effectiveness of this nutritional therapy.
Disclosure of Interest: None Declared.

PH-P359
THE EFFECT OF ANTI-THYMOCYTE GLOBULIN ON CLINICAL OUTCOMES IN PATIENTS RECEIVED MYELOABLATIVE CONDITIONING WITH BUSULFAN AND FLUDARABINE FOR ALLOGENEIC TRANSPLANTATION

D. S. Hong[1,], S. K. Park[1], J. H. Won[2], S. H. Kim[1], J. Yun[1], H. J. Kim[1], K. H. Kim[2], S. B. Bae[3], H. S. Park[2]*
[1]Hematology/Oncology, Soonchunhyang Univeristy Bucheon Hospital, Bucheon, [2]Hematology/Oncology, Soonchunhyang University Hospital, Seoul, [3]Hematology/Oncology, Soonchunhyang University Chonan Hospital, Chonan, Republic Of Korea

Introduction: Fludarabine has been used in combination with standard doses of intravenous injection of busulfan, thus reducing the toxicity previously observed with cyclophosphamide/busulfan regimens. However, some studies have documented that fludarabine based regimen was associated with an increased risk of relapse, especially in patients with active disease at the time of transplantation. However, it is unclear whether the additional use of anti-thymocyte globulin (ATG) to decrease the risk of acute graft versus host disease (GVHD) leads to improved clinical outcomes in this condition.
Materials (or patients) and Methods: We evaluated the impact of anti-thymocyte globulin (ATG), to decrease the incidence of acute graft versus host disease, on the clinical outcome including transplant related mortality and relapse rate in patients received myeloablative conditioning based on fludarabine and busulfan. Donors were fully HLA matched (6 out of 6 match for sibling donors). The dose of ATG was 4.0 ~ 6.0 mg/kg body weight for 2 or 3 consecutive days.
Results: Totally 66 patients undergoing allogeneic hematopoietic stem cell transplantation using peripheral blood stem cells were evaluated. The median follow-up time was 36 months. The incidence of moderate to severe acute GVHD (≥ grade 2) was not significantly changed by the addition of ATG (ATG group; 27.8% versus non-ATG group; 29.2%). Transplant related mortality seemed to be lower in cases treated with ATG. The incidence of relapse was significantly higher in patients received ATG (ATG group; 38.9% versus non-ATG group; 18.8%). 5 year disease free survival seemed to be lower in ATG group and 5 year overall survival was not significantly different between two groups.
Discussion: Conclusively, adding ATG to fludarabine based conditioning may not guarantee significant benefit of clinical outcomes in a setting of transplantation from a sibling donor. Therefore, a careful consideration of the use of ATG based on the patient's condition and the risk factors of the transplantation setting should be made.
Disclosure of Interest: None Declared.

PH-P360
VENTILATION HETEROGENEITY OF PERIPHERAL AIRWAYS (SACIN*VT) ASSESSED BY NITROGEN MULTIPLE BREATH WASHOUT (N2-MBW) IS AN ACCURATE AND SENSITIVE MARKER OF BRONCHIOLITIS OBLITERANS – THE SAN RAFFAELE EXPERIENCE IN HSCT SETTING

F. De Giacomi[1], A. Orsini[2], E. Guggiari[2], C. Corti[2], B. Calcaterra[1], A. Forcina[2], T. Perini[2], S. Piemontese[2], F. Pavesi[2], G. Levati[2], R. Milani[2], J. Peccatori[2], M. Bernardi[2], A. Assanelli[2], C. Bonini[2], F. Ciceri[2], G. Cremona[1], M. Lupo-Stanghellini[2,]*
[1]Unit of Respiratory Medicine, [2]Bone Marrow Transplantation Unit, Ospedale San Raffalele, Milan, Italy

Introduction: Chronic graft-versus-host disease (c-GVHD) is an immune-mediated disorder that occurs frequently after allogeneic hematopoietic cell transplantation (HCT). C-GVHD most often involves skin but lung can be involved and it is recognized as the major risk factor for reduced lung function. Bronchiolitis obliterans (BO) is a severe pulmonary manifestation characterized by a nonspecific inflammatory injury and is strongly associated with c-GVHD, suggesting that BO is a pulmonary manifestation of c-GVHD. BO initially affects terminal and respiratory bronchioles, a region largely unexplored by spirometry, which is only altered in advanced disease. In contrast, the Impulse Oscillation System (IOS) and the nitrogen multiple breath washout (N2-MBW) are techniques characterized by a high sensitivity to peripheral airway changes and potentially more suited to early detection of small airways disease.
Materials (or patients) and Methods: In a cross sectional study, a total of 161 patients (pts), divided into 4 groups: healthy controls (41), HCT candidates (47), HCT recipients (65) and pts with chronic obstructive pulmonary disease (COPD; *n*=8), were assessed by IOS, N2-MBW, spirometry, body plethysmography and diffusing capacity for carbon monoxide (DLCO) in order to describe respiratory function changes in post-HCT pts without pulmonary GvHD and in order to characterize the pattern of peripheral airway changes in BO.
Results: All subjects were able to perform IOS and N2-MBW without difficulty. HCT, even without respiratory complications, does not affect spirometry but appears to cause an increase in air trapping, a reduction in DLCO and enhanced ventilation inhomogeneity both in conductive (Scond*VT) and acinar (Sacin*VT) airways.
In the cohort of transplanted pts 33 were diagnosed with c-GvHD, 15/33 categorized as severe and 8/15 presented lung involvement. Immune-reconstitution analysis show no difference across the 3 sub-populations. Pts with lung GvHD were characterized by further DLCO reduction, increase in oscillometric indices sensible to peripheral airways involvement and a further threefold increase in Sacin*VT. Compared to patients with BO, COPD patients with the same degree of spirometric obstruction (FEV1/FVC<0.7, FEV1 50% predicted) showed only half the increase in predicted Sacin*VT (*P*0.03).

	Control		No BOS			BOS		
	M	SD	M	SD	p	M	SD	p
RV/TLC %	97	14	109	24	0.044	144	22	<0.001
DLCO %	96	8	80	16	0.002	49	11	<0.001
Scond*VT [l]	0.02	0.01	0.03	0.02	<0.001	0.02	0.01	<0.001
Sacin*VT [l]	0.05	0.02	0.13	0.09	<0.001	0.37	0.20	<0.001
Z5Hz [$cmH_2O/(l/s)$]	3.07	0.67	3.49	1.29	NS	4.93	2.46	0.017
DR5-20Hz [$cmH_2O/(l/s)$]	0.30	0.23	0.56	0.68	NS	1.25	0.96	0.016
X5Hz [$cmH_2O/(l/s)$]	-0.95	0.21	-1.13	0.77	NS	-2.27	1.98	0.002
fR [1/s)]	10.63	2.54	13.19	4.86	NS	20.34	6.39	<0.001
$R_{peripheral}$ [$cmH_2O/(l/s)$]	1.77	0.80	2.09	1.46	NS	3.70	3.06	0.026

Discussion: At a cut off of 321% of predicted value, Sacin*VT could distinguish the subjects with BO from pts without lung impairment post HCT with good accuracy (87%), sensibility (87.5%) and specificity (89.5%). IOS and N2-MBW are simple tests able to detect changes following HCT as well as those specific to BO. Further exploitation of this technique could provide improvement in diagnosis of lung GvHD in order to start appropriate therapy promptly, minimize symptoms and prevent irreversible organ damage.
Disclosure of Interest: None Declared.

PH-P361
PROGNOSTIC FACTORS AND OUTCOMES OF HAEMATOLOGICAL PATIENTS WITH ICU ADMISSION DURING THE 100 FIRST DAYS OF AUTOHSCT

I. Vazquez Fernandez[1,], R. Del Campo[1], J. Borras[1], P. Romero[1], A. Cladera[1], D. Gomez[1], E. Gonzalez[1], J. M. Guerra[1], M. Mascaro[1], F. Serra[1], J. J. Bargay[1]*
[1]Hematology, Hospital Son Llàtzer, Palma de Mallorca, Spain

Introduction: ICU clinicians are often reluctant to admit haematological patients due to the assumption of poor prognoses, as the survival of this patients has improved in recent years due to the advances in chemotherapy regimens and in order to achieve better survival rates, we consider crucial to identify prognostic factors which may predict their outcomes during their Intensive Care Unit (ICU) admission.
Materials (or patients) and Methods: Evaluate the prognostic factors and long-term survival of patients with haematological malignancies who were transferred to ICU due to a life-threatening complication during the first 100 days of autoHSCT. We performed a retrospective analysis between 2002 and 2012.
Results: From a total of 144 patients, we have registered 11 (7.5%) transferred to the ICU in 13 episodes. Six of them were male and 5 female, the average age at the diagnostic was 58 years (25-66) and the average days to admission were 8 days (5-100) from the infusion. The haematological underlying diseases were: 4 NHL, 4 MM, 1 AML, 1 HL and 1 AL. Those patients are the 7% of lymphoma, 8% of myeloma, 6% of leukemia, 6% of Hodgkin's lymphoma and 50% of amyloidosis of the global of autoHSCT performed during this period.
Intensive therapy regimens consisted of 5 patients with melfalan, 5 with BEAM and 1 with BuCy. Most of them in response to their haematological disease at the time of infusion (91%; 6CR, 4PR). Just one patient had received multiple transfusions.
The most common reason precipitating the ICU transfer were septic status, in 38% (5/13) of patients due to respiratory and in the same number of cases due to central venous catheter sepsis. We obtained microbiological isolates in 8 cases (61.5%). Seven of them were bacterial (50% GNB, 50% GPB) and one viral.
The average rate of neutrophils at the admission was $0x10^9$/L (0-8.2). Analitic data associated to infection were reviewed, aiming high levels of C-Reactive Protein (CRP) in 100% cases (12/12) of available data (12/13) and procalcitonin in 86% cases (6/7) of available data (7/13), with an average APACHE score of 15.5 points (5-23). On the other hand, we obtained a low acute renal failure rate among our patients (just 3 episodes, 23%).
During the first 24h of admission, in 4 occasions (30%) orotracheal intubation was needed, just one patient required non-invasive ventilation, in 8 patients (61%) vasoactives drugs were started and just in one patient continuous venovenous hemodiafiltration was performed.
The median ICU stay was 11 days (4-84). 5/11 patients died in the ICU (45,5%) with a median of 36 days (9-84) since de admission day. The death cause in 2/5 (40%) of patients was hemorrhagic complication and in 3/5 (60%) it was secondary to septic process.
Discussion: Our ICU admission rate is low with a mortality intra-ICU rate of 45.5%, both of them similar to the ones reported on literature. Neither the type of haematological malignancy or the status of the disease seem to be related to a higher rate of transfer to ICU, although our limited number of patients make it not completely assessable.
Our patients have income gravity data and medium APACHE scores, 30% of episodes are requiring orotracheal intubation and in more than half vasoactive drugs within the first 24h of admittance. Severe neutropenia and high levels of procalcitonin and CRP should be considered as heavy variables when assessing the severity of the infection.
Disclosure of Interest: None Declared.

PH-P362
COMPARISON OF FOUR-TIMES-DAILY VERSUS ONCE-DAILY INTRAVENOUS BUSULPHAN AS PART OF THE CONDITIONING THERAPY FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

C. Martinez-Mugica[1], A. J. Gonzalez[2], V. Dueñas[2], J. Solano[2], P. Palomo[2], L. Gutierrez[2], A. P. Gonzalez[2], S. Gonzalez[2], C. Rayón [2], C. Vallejo[2,]*
[1]Pharmacy Department, [2]Hematopoietic Transplant Unit of Asturias, Hospital Universitario Central De Asturias, Oviedo, Spain

Introduction: The aim of this study is to compare the safety of two administration schedules of intravenous busulphan (4-times-daily *versus* once-daily dosing) as part of the conditioning therapy for HSCT.
Materials (or patients) and Methods: We studied all the patients who received intravenous busulphan four times a day (BU-4 group) or once a day (3 hours infusion) (BU-1 group) during the conditioning therapy for HSCT at our Center during the last six years. In total, 66 patients received intravenous busulphan during the period of the study. Acute leukemias or myelodysplastic syndromes were the most common indications for HSCT (83.3% of the patients). Forty patients underwent allo-HSCT (half of them from unrelated donor) and 26 auto-HSCT. The SC source was PBSC in 47 cases, BM in 17, and UCT in 2. The distribution of patients

	Total (N=66)	BU-4 (N=39)	BU-1 (N=27)
Age [median (range)] (years)	47 (1-65)	39 (1-65)	53 (8-65)
Gender (male / female)	34 / 32	22 / 17	12 / 15
Disease (acute leukemia-MDS / others)	55 / 11	29 / 10	26 / 1
Type of transplant (allo / auto)	40 / 26	14 / 25	26 / 1
Conditioning (BEA, BuCy, BuFlu, BuMel)	18 / 14 / 28 / 6	17 / 11 / 5 / 6	1 / 3 / 23 / 0
Hospital stay [median (range)] (days)	30 (15-67)	29 (15-53)	30 (23-67)
Sinusoidal occlusive syndrome	4 (6.1%)	3 (7.7%)	1 (3.7%)
HSCT survival (alive at hospital discharge)	65 (98.5%)	38 (97.4%)	27 (100%)

between the treatment groups was: 39 patients in BU-4 group and 27 patients in BU-1 group (see table).
Results: The median hospitalization stay was 30 days (15-67), with no differences depending on the busulphan pattern of administration. Recovery of neutrophils and platelets counts varied depending on the conditioning regimen employed, but not on the two different schedules of busulphan. The mean requirement of platelet and packed red cells (PRBC) transfusion was also similar in both groups (PRBC: 5,54 in BU-4 group *vs* 5,04 in BU-1 group; Platelet concentrates: 9,8 in BU-4 group *vs* 8 in BU-1 group). Sinusoidal occlusive syndrome (SOS) was developed in 4 cases (6.1%), 3 of them in patients who received BuCy conditioning regimen. Although there were no significant differences in the incidence of SOS between the two groups, there was a trend to be superior in Bu-4. There were no differences in transplant mortality between the two group of patients (see table).
Discussion: This study shows that safety of once-daily administration of intravenous busulphan is at least similar to traditional 4-times-daily. Considering the advantages for the hospital Pharmacy and for the nurses of once a day schedule, it might be considered the method of choice for the administration of intravenous busulphan.
Disclosure of Interest: None Declared.

PH-P363
IMPACT OF SMOKING ON OVERALL SURVIVAL, NON RELAPSE MORTALITY AND SECOND CANCERS AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES

F. Spina[1,], C. De Philippis[1,2], L. Farina[1], V. Montefusco[1], A. Dodero[1], P. Corradini[1,2]*
[1]Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori, [2]Università degli Studi di Milano, Milan, Italy

Introduction: The impact of smoking on health has been extensively studied in epidemiology. This retrospective monocentric study aimed at understanding whether smoking affects the survival outcomes of hematologic patients undergoing reduced intensity allogeneic stem cell transplantation (RIC alloSCT).
Materials (or patients) and Methods: We searched for information on the smoking history (packs-year and duration of exposure) of 272 patients who received RIC alloSCT between 2001 and 2011 in our division, reviewing the clinical charts and by telephone interviews. We analyzed the impact of smoking and hard smoking (defined as smoking >=1 pack per day) on non-relapse mortality (NRM) by Cumulative Incidence method, on overall survival (OS) and progression free survival (PFS) by log-rank method. We ran a Cox multivariate analysis for NRM, OS and PFS using pre-transplant disease status, donor type, and smoking as covariates.
Results: We had complete data of 172 patients. All the patients received RIC alloSCT for lymphoma (58%), multiple myeloma (26%), or acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) (16%). The median age at alloSCT was 49 years (range, 18-66). Donor were HLA-identical siblings (45%), mismatched siblings (3%), unrelated (41%), or haploidentical (11%). At alloSCT, 54% of patients were in complete response (CR), 33% in partial response, 3% of patients were stable and 10% were in progression. Forty percent of patients were former smokers (21% hard smokers), 8% were smokers at admission (5% hard smokers). The exposure to smoking was a median of 1 pack per day (range, 0.1-3) for a median of 16 years (range, 1-40), and smokers had quit smoking by a median interval of 5 years before transplant (range, 0-40).
The median follow-up after alloSCT was 6.3 years (range, 0.4-11.4). The NRM at 100 days was 5%, at 1 year 9%, at 5 years 12%. Five-years OS and PFS were 76% and 49%. In univariate analysis, smoking and hard smoking increased NRM (P=0.017 and P=0.020), and reduced the OS (P=0.035 and P=0.003, respectively). The multivariate analysis showed that smoking increased the NRM by a factor of 3.6 (P=0.009), and significantly impacted OS increasing the risk of death by a factor of 1.8 (P=0.044). Overall survival was also impacted by alternative donor (P=0.018) and pre-transplant disease not in CR (P<0.001). Hard smokers had a more striking reduction of OS, with an increased risk of death by a factor of 2.8 (P=0.002) and an increased risk of NRM by a factor of 3.5 (P=0.016). Smoking or hard smoking did not affect PFS, which was impacted by disease status (P<0.001).
Six patients had a second cancer after alloSCT (3%), a patient was a non-smoker (breast carcinoma), 5 patients were smokers or former smokers (AML, MDS, bladder carcinoma, tongue carcinoma and squamous-cell skin carcinoma). The crude incidence of second cancer was 1% for non-smokers and 6% for smokers (trend, P=0.080).
Discussion: Smoking is a significant independent factor that impacts NRM and OS in patients undergoing a RIC alloSCT, and it may increase the incidence of second tumors. Smoking should be considered a comorbid condition before alloSCT. Aggressive anti-smoking campaigns should be undertaken to decrease this avoidable risk.
Disclosure of Interest: None Declared.

PH-P364

EXTRAMEDULLARY RELAPSE OF ACUTE LEUKEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: DIFFERENT CHARACTERISTICS BETWEEN ACUTE MYELOID LEUKEMIA AND ACUTE LYMPHOBLASTIC LEUKEMIA

X. Tang[1,], L. Ge[1], A. Sun[1], X. Zhu[1], D. Wu[1]*
[1]Hematology, The First Affiliated Hospital of Soochow University, Jiangsu Institute of Hematology, Suzhou, China

Introduction: Extramedullary relapse (EMR) of Acute Leukemia (AL) following allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a contributor to post-transplant mortality and remains a poorly understood, especially the different characteristics of EMR between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) patients. In order to investigate the incidence, risk factor and clinical outcome of EMR for AML and ALL, we performed a retrospective analysis 362 patients with AL who underwent allo-HSCT at the First affiliated Hospital of Soochow University from January 2001 through March 2012.
Materials (or patients) and Methods: We retrospectively studied 362 patients with AL including including 208 AML patients, 147 ALL patients and 7 hybrid acute leukemia (HAL) patients who underwent allo-HSCT at our center during these 10 years.
Results: 1.The 10-year cumulative incidence of overall relapse of AL was 27.0%, EMR was 7.9%. Compared with AML, ALL patients had a higher incidence of EMR (12.9% vs 4.6%, *P*=0.009). 2. EMR sites of AL post-HSCT included central nervous system (CNS) (*n*=18), testis (*n*=5), skin (*n*=2), soft tissue (*n*=2), bone (*n*=2), lymph nodes (*n*=1), nasopharynx (*n*=1), and the peritoneum (*n*=1). Five of 26 (19.2%) patients presented with EMR in multiple sites. The most common type of EMR in ALL was CNSL. 3. Multivariate analysis results showed that the risk factors of EMR for AML patients included: advanced disease status at HSCT, hyperleukocytosis at diagnosis, a history of extramedullary (EM) leukemia before HSCT and conditioning regimen (total body irradiation [TBI]–based). While the risk factors of EMR for ALL patients included hyperleukocytosis at diagnosis, adverse cytogenetics, and peripheral blood stem cell (PBSC) as stem cell source. 4. The prognosis of EMR of AL was poor. The 3-year overall survival (OS) of isolated EMR and EMR with concurrent BMR was 18.2% and 8.0% respectively. EMR of ALL is associated with better survival than that of AML.
Discussion: Compared with AML, ALL patients had a higher cumulative incidence of EMR post transplantation. PBSC as stem cell source was a independent risk factor for EMR in ALL patients but not in AML patients, this maybe suggested that the GVL effect was less effective in EM sites of ALL patients.
Disclosure of Interest: None Declared.

PH-P365

BUDGET IMPACT ANALYSIS OF INTRAVENOUS VERSUS ORAL BUSULFAN FOR CONDITIONING PRIOR TO HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ADULT PATIENTS – A GERMAN HEALTH CARE PROVIDERS' PERSPECTIVE

K. Christa Berger[1,], D. Schopohl[1], H. Ostermann[1]*
[1]Department of Haematology/Oncology, University Hospital of Munich, Munich, Germany

Introduction: Busulfan (BU) frequently used for conditioning prior to hematopoietic stem cell transplantation (HSCT) is available in an intravenous (IV) and an oral (O) formulation. IV-BU is advantageous due to controlled administration, exact dosing and reduced adverse events. So far, German economic data including the effects of IV-BU on costs of adverse events are not available. To determine the economic impact of IV-BU versus O-BU in adult patients undergoing HSCT from a German health care providers' perspective.
Materials (or patients) and Methods: A Budget-Impact Model (BIM) was developed with Microsoft Excel 2010®. Literature was searched for data comparing IV-BU versus O-BU (combination of BU with cyclophosphamide) regarding risks for oral mucositis (MUC), infection with MUC and hepatic veno-occlusive disease (HVOD). German cost data were taken from the literature and tariff lists. Sensitivity analyses were conducted assuming minus/plus 25% of risks of all adverse events for O-BU and IV-BU.
Results: Model calculations include costs for MUC Grade 1-2/3-4, for documented infection without/with MUC, for moderate and severe HVOD without/with multiorgan failure, and drug costs for conditioning with IV-BU or O-BU. Base case: cost-savings with IV-BU of € 584 per patient considering all adverse events and of € 207 per patient regarding HVOD only. Worst case scenario in the sensitivity analyses (minus 25% of adverse events' risks for O-BU, plus 25% for IV-BU): additional costs with IV-BU of € 2,757 per patient considering all adverse events and of € 2,307 per patient regarding HVOD only. Treatment of HVOD with defibrotide and treatment of multiorgan failure during severe HVOD are major cost-drivers during sensitivity analyses.
Discussion: IV-BU is dominant compared to O-BU considering the risk and subsequent treatment costs for MUC and HVOD. Additional costs of IV-BU with sensitivity analyses appear relatively modest in the context of HSCT. For a comprehensive economic evaluation additional evidence on clinical outcomes e.g. on the effects of IV-BU on graft-versus-host disease, mortality and patient reported outcomes are necessary
Disclosure of Interest: K. Berger Conflict with: Pierre Fabre, Germany, D. Schopohl: None Declared, H. Ostermann Conflict with: Pierre Fabre, Germany.

PH-P366

TREATMENT OF RELAPSED ACUTE LEUKEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION BY SALVAGE CHEMOTHERAPY WITH LOW-DOSE CYTARABINE AND ACLARUBICIN COMBINED WITH GRANULOCYTE COLONY-STIMULATING FACTOR PRIMING: MORE EFFECTIVE AND LESS TOXIC?

X. Tang[1,], H. Zhou[1], A. Sun[1], X. Zhu[1], D. Wu[1]*
[1]Hematology, The First Affiliated Hospital of Soochow University, Jiangsu Institute of Hematology, Suzhou, China

Introduction: Recurrence is a major cause of treatment failure after allogeneic hematopoietic stem cell transplantation (allo-HSCT) for acute leukemia, and subsequential treatment options are very limited. We evaluate the efficacy and toxicity of cytarabine and aclarubicin combined with granulocyte colony-stimulating factor priming (CAG regimen), consisting of concurrent use of granulocyte colony-stimulating factor (G-CSF) with low-dose cytarabine and aclarubicin, as a salvage therapy for acute leukemia patients who relapsed after allo-HSCT.
Materials (or patients) and Methods: Fifty-nine patients (32 male and 22 female) with acute leukemia, with a median age of 27 years, relapsed post allo-HSCT and received salvage chemotherapy. Twenty seven patients received CAG regimen while 32 patients received non-CAG regimen such as intensive chemotherapy.
Results: The overall response rate (ORR) of CAG and non-CAG groups were significant different (55.6% vs 28.1%, *P* = 0.033). With regard to disease type, comparing with non-CAG group, ORR of AML patients in CAG group was significantly higher (64.3% vs 26.7%, *P* = 0.025). However, ORR of acute lymphocytic leukemia (ALL) in CAG group was similar as that in non-CAG group (50% and 41.2%, respectively; *P* = 0.471). Median overall survival (OS) from the starting of CAG chemotherapy and 2-year OS of CAG group were 9 (1-27) months and 16.1%. Meanwhile, median OS and 2-year survival of non-CAG group were 4 (1-49) months and 8.8%. Moreover, the median duration of neutropenia and thrombocytopenia of CAG group were significantly shorter than that of non CAG group, 6 (1-12) vs 11 (5-28)days (*P*=0.000) and 8(1-14) vs 14 (7-35) days (*P*=0.000). For the patients who received donor lymphocyte infusion (DLI[JW1]) as a subsequential therapy, two-year OS of CAG and non-CAG group were 17.2% and 12.5%, respectively (p[JW2] =0.577). Treatment related mortality (TRM) was seen in 2 cases in CAG group compared with 10 cases in non-CAG group. For CAG group, impact on overall response rate was significantly associated with leukocyte level, and medullar blast percentage[JW3] at relapse (*P* = 0.005 and *P* = 0.000 respectively).

Furthermore, multivariate analysis showed response to chemotherapy was the only factor that correlated with better survival (*P*=0.032, HR 0.461, 95% CI (0.227, 0.937)).
Discussion: CAG regimen as a salvage chemotherapy for relapsed acute leukemia post allo-HSCT could effectively reduce tumor burden with mild toxicity, especially for hypoplastic acute leukemia patients. For certain relapsed acute leukemia patients post allo-HSCT, CAG regimen may be an optimal choice as the bridge therapy followed by DLI or second stem cell transplantation (SCT).
Disclosure of Interest: None Declared.

PH-P367
INCIDENCE AND EVOLUTION OF PTLD IN 1370 TRANSPLANTS BETWEEN 1998 AND 2013. SINGLE CENTER EXPERIENCE

E. Garcia Torres[1,], D. Buenasmañanas[1], G. Rodriguez[1], J. Serrano[1], R. Rojas[1], L. Quintana[1], A. Rodriguez Villa[1], C. Martin[1], J. R. Molina[1], P. Gomez[1], J. Sanchez García[1]*
[1]Hematology, University Hospital Reina Sofía, Córdoba, Spain

Introduction: Organ transplantation is an increasingly used medical procedure for treating otherwise fatal end stage organ diseases with 107,000 transplants performed worldwide in 2010. Post-transplant lymphoproliferative disorders (PTLDs) are serious, life-threatening complications of solid-organ transplantation (SOT) and bone marrow transplantation leading to a high mortality (30–60%). The incidence of PTLD, ranging from 1% to 20%, clearly relates to the type of transplanted organ, intensity of immunosuppression (IS), underlying disease, age, viral infections including EBV, cytomegalovirus and hepatitis C virus (HCV).
Materials (or patients) and Methods: A retrospective study of 48 patients diagnosed with PTLDs from 1998 to 2013, in our center. We analyzed the incidence, clinical features, and outcome in a 48 patients diagnosed with PTLDs in our unit between 1998 to 2013. The data analysis was performed with the SPSS 17.1 program.
Results: We analyzed 1340 transplants procedures performed in our Center during the study period. 48 patients (3.5%) developed PTLDs. The highest incidence 11% was reported in lung transplantation. A total of 48 subjects were analyzed in the study, 69% males, 31% females, 31% children. 27.1% (*n*=13) received pulmonary transplant, 20.8% (*n*=10) renal, 20.8% (*n*=10) hepatic, 16.7% (*n*=8) allogeneic bone marrow transplantation, 10.4% (*n*=5) heart, 2.1% (*n*=1) renal-heart and 2.1 (*n*=1) lever-renal. The median age at transplant was 36 years (1-65). The mean time between diagnosis and transplant was 34 months (2-180). 27% (n 13) developed PTLD early (<1 year). Histological classification and immunophenotype: 92% were CD20+ and 63% EBER+. The main subtype was monomorphic PTLDs (M-PTLD) (58%) and the most common was DLBCL (47%). 80% had EBV active infection (demonstrated by PCR) after transplantion. Calcineurin inhibitor (tacrolimus *n*= 34, 71%) was the main immunosuppression regimen used, either as monotherapy or with corticosteroids (50%, *n* = 24). After the diagnosis of PTLD 84% of patients reduced immunosuppression, and 31% (*n*=15) presented signs of transplanted organ. 69% of patients received specific PTLD treatment. Several therapeutic approaches were currently used. 50% received Rituximab, 27% chemotherapy with Rituximab, 16% surgery and 7% other treatment. Median follow-up was 18 months (0- 117meses). 1-year and 2-year was 56% and 50 ± 8% respectively. The response rate to standard therapy was 65%. The immunosuppression was discontinued in 29 patients and the survival was higher in this group (*P* = 0.008). The presence of M-PTLD and late-onset was associated with a worse prognostic, although it was not statistically significant (*P* = 0.56) (ns). We have not found any association between OS and the time to relapse, immunosuppression regimen, staging, presence of EBER+ or therapy administered. The use of Rituximab was associated with a higher OS, without statistical significance (ns).
The results are shown in the following table.
Discussion: In our study we didn´t find significant difference in OS between histological factors, staging, EBER + and treatment regimen used. The presence of M-PTLD and late-onset was associated with a worse prognostic and the use of Rituximab was associated with a higher OS, in both cases without statistical significance (ns). Discontinue immunosuppression was associated with a better overall survival (*P*=0.008).
Disclosure of Interest: None Declared.

PH-P368
AVASCULAR NECROSIS AFTER ALLOGENEIC TRANSPLANTATION HEMATOPOIETIC PROGENITORS: SINGLE-CENTER EXPERIENCE

A. Melero[1,], M. Blanquer[1], A. Sánchez-Salinas[1], J. M. Moraleda[1], J. Monserrat[1], M. J. Majado[1], V. Cabañas-Perianes[1]*
[1]Hematology, University Clinical Hospital Virgen de la Arrixaca, Murcia, Spain

Introduction: Fortunately, the number of long-term survivors after allogenic stem cell transplant (allo-SCT) is growing. Thus, attention increasingly focuses on late complications such as avascular necrosis (AVN). AVN affects negatively quality of life and requires hip replacement when evolves. The main factor risk described is post-SCT long-lasting high-dose steroids.
Materials (or patients) and Methods: We reviewed the number of patients diagnosed of symptomatic AVN after allo-SCT in our centre between 2008 to 2013. Disease, conditioning regimen, type of allogeneic transplant, acute and chronic graft versus host disease (GVHD) and time and dose steroids were analyzed.
In cases of GVHD, the initial dose prednisone was 1.5 mg/kg/24h. AVN was diagnosed by nuclear magnetic resonance imaging (MRI) following had joint pain complaints. Patients were treated with calcium, vitamin D, zoledronic acid and were assessed by the Traumatology Service for autologous bone marrow mononuclear cells therapy indication.
Results: 81 medical records were reviewed. The number of patients with symptomatic AVN was 5 (6.17%). All of them had acute GVHD which required treatment with high doses of corticosteroids.
Discussion: All patients with symptomatic AVN required post-SCT long-lasting high-dose steroids, fact that supports other publications that consider steroids the main risk factor. However, the incidence in our center has been lower than in other works.
Despite cell therapy is not the treatment of choice of AVN, in several published series and in our experience this treatment prevents the collapse of the femoral head in the early stages of AVN and avoids the need for inserting a prosthesis.
Disclosure of Interest: None Declared.

PH-P369
RISK SCORE FOR OUTCOME AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDHOOD

C. Arndt[1], I. Wolff[1], J. F. Beck[1], B. Gruhn[1,]*
[1]Department of Pediatrics, Jena University Hospital, Jena, Germany

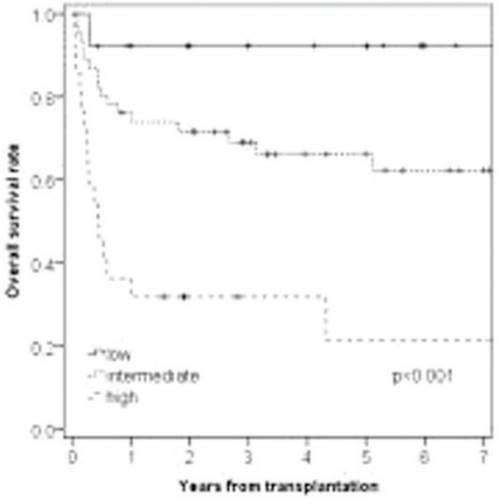
Introduction: The effects of certain risk factors on the survival of adults undergoing allogeneic hematopoietic stem cell transplantation (HSCT) have been the subject of research for many years. The impact of graft source, donor type, and iron parameters like ferritin has already been examined closely. However, observations of pediatric populations considering those factors remain rare and no score in this regard for children with HSCT is available yet.
Materials (or patients) and Methods: We retrospectively analyzed the effects of patient age, patient sex, disease risk, donor age, recipient-donor sex match status, donor HLA match, graft source as well as ferritin, albumin, total bilirubin, C-reactive protein (CRP), aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transpeptidase (GGT), cholinesterase (CHE), and lactate dehydrogenase (LDH) taken at the time of transplantation on the 5-year-overall survival of 132 children undergoing allogeneic HSCT between 2001 and 2011 in a single center. The graft source was either bone marrow (*n*=82) or peripheral blood stem cells (*n*=50). The patients had the following underlying diseases: ALL (*n*=44), AML (*n*=29), CML (*n*=5), myelodysplastic syndrome (*n*=16),

[PH-P368]

number of patients	Disease	conditioning regimen	type of allo-SCT	Prophylaxis GVHD	GVHDa: -localization -day post-SCT	Total dose (TD) prednisone/Kg and long-term treatment (days)	Time of ocurrence symptomatic AVN after trans-plant (months)	Localization of AVN	Cell therapy
1	Ewing sarcoma metastatic. Father haploidentical CD3/ CD19 depletion. Early failure marrow graft	ICT-Fludarabine-ATG	Non myelo-ablative HLA identical syster	CsA and MMF	-cutaneous grade 3 -+20 and +65	-TD 7132 mg -208 days	7	Right femoral head	No (1)
2	Limphoma lympho-blastic T stage IV-A	Flu-Bu-Thiotepa-MPD	Haploidentical CD3/CD19 depletion from her mother	CsA and MTX	-cutaneous grade 2 -+30	-TD 2025 mg. -60 days	15	Bilateral Knees	No (2)
3	Myelodysplastic syndrome high risk. HLA identical syster. Early relapse	ICT-Cy	Unrelated donor	CsA and MTX	cutaneous grade 3 -+30 and +200	-TD 5100 mg -100 days	24	Left femoral head	Yes
4	B acute lympho-blastic leukemia high risk	ICT-Cy	HLA identical sister	CsA and MTX	-cutaneus and G-I grade 3 -+32,+105 and +112	-TD 4192 mg -300 days	10	Left femoral head	No (2)
5	Aplastic anemia unresponsive inmunosupressive treatment	Flu-Cy-ATG	HLA identical sister	CsA and MMF	-G-I grade 2 - +70 and +148	-DT 5880 mg -240 days	7 and 50	Bilateral femoral head Left humeral head	Yes (bilateral femoral head). No (1; humeral head)

1. Traumatology evaluation is pending. 2. Established AVN.

non-Hodgkin lymphoma (*n*=7), solid tumor (*n*=4), severe aplastic anemia (*n*=7), myelofibrosis (*n*=2) and genetic disease (*n*=18). Conditioning regimen was myeloablative in all cases. The disease risk was formed by dividing the patients into two groups according to their clinical risk. Patients with genetic disease, severe aplastic anemia, refractory cytopenia, myelofibrosis, leukemia and lymphoma in first or second remission as well as chronic myeloid leukemia in chronic phase were low risk, while patients with solid tumor, advanced myelodysplastic syndrome as well as leukemia and lymphoma in more than second remission or in relapse were high risk. For statistics we used Kaplan-Meier-method for univariate analysis and Cox regression for multivariate analysis.



Results: In univariate analysis, 5-year-overall survival decreased significantly in patients with high disease risk (38.3% versus 74.7%, *P*<0.001), peripheral blood stem cells as graft source (47.1% versus 72.2% for bone marrow, *P*=0.001) as well as in patients with ferritin >1500 µg/L (40.8% versus 78.8%, *P*<0.001), CRP >10 mg/L (54.6% versus 69.4%, *P*=0.017), LDH >6 µmol/L·s (22.2% versus 66.8%, *P*=0.001), GGT >1 µmol/L·s (43.2% versus 67.9%, *P*=0.032) and CHE <60 µmol/L·s (35.7% versus 70%, *P*=0.002). Other factors did not show a significant correlation. We subsequently developed a score of those parameters that were significant in multivariate analysis, i.e. disease risk (HR=3.744, *P*=0.035), ferritin (HR=6.860, *P*=0.002) and CHE (HR=4.556, *P*=0.043), dividing the patient population into three groups: low with no risk factor, intermediate with one risk factor and high with two or three risk factors. For this score we found a 5-year-overall survival of 92.3% for the low risk group, 66.2% for the intermediate risk group and 17.4% for high risk group (*P*<0.001).

Discussion: Our data show that disease risk, ferritin, and CHE are factors that decisively influence the prognosis after allogeneic HSCT in children. They should be evaluated in further trials as well as our proposed risk score. The characteristics that showed up significant in univariate but not in multivariate analysis appear to have an influence as well and might show a stronger correlation in larger trials.

Disclosure of Interest: None Declared.

PH-P370

IMPACT OF GLUTATHİONE S-TRANSFERASE AND CYTOCHROME P450 POLYMORPHİSMS ON CLINICAL OUTCOME AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TANSPLANTATION

H. Ugur Bozbey[1], S. Kalayoglu-Besisik[1,], S. Usta Akgul[2], F. Oguz[2], D. Sargın[1]*

[1]Internal Medicine, Hematology, [2]Medical Biology, Istanbul University, Istanbul Medical Faculty, Istanbul, Turkey

Introduction: It is important to prevent the formation of free radicals formed as consequence of inflammatory processes, directly or indirectly, in patients undergoing stem cell transplantation. Glutation-S-Transpherase (GST) and Cytochrome P-450 (CYT P-450) enzymes are detoxification enzymes that play a role in and are responsible for reduction of glutation by conjugating reactive chemicals. In this way, in the future it seems to be possible to make a prediction in terms of preventing possible complications by evaluation of gene polymorphism. We aimed to study the relationship between GST and CYT P-450 gene polymorphisms, and sinusoidal obstruction syndrome (SOS), mucositis, liver toxicity, febrile neutropenia, graft versus host disease (GVHD) and transplant related mortality, which are tightly related to the clinical course after HLA matched allogeneic bone marrow transplantation.

Materials (or patients) and Methods: We included 62 allogeneic stem cell transplanted patients and their healthy donors as controls group in our study. Polymerase chain reactions were used to detect GSTM1 and GSTT1 polymorphisms, whereas PCR-RFLP (restriction fragment length polymorphism), for GSTP1 polymorphism and CYP1A2(C734A)(intron 1) and CYP2E1(5'-).

All major transplant related complications were recorded.

Results: Compared to GSTM1(-) polymorphisim, patients with GSTM1(+) polymorphmisim were mostly in remission at transplantion (P= 0,001). Among early complications such as SOS, mucositis, liver toxicity, febrile neutropenia, GVHD there was no significant difference between GSTM1, GSTT1 null genotype, GSTP1 and CYP1A2, CYP2E1 mutant allel (P>0.005). There was a trend to increase for SOS in patients with GSTT1 (-) genotype and CYP12A C which was not found statistically significant. In patients with GSTM1 (+) genotype severe GVHD (≥2 grade) seemed to be lower but statistically not significant. In addition, a numerical but not statistical significant association was between graft failure and GSTM1 (-) GSTT1 (-) genotype. On the other hand, patients with GSTP1 GG genotype seemed to experience more severe GVHD compared to the patients with GSTP1 AA genotype which could not documented as statistically significant. Similar status was for patients with CYP1A2 C allel compared to CYP1A2 AA wild genotype.

Discussion: GSTA1, GSTP1, GSTM1 and CYT P-450 genotyping prior to allogeneic hematopoetic stem cell transplantation may allow better prediction of the outcome and the need for intervention as thereby improving clinical outcome.

Disclosure of Interest: None Declared.

PH-P371

CARDIOVASCULAR RISK FACTORS AND BODY HABITUS IN LONG TERM SURVIVORS OF STEM CELL TRANSPLANTATION

T. Erblich[1,], J. Apperley[2], J. Todd[2], N. Salooja[2]*

[1]Endocrinology, Hammersmith hospital, [2]Haematology, Hammersmith hospital, Imperial, London, United Kingdom

Introduction: Following stem cell transplantation (SCT) patients are at increased risk of accumulating cardiovascular risk factors (CVRF) and developing a metabolic syndrome compared to the normal population. These parameters are associated with an increase risk of cardiovascular death. In the general population there is an association between the metabolic syndrome and obesity and the latter has also been implicated as a risk factor for cardiovascular complications after transplantation. In the UK the prevalence of obesity is relatively high with 2010 data from NHS National Statistics classifying 26% of adults over the age of 16 as obese. It is our impression, however, that the majority of our patients post SCT are non-obese despite their tendency to accumulate CVRF.

Materials (or patients) and Methods: In this retrospective study we have reviewed case note data from all patients attending a monthly dedicated clinic for long term (>10 years) survivors of SCT over the time course of one year (Jan–Dec 2013). Data was collected on the frequency of dyslipidaemia, hypertension, diabetes or overt ischaemic cardiac disease. In addition indicators of obesity were collected including body mass index (BMI), and waist-hip ratios. Obesity was indicated by BMI>30 kg/m^2 and overweight defined as a BMI of 25-29 kg/m^2. A waist-hip ratio >1 was considered abnormal.

Results: 53 patients (29 male) were evaluated a median of 21 years (range 10.9-34y) post SCT. The sources of stem cells were sibling (n=37), unrelated (n=12), haplo-identical (n=1) and syngeneic (n=3). Underlying disease was as follows: CML n=42, AML n=5. ALL n=3, AA n=3.

One patient had overt ischaemic cardiac disease and a further 30/53 patients had at least one cardiovascular risk factor (CVRF). Fifteen had one CVRF (11 dyslipidaemia, 4 hypertension), ten had two CVRF (8 dyslipidaemia plus hypertension) and five had 3 CVRF (dyslipidaemia, hypertension and diabetes).

Data on BMI was available for 45/53 (85%) with 5/45 (11%) defined as obese and 14 (31%) overweight. These data are lower than for the normal population. 39/53 (74%) had waist/hip ratios measurements. and7/39 (18%) had abdominal obesity. The relationship between these parameters and cardiovascular risk factors is shown in Table 1.

Table 1: Association of indicators of obesity with number of cardiovascular risk factors (CVRF)

	Obese (BMI>30)	Overweight (BMI 25-29)	Median BMI (range)	Median Waist/height (range)	Waist/height>1
No CVRF N=21 data on 15	none	30% n=5	22.7 (17.9-25.5)	0.9 (0.78-1.2)	7%
1 CVRF N=16 Data on 14 for BMI Data on 11 for W/H	14% n=2	43% n=6	24.5 (19-31)	0.9 (0.8- 1.1)	9%
2 CVRF N=10 Data on 9 for BMI 7 W/H	22% n=2	67% n=5	26.6 (20.7 – 31.6)	0.9 (0.83-1.1)	28.5%
3 CVRF N=5 Data on 4	25% n=1	75% n=3	27.3 (23.6-31.1)	1.05 (0.9-1.2)	50%

Discussion: The prevalence of cardiovascular risk factors was high at 53%. Despite this the prevalence of obesity was approximately half that of the general adult population. Data in Table 1 does indicate a relationship between BMI, waist –hip ratios and the number of CVRF. Although the numbers are small the percentage of patients who are obese, overweight or have a raised waist-hip ratio are increased in patients with 3 CVRF compared to those with less. These data suggest that recommendations for ideal weight may be different in the post-transplant population compared to the normal population.
Disclosure of Interest: None Declared.

PH-P372
AGE RELATED INCREASE IN THE INCIDENCE OF SECOND SOLID MALIGNANCY (SSM) AFTER HAEMATOPOETIC STEM CELL TRANSPLANTATION

J. Murray[1,], J. Tomlins[1], A. Leather[1], M. Dennis[1], J. Cavet[1], T. Somervaille[1], A. Bloor[1], S. Kulkarni[1]*
[1]Haematology, The Christie NHS Foundation Trust, Manchester, United Kingdom

Introduction: As the population of long term transplant survivors is increasing, long term complications and quality of life are becoming increasingly important issues. Risk of developing second solid tumours is higher in the long term survivors. This analysis was carried out to identify the risk in a single centre.
Materials (or patients) and Methods: From February 1973 to November 2013, 1983 patients (median age: 45yr., range: 14-76 yr.; M:1259, F:724) received stem cell transplants for haematological malignancies (Ac. Leuk: 507, Chr. Leuk: 97, lymphoma:645, myeloma:621, solid tumours:113). Donor was allogeneic (*n*=528) or autologous (*n*=1455) and conditioning was with (*n*=556) or without TBI (*n*=1427). Donor was sibling (*n*=302), matched unrelated (*n*=220) or cord blood (*n*=6). Source of stem cell was marrow (*n*=322), PBSC (*n*=1627), both (*n*=28) or cord blood (*n*=6). GVH prophylaxis included Campath in 203 cases. Of all the patients 1774 received single transplant but 209 received more than one transplant. Data was analysed as of 01/12/2013 using competing risk methods with death as competing risk for developing second cancers.
Results: Patients follow-up was more than 10 years in 382 cases (19%), between 5 to 10 years in 328 (17%), 1 to 5 years in 667 (34%) and less than 1 year in 606 cases (31%). Second solid cancers developed in 70 patients with the incidence of 1% at 5yr (95% CI: 0.5-1.6), 2.2% at 10 yr (95% CI: 1.6-3.3),4.8% at 15yr (95%CI: 3.6-6.8) and 8% (95% CI: 5.9-10.5)at 20 years. Site of second malignancy was brain (*n*=2), breast (*n*=15), cervix (*n*=3), GIT (*n*=11), genitourinary (*n*=9), lung (*n*=3), skin (*n*=17), head & neck (*n*=7), thyroid (*n*=3), non EBV related lymphoma (*n*=3). In univariate analysis 10 yr. probability of developing SMN was not influenced by gender, stage of disease, primary diagnosis, type of HSCT, use of TBI, type of donor or year of transplant. It was significantly higher with use of PBSC (1.4% vs. 2.6%, *P*=0.02) and age above 65yr. (1.5% vs. 11%, *P*=0.001). In multi-variate analysis age above 65yr. (RR: 1.8, 95% CI: 1.1-2.9, *P*=0.02) and PBSC (RR:9.4, 95% CI:1-99, *P*=0.05) were independently associated with increased risk of SMN. 19 patients have died due to SMN (27%) and significantly shorter with Gi, genitourinary and lung cancers. Analysis will be extended to identify the role of gvhd, TBI dose, fractionation and use of additional radiotherapy on the incidence of organ specific second solid malignancies.
Discussion: This single centre analysis shows that the risk of developing SMN increases with longer follow-up and the survival is poor. Long term survivors of stem cell transplants need follow-up probably for life in speciality clinics. Early detection, surveillance and advice regarding avoidance of known carcinogens should be encouraged through patient education.
Disclosure of Interest: None Declared.

Graft-versus-Host Disease – Clinical

PH-P373
PROMISING OUTCOMES FROM INTRA-ARTERIAL STEROID INFUSIONS IN PATIENTS WITH TREATMENT-RESISTANT ACUTE GASTROINTESTINAL GRAFT-VERSUS-HOST DISEASE

M. Nishimoto[1,], H. Koh[1], H. Nakamae[1], A. Hirose[1], M. Nakamae[1], T. Nakane[1], Y. Hayashi[1], H. Okamura[1], T. Yoshimura[1], S. Koh[1], S. Nanno[1], Y. Nakashima[1], T. Takeshita[2], A. Yamamoto[2], Y. Sakai[2], N. Nishida[2], T. Matsuoka[2], Y. Miki[2], M. Hino[1]*
[1]Hematology, [2]Department of Radiology, Graduate School of Medicine, Osaka City University, Osaka, Japan

Introduction: Acute graft-versus-host disease (aGVHD) remains the major cause of non-relapse mortality (NRM) following allogeneic hematopoietic cell transplantation. The prognosis of patients with steroid-refractory aGVHD is very poor, although several salvage treatments such as mycophenorate mofetil (MMF) and antithymocyte globulin have been tried.
It is speculated that a high topical concentration of corticosteroids for gastrointestinal (GI) GVHD may enhance the effect and overcome refractoriness caused by down-regulated steroid receptor as occurs in cases of ulcerative colitis. Two prospective studies reported the efficacy and safety of intra-arterial steroid infusions (IASI) for severe GI GVHD (Shapira *et al*, 2002; Weintraub *et al*. 2010). However, despite promising responses seen in these studies, it is unclear whether or not IASI improves survival. We therefore prospectively examined the efficacy and safety of IASI, and compared the outcomes with those of historical controls.
Materials (or patients) and Methods: We assessed consecutive patients with hematological disorders who developed systemic steroid-therapy refractory acute GI GVHD and who had signed written informed consent at our institution between 2008 and 2012. Patients with GVHD involving multiple organs were excluded. The control group consisted of 14 consecutive patients between 2001 and 2008 who had received second-line treatment including increased dose of steroids, MMF and infliximab. The primary endpoint was set as treatment response rate of aGVHD at day 28. Enrolled patients were treated with infusions of 2 mg/kg methylprednisolone into the superior and inferior mesenteric arteries for lower GI GVHD, and/or 1 mg/kg methylprednisolone into gastroduodenal and left gastric arteries for upper GI GVHD.
Results: A total of 19 transplant subjects aged 31-67 years (median 52) were enrolled. Seventeen patients received myeloablative conditioning. Donor sources consisted of HLA-matched related peripheral blood (rPB) (*n*=2), haploidentical rPB (*n*=6), unrelated bone marrow (*n*=8) and cord blood (*n*=3). Of 18 evaluable subjects, fourteen (78%) showed an overall response at 28 days, twelve (67%) achieved a complete response and two (11%) achieved partial response after a median of one IASI (range 1μ4). With a median follow-up of 23 months (range 7μ56), 1-year NRM was significantly lower and 1-year OS tended to be higher in the study group than in controls (11% versus 50%, Gray's test, *P*=0.046; 67% versus 36%, Log-rank test, *P*=0.106, respectively). There were no serious complications related to IASI.
Discussion: Consistent with previous reports, promising responses were also observed in this study. Of note, our data suggests the possibility that IASI can improve OS via a decrease of NRM in patients with systemic steroid-therapy refractory acute GI GVHD. The favorable responses to GVHD without worsening infection-related complications might contribute to a decrease of NRM since IASI might enhance response to GVHD and prevent untoward effects related to systemic immunosuppressive treatment. In the future, a larger adequately powered prospective study will be required to validate our results.
Disclosure of Interest: M. Nishimoto: None Declared, H. Koh Conflict with: Pfizer, H. Nakamae Conflict with: Pfizer, A. Hirose: None Declared, M. Nakamae: None Declared, T. Nakane: None Declared, Y. Hayashi: None Declared, H. Okamura: None Declared, T. Yoshimura: None Declared, S. Koh: None Declared, S. Nanno:

None Declared, Y. Nakashima: None Declared, T. Takeshita: None Declared, A. Yamamoto: None Declared, Y. Sakai: None Declared, N. Nishida: None Declared, T. Matsuoka: None Declared, Y. Miki: None Declared, M. Hino Conflict with: Pfizer.

PH-P374

GALECTIN-1 AS A NOVEL PROGNOSTIC BIOMARKER IN HAEMATOLOGIC MALIGNANCIES TREATED WITH NON-MYELOABLATIVE HAEMOPOIETIC STEM CELL TRANSPLANTATION

I. Petruskevicius[1,], M. Ludvigsen[2], P. Martin Hjornet Kamper[1], R. Møller Hjortebjerg[3], B. Samuelsen Sørensen[4], B. Nielsen[1], B. Honoré[2], F. Annibale d´Amore[1]*

[1]Haematology, Aarhus University Hospital, [2]Biomedicine, Aarhus University, [3]Clinical Medicine, [4]Immunology, Aarhus University Hospital, Aarhus, Denmark

Introduction: Galectin-1 (Gal-1), a glycan-binding protein belonging to the growing family of animal lectins has an important role in immune cell activation, differentiation and homeostasis. Multiple experimental models have shown the role of Gal-1 in suppressing chronic inflammation and autoimmunity. Gal-1 therapy demonstrated immunoregulatory properties in a murine model of graft-versus-host disease (GvHD). However, little is known about the possible impact of Gal-1 on outcome and occurrence of graft-versus-host disease in humans receiving non-myeloablative haemopoietic stem cell transplantation (NM-HSCT).

Materials (or patients) and Methods: The purpose of this study was to investigate the impact of Gal-1 serum levels measured before NM-HSCT on i) incidence and severity grade of acute and chronic GvHD, ii) progression free (PFS) and overall survival (OS), iii) transplant related mortality (TRM) in patients with different haematologic malignancies (HM).

Fifty-eight patients with haematologic malignancies treated with NM-HSCT between Mar 2009 and Dec 2012, were included. Median age was 57 (range 17-71). There were 37 males (64%) and 21 females (36%). A total of 35 patients (60%) were treated for myeloid and 23 (40%) for lymphoid malignancies. All patients were conditioned with fludarabine 90 mg/m^2 and total body irradiation (TBI) 2-4 Gy. Postgrafting immunosuppression consisted of calcineurin inhibitor and mycophenolate mofetil. Seventeen patients (30.3%) had HLA identical sibling donors, 35 (60.3%) matched unrelated donors (10/10 match) and 6 (10.3%) mismatched unrelated donors (9/10 match). Peripheral blood haematopoietic stem cells mobilised with granulocyte-colony stimulating factor (G-CSF) were used as stem cell source. The median follow-up was 428 days (range 73-1238).

Acute GvHD (aGvHD) was defined as a complex of symptoms occurring within 100 days after transplantation and graded according to international consensus criteria.

GvHD developing after day 100 was considered as chronic GvHD (cGvHD) and graded as (i) none/not needing systemic therapy (NNST) or (ii) needing systemic therapy (NST).

Serum samples frozen at the time of tissue typing before NM-HSCT were used to perform Gal-1 analysis. Serum galectin-1 levels were assessed according to a standard time-resolved immunofluorometric assay (TRIFMA) protocol.

Results: High levels – defined as the 50% above the median level - of Gal-1 correlated with a higher incidence of cGvHD NST. After 8 months the incidence of cGvHD in patients with low vs. high Gal-1 levels was 57.5 % (95 % CI: 38.7-76.3) and 86.1 %, (95 % CI: 71.3-1.00), respectively (P=0.019). High levels of Gal-1 seemed also associated with improved OS, HR 0.40 (0.15-1.00), P=0.051. The 1-year TRM was lower in patients with high vs. low Gal-1 levels, i.e. 5.9 % (95 % CI:-0.7-19.0) vs. 33 % (95 % CI: 14.8-51.3), respectively (P=0.018). In a multivariate model adjusting for the type of donor (unrelated vs. sibling), the association between high levels of Gal-1 and a favourable impact on OS (P=0.003) and PFS (P=0.032) was further enhanced. There were no correlations between Gal-1 levels and aGvHD (P=0.422).

Discussion: Gal-1 is a biomarker able to predict (i) incidence of cGvHD, (ii) outcome and (iii) risk of TRM in patients with HM undergoing NM-HSCT. Confirmation of these findings in an independent data set is warranted.

Disclosure of Interest: None Declared.

PH-P375

COMPARISON OF ALARM RATES, TREATMENT TIMES, AND BUFFY COAT COLLECTIONS OBTAINED WITH SOFTWARE VERSION 4.1 VERSUS 3.0 OF THE THERAKOS® CELLEX® PHOTOPHERESIS SYSTEM, WHEN USED TO TREAT ADULT AND PAEDIATRIC PATIENTS WITH CHRONIC GRAFT-VERSUS-HOST DISEASE

H. Denney[1,], R. Whittle[1], M. Foster[1], T. Maher[2], R. Goodgrove[2], L. Robertson[2], C. Swift[2], J. Mayo[2], F. Hammerton[2], A. Alfred[1], P. Taylor[1]*

[1]Haematology/Photopheresis, [2]Photopheresis, Rotherham NHS Foundation Trust, Rotherham, United Kingdom

Introduction: Extracorporeal photopheresis (ECP) Software Version 4.1 replaced V3.0 in 2012 for the THERAKOS® CELLEX® Photopheresis System Instrument, with the introduction of variable centrifuge speeds that adjust to the collect flow rate, aimed at reducing heat build-up in the centrifuge chamber, and improved temperature monitoring. We compared the buffy coat collections, alarm rates and durations of treatments performed using CELLEX® instruments running V4 software with those that used V3.

Materials (or patients) and Methods: ECP was performed on 2 consecutive days for each treatment cycle. 196 V3 treatment cycles (from 54 adults and 10 children) in 3 months were compared with 192 V4 cycles (from 61 adults and 9 children) conducted a year later. Treatments utilising a blood prime (BP) were analysed as a separate group. BPs were used with the majority of the paediatric treatments, and infrequently with 4 adults. Analyses of treatment times were split into single- and double-needle modes. A separate analysis was done of 23 adults who received 1 treatment cycle that included a different software version on Day 1 to Day 2, to enable a direct comparison of V3 and V4. Total white blood cell (WBC) counts and cell differentials were recorded from samples taken from the cellular harvest obtained during treatment, and used to calculate the cell dose returned to the patient.

Results: Treatments in single-needle mode without a BP took significantly longer using software V4 than V3 (P<0.0001, V3 n=202 treatments, mea$n$=136 min; V4 n=210, mea$n$=146 min), and for double-needle mode treatments with a BP (P<0.0001, V3 n=60, mea$n$=137 min; V4 n=37, mea$n$=158 min). No significant difference was found in treatment times when double-needle mode was used without a BP (V3 n=95, mea$n$=115 min; V4 n=96, mea$n$=117 min). No difference in treatment duration was found for the 23 adults who received paired V3 and V4 treatments. No significant difference was found in WBC, neutrophil, lymphocyte, or monocyte cell doses in any of the groups. The frequency of red cell pump alarms was significantly lower with V4 than with V3, in both the BP group (P<0.0022, 26% vs 58%, V3 n=62, V4 n=38), and the non-BP group (P<0.0004, 7.4% vs 16.4% V3 n=317, V4 n=338). The frequency of system pressure alarms was also significantly lower with V4 than with V3 in the non-BP group (P<0.0001, 1.2% vs 11.6%). No significant differences were found between alarm rates in the paired treatment group, but there was a trend for fewer alarms with V4. Interestingly, a significantly higher bag collection volume was observed with V4 than with V3, for the BP, and non-BP, treatments (P<0.0001).

Discussion: We found that when using the CELLEX® device with V4 software, the incidence of red cell pump and system pressure alarms was reduced compared to with V3, during the 3 month periods examined. The improved regulation of flow rates may account for this, and the variation of the centrifuge speed as a function of the flow rate may prevent packing of red blood cells which can cause alarms during treatments with a BP. Treatment durations were longer with V4, which may result from the increased frequency of pauses observed during treatment. The cellular harvest returned to the patient appears to be unaffected by the software upgrade.

Disclosure of Interest: None Declared.

PH-P376

ADOPTIVE TRANSFER OF ALLOGENEIC REGULATORY T CELLS INTO PATIENTS WITH CHRONIC GRAFT-VERSUS-HOST DISEASE

S. Tuve[1,], A. Theil[2], U. Oelschlägel[1], A. Maiwald[1], D. Döhler[1], D. Ossmann[1], J. M. Middeke[1], N. Shayegi[1], K. Trautmann-Grill[1], M. von Bonin[1], U. Platzbecker[1], J. Schetelig[1], G. Ehninger[1], E. Bonifacio[2], M. Bornhäuser[1]*

[1]Medical Clinic I, University Hospital Dresden, [2]Center for Regenerative Therapies, Technische Universität Dresden, Dresden, Germany

Introduction: Treatment of chronic graft-versus-host disease (cGvHD) after allogeneic hematopoietic blood stem cell transplantation (HCT) remains a challenge. Mouse models indicate that adoptive transfer of regulatory T cells (Treg) may suppress GvHD while preserving graft-versus-leukemia (GvL) reactions. In this study we aimed to develop a protocol for the efficient isolation and *in vitro* expansion of regulatory T cells and to conduct the first trial testing the toxicity and therapeutic efficacy of Treg-infusion in five patients with otherwise treatment-refractory cGvHD.

Materials (or patients) and Methods: Allogeneic Tregs were isolated from unstimulated leukapheresis products of the corresponding HLA-matched donors by Ficoll density centrifugation following magnetic activated bead sorting (MACS). To increase the amount and purity regulatory T cells were cultivated in the presence of rapamycin, IL-2 and anti-CD3/anti-CD28 beads for 7–12 days. Purity and functionality of Tregs was assessed during the purification and expansion process using suppression assays and flow cytometry. Tregs were infused after a median time of 35 month (range 26-34month) after HCT. The kinetic and suppressive capacity of regulatory T cells in the peripheral blood was monitored weekly after adoptive transfer using flow cytometry and clinical grading of GvHD organ manifestations. 3/5 patients received low-dose IL-2 for 8 weeks after Treg infusion in order to support Treg expansion.

Results: Final products contained regulatory T cells with a mean purity of 84.7% (of total cells; range: 77.7% - 94%) and a mean quantity of $2.4x10^6$ Tregs per kg BW (range: $0.52x10^6$ – $4.45 x10^6$). All isolated cell products showed *in vitro* suppressive activity. Transfusion was well tolerated by all patients. Upon transfusion two of five patients showed a clinical response with improvement of cGvHD symptoms. The other three patients showed stable cGvHD symptoms for up to 21 month. In 3/5 patients immunosuppressive treatment could be reduced. Increased counts of Tregs were detectable in 4/5 patients upon Treg infusion. Suppression of activation marker expression on CD8 T cells was observed in 3 of 5 patients. Transfusion was well tolerated by all patients. With a median follow up time of 19 month after infusion no relaps of hematologic malignancy occurred. However, one patient developed malignant melanoma and another patient Bowen´s skin cancer 4 month and 11 month after Treg infusion, respectively.

Discussion: This data show (i) a feasible and reproducible approach of isolating functional Tregs in high quantity and purity for clinical application and (ii) opportunities and risks of adoptive Treg transfer into patients with chronic GvHD.

Disclosure of Interest: None Declared.

PH-P377

HAPLOIDENTICAL STEM CELL TRANSPLANTATION (HAPLO-HSCT) WITH HIGH DOSE CYLOPHOSPHAMIDE POST-TRANSPLANT (PT-CY) AS GVHD PROPHYLAXIS IN HIGH RISK HEMATOLOGIC MALIGNANCIES: MULTICENTRIC SPANISH EXPERIENCE

J. Gayoso[1,], P. Balsalobre[1], M. Kwon[1], M. Jesús Pascual[2], C. Castilla[3], D. Serrano[1], L. López-Corral[4], J. Antonio Pérez-Simón[5], A. Bermúdez[6], M. Rovira[7], A. Pérez-Corral[1], J. Anguita[1], I. Heras[3], C. Solano[8], A. Figuera[9], M. Antonia Sampol[10], C. Ferrá[11], P. Herrera[12], M. Sonia González[13], P. Montesinos[14], I. Buño[1], J. Luis Díez-Martín[1] and on behalf of GETH (Grupo Español de Trasplante Hematopoyético)*

[1]Hematology, HGU Gregorio Marañón, Madrid, [2]Hematology, HU Carlos Haya, Málaga, [3]Hematology, HU Morales Messeguer, Murcia, [4]Hematology, HU Clínico, Salamanca, [5]Hematology, HU Virgen del Rocío, Sevilla, [6]Hematology, HU Marqués de Valdecilla, Santander, [7]Hematology, HU Clínic, Bacelona, [8]Hematology, HU Clínico, Valencia, [9]Hematology, HU La Princesa, Madrid, [10]Hematology, HU Son Espasses, Palma de Mallorca, [11]Hematology, HU Can Ruti, Badalona, [12]Hematology, HU Ramón y Cajal, Madrid, [13]Hematology, HU Santiago, Santiago de Compostela, [14]Hematology, HU La Fé, Valencia, Spain

Introduction: Allogeneic transplantation is the only curative option for patients with high risk hematologic malignancies. Only one third of them have an HLA identical donor and around 60-70% will find an unrelated donor, that´s why HAPLO-HSCT offers a therapeutic option to most of these patients with the advantages of quick availability, easy programation and logistics, and a committed donor.

Materials (or patients) and Methods: We retrospectively evaluate the results of HAPLO-HSCT with reduced conditioning or myeloablative regimens and GVHD prophylaxis based on PT-CY (50 mg/kg on days +3 and +4) and a calcineurin inhibitor plus mycophenolate from day +5 performed in GETH centers.

Results: From Dec-2007, 80 HAPLO-HSCT have been done in 14 centers. Median age was 37 years (16-66), 67.5% were males and all were in advanced phases of their disease or presented high risk features (29 Hodgkin´s, 22 AML, 9 ALL, 8 MDS, 5 NHL, 4 myeloma and 2 myelofibrosis). Previous HSCT has been employed in 65%, autologous in 38 and allogeneic in 15 (5 siblings, 3 unrelated and 7 cord blood transplants), and in 35% the HAPLO-HSCT was their first transplant. Disease status at HAPLO-HSCT was CR in 45%, with persistent disease in 55%. Bone marrow was the graft source for 51% and peripheral blood for 49%, non T-cell depleted in all cases. The haploidentical donor was the patient´s mother (21), father (7), brother/sister (35) or offspring (17). Non-myeloablative conditioning was employed in 77.5% and myeloblative in 22.5%. Median neutrophils engraftment was reached at day +18 (13-45) and platelets >50K at day +27 (11-150). Main toxic complications were grade II-III mucositis in 36%, febrile neutropenia in 75% and CMV reactivations in 62%, with a transplant related mortality rate of 12.5% at day +100 and 19% at 6 months post-transplant. Acute GVHD grade II-IV affected to 24/73 patients at risk (33%), with grade III-IV in 10/73 (14%). Chronic GVHD was present in 12/51 (24%), being extensive in 6/51 (12%). After a median follow-up of 9 months (0.3-49), 26/80 patients have died due to relapse in 13, infections in 10 and GVHD in 3 cases. Event-free survival and overall survival at 1 year were 48% and 60% respectively. Immune reconstitution was fast and complete in those evaluated.

Discussion: HAPLO-HSCT with PT-CY is a useful tool in the treatment of high risk hematologic malignancies, rendering long-lasting remissions with limited toxicity, low GVHD incidence and early immune reconstitution.

Disclosure of Interest: None Declared.

PH-P378

RISK FACTORS FOR STEROID-REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FROM MATCHED RELATED OR UNRELATED DONORS

C. Calmettes[1,], S. Vigouroux[1], R. Tabrizi[1], A. Pigneux[1,2], T. Leguay[1], K. Bouabdallah[1], M.-S. Dilhuydy[1], C. Duclos[1], A. Lascaux[1], P.-Y. Dumas[1], S. Dimicoli-Salazar[1], G. Marit[1,2], N. Milpied[1,2]*

[1]Hematologie et thérapie cellulaire, CHU Bordeaux, [2]Université Bordeaux Segalen, BORDEAUX, France

Introduction: The standard risk factors for acute graft-versus-host disease (aGVHD) after allogeneic stem cell transplantation (allo-SCT) from related or unrelated donors are well defined and include HLA mismatch or unrelated donor, older recipient age, and female donor for male recipient (FM). The steroid-refractory (SR) forms of aGVHD are important to consider because they often have a major deleterious impact on transplant outcome. Unfortunately, the specific risk factors for SR aGVHD are less clearly defined. To characterize these risk factors after allo-SCT from matched related or unrelated donors, we undertook a retrospective analysis of adult patients transplanted at our center between 01/01/2000 and 12/31/2012.

Materials (or patients) and Methods: Steroid-refractory aGVHD was defined as aGVHD progressing after 3 days of treatment, or unchanged after 7 days, or in incomplete response after 14 days. GVHD occurring after donor lymphocytes infusion were excluded. Cumulative incidences (CI) were used for SR aGVHD in a competing risk setting with death as a competing event. The Gray test was used to compare CI curves.

Results: Six hundred and thirty four patients were identified and included in the present study. The median age was 50 years (18-67). Diseases were AML (*n*=230), ALL (*n*=104), myeloma (*n*=80), NHL (*n*=74), Hodgkin's disease (*n*=18), MDS (*n*=47), CLL (*n*=29), CML (*n*=18), aplastic anemia (*n*=19), and MPS (*n*=15). Status at transplant were CR1 or PR1 or chronic phase (*n*=260), > CR1 or PR1 (*n*=237), refractory (*n*=101), or untreated (*n*=36). Conditioning regimens were RIC (*n*=405) or MAC (*n*=229). Rabbit ATG was administered to 327 patients, of whom 298 received a RIC regimen. Donors were MRD (*n*=360) or MUD (*n*=274). Sources of stem cells were PB (*n*=452), BM (*n*=177), missing data (*n*=5). The prophylaxis of GVHD was CsA+metho for 339 patients.

In the whole population, 71 patients presented a SR aGVHD at a median time of 29 days (8-137) after transplant, representing a CI of 11.2% ± 1.2%. Their OS at 1 year post-transplant was 27% ± 5%. In univariate analysis, the risk factors for SR aGVHD were MAC (*P*=0.02), MUD (*P*=0.02), no ATG (*P*=0.01), and a trend for FM (*P*=0.07). Other variables considered in univariate analysis were recipient age (*P*=0.6), female *vs* male donor (*P*=0.6), recipient CMV status (*P*=0.7), status at transplant (*P*=0.2), PB *vs* BM graft (*P*=0.9), number of CD34+ cells (*P*=0.4), and GVHD prophylaxis (*P*=0.6). In multivariate analysis, the risk factors for SR aGVHD were MUD (HR=2.5, 95%CI: 1.5-4.1, *P*=0.0003), FM (HR=2, 95%CI: 1.2-3.4, *P*=0.008), and no ATG (HR=2.1, 95%CI: 1.3-3.4, *P*=0.002). Patients were then divided into 3 groups. The CI of SR aGVHD was 2.7% ± 1.6% in the low-risk group (no MUD + no FM + ATG; *n*=112), 21.8% ± 3.4% in the high-risk group (MUD + FM +/- ATG, or MUD + no FM + no ATG; *n*=147), and 9.6% ± 1.5% in the intermediate-risk group (*n*=375); $P=10^{-6}$.

Discussion: We conclude that MUD, FM, and no ATG were independent risk factors for SR aGVHD in adult patients after allo-SCT from MRD or MUD. To avoid a high risk of SRaGVHD, our study suggest that in unrelated transplants, one should avoid a female donor for a male recipient and use ATG if the recipient is a female or if both recipient/donor are males.

Disclosure of Interest: None Declared.

PH-P379

EXTRACORPOREAL PHOTOPHERESIS FOR GRAFT-VERSUS-HOST DISEASE: THE ROLE OF PATIENT-, TRANSPLANT-, DIAGNOSTIC CRITERIA AND HEMATOLOGICAL VALUES ON RESPONSE. RESULTS FROM A LARGE SINGLE CENTER STUDY

M. Berger[1,], R. Albiani[2], B. Sini[2], F. Fagioli[1]*

[1]Pediatric Onco-Hematology and Stem Cell Transplant Division, [2]Immunohematology and Transfusional Medicine, Regina Margherita Children's Hospital, Turin, Italy

Introduction: Graft-versus-host disease (GVHD) remains a major problem following HSCT. Aim of this study was to ascertain the role of Extracorporeal Photopheresis (ECP) in 76 patients with steroid refractory or dependent acute and chronic GVHD (aGVHD and cGVHD).

Materials (or patients) and Methods: A total of 37 patients were treated for aGVHD (median age 12 years, median follow-up for surviving patients 4 yrs [2 months-12 yrs]) and 39 patients for cGVHD (median age 25 years, median follow-up for surviving patients 4 yrs [1 month-12 yrs]). Patients were treated with ECP on two consecutive days at weekly intervals for the first month, every two weeks during the second and third months, and then at monthly intervals for a further three months. Briefly, patients with aGvHD were ruled out from the ECP protocol if they had: a) completed their planned 22 procedures, b) had aGvHD progression under ECP, c) had GVHD response but interrupted their treatment early on given their high risk of relapse. Patients with cGvHD were ruled out from ECP therapy if they reached complete response (CR), partial response (PR), or minor response (MR) following the 22 planned ECP procedures or before if they had aGvHD progression under ECP.

Results: The crude aGVHD response was 67% and the complete aGVHD Free Survival was 50% (95% CI, 25-70). The crude cGVHD response was 77% while the cGVHD Free Survival was 34% (95% CI 21-47). Among aGVHD patients, a better aGVHD Free Survival was associated to aGVHD lower grade (grade II 78% [95% CI, 62-100], grade III 36% [95% CI, 17-79] and grade IV 0%], *P*=<0.00) and donor type (MFD 41% [95% CI, 23-73], MUD 57% [95% CI, 36-90] and Haplos 100%, *P*=0.02). The Transplant-Related Mortality (TRM) was associated to stem cell source (*P*=0.01) and aGVHD grading (*P*<0.00) and finally the relapse incidence was significantly higher for patients without visceral organ involvement (*P*=0.04). Among the cGVHD patients the cGVHD Free Survival was significantly associated with the female gender (55% [95% CI, 31-99] vs. 24% [95% CI, 13-46, *P*=0.01]) and with the limited form according to Seattle classification (58% [95% CI, 36-94] vs. 19% [9-42], *P*=0.002). For TRM and RI no factors were identified as predictors.

Discussion: No role of hematological value or aphaeretic cell count were found in our patient cohorts, but a trend for improved outcome for patients with higher aphaeretic yield was observed. Larger studies are warranted to support the cell dose effect.

Disclosure of Interest: None Declared.

PH-P380

IMPACT OF FEMALE DONOR ON ALLOGENEIC STEM CELL TRANSPLANTATION OUTCOME OF MALE RECIPIENT AFTER T-CELL DEPLETION BY RABBIT ANTITHYMOCYTE GLOBULIN

C. Calmettes[1,], S. Vigouroux[1], R. Tabrizi[1], A. Pigneux[1,2], T. Leguay[1], K. Bouabdallah[1], M.-S. Dilhuydy[1], C. Duclos[1], A. Lascaux[1], P.-Y. Dumas[1], S. Dimicoli-Salazar[1], G. Marit[1,2], N. Milpied[1,3]*

[1]Hematologie et thérapie cellulaire, CHU Bordeaux, [2]Université Bordeaux Segalen, BORDEAUX, France, [3]Hematologie et thérapie cellulaire, Université Bordeaux Segalen, BORDEAUX, France

Introduction: The female donor/male recipient combination (FM) increases the risks of graft-versus-host disease (GVHD) and non-relapse mortality (NRM) after allogeneic stem cell transplantation (allo-SCT), with a possible deleterious impact on overall survival (OS) [Gahrton G. *Best Pract Res Clin Haematol*. 2007 Jun;20(2):219-29]. Most patients in these studies received T-cell replete transplants. As a consequence, and because antithymocyte globulin

(ATG) reduces the risk of GVHD, the impact of FM in the specific setting of allo-SCT with ATG remains poorly defined.
Materials (or patients) and Methods: To explore the impact of FM in the ATG setting, we undertook a retrospective analysis of male adult patients transplanted with rabbit ATG at our center between 01/01/2000 and 12/31/2012. Overall survival and disease-free survival (DFS) were calculated using the Kaplan-Meier estimate, with comparisons by the log-rank test. Cumulative incidences (CI) were used for NRM, relapse (REL), and GVHD in a competing risks setting. MUD were matched at the allele level for HLA-A, B, C, DRB1, DQB1.
Results: 212 male patients were identified and included in the present study. The median age was 56 years (18-67). Diseases were AML (*n*=61), ALL (*n*=16), NHL (*n*=36), Hodgkin's disease (*n*=10), myeloma (*n*=37), MDS (*n*=26), aplastic anemia (*n*=7), CLL (*n*=11), CML (*n*=1), and MPS (*n*=7). Status at transplant were CR1 or PR1 or chronic phase (*n*=74), > CR1 or PR1 (*n*=83), refractory (*n*=34), or untreated (*n*=21). Conditioning regimens were RIC (*n*=195) or MAC (*n*=17). Donors were MRD (*n*=110) or MUD (*n*=102). Eighty-eight patients were transplanted with a FD. Source of stem cells were PB (*n*=193), BM (*n*=18), missing data (*n*=1). Prophylaxis of GVHD consisted of CsA+metho for 84 patients.
The median follow-up was 42 months (5-149). In univariate analysis, the 3-year OS and DFS in FD *vs* MD groups were 48% ± 6% *vs* 56% ± 5% (*P*=0.3) and 42% ± 5% *vs* 50% ± 5% (*P*=0.4), respectively. The 3-year NRM and REL in the same groups were 25% ± 5% *vs* 19.6% ± 4% (*P*=0.4) and 30% ± 5% *vs* 31% ± 4% (*P*=0.9), respectively. The CI of aGVHD II-IV, aGVHD III-IV, SR aGVHD, and extensive chronic GVHD in FD *vs* MD groups were 37.5% ± 5% *vs* 33% ± 4% (*P*=0.5), 22.7% ± 4% *vs* 17% ± 3% (*P*=0.3), 17.2% ± 4% *vs* 8.3% ± 3% (*P*=0.049), and 20% ± 4% *vs* 22.5% ± 4% (*P*=0.9), respectively. Other variables considered in univariate analysis for SR aGVHD were recipient age (*P*=0.9), recipient CMV status (*P*=0.7), disease status (*P*=0.9), RIC *vs* MAC (*P*=0.16), MRD *vs* MUD (*P*=0.09), PB *vs* BM graft (*P*=0.4), and GVHD prophylaxis (*P*=0.2). In multivariate analysis, the risk factors for SR aGVHD were FD (HR=3.1, 95%CI: 1.2-7.9, *P*=0.01) and MUD (HR=2.9, 95%CI: 1.1-7.1, *P*=0.02). The CI of SR aGVHD were 32.3% ± 9.7% in the FD + MUD group (*n*=29), 9.7% ± 3.8% in the MD + MUD group (*n*=73), 10.7% ± 4.1% in the FD + MRD group (*n*=59), and 6.5% ± 3.7% in the MD + MRD group (*n*=51); *P*=0.004.
Discussion: We conclude that FD was an independent risk factor for SR aGVHD after allo-SCT with rabbit ATG in male adult patients mostly transplanted with PB after RIC. There was no impact of FD on aGVHD II-IV or III-IV, chronic GVHD, NRM, REL or survival. Finally, the absence of impact of FD on NRM and survival should be interpreted with caution given the retrospective design of the study and because we cannot exclude a possible limiting effect of an insufficient number of patients.
Disclosure of Interest: None Declared.

PH-P381
A PROSPECTIVE COMPARATIVE STUDY OF EXTRACORPOREAL PHOTOPHERESIS PERFORMED USING THE OPEN-LOOP COBE® SPECTRA OR SPECTRA OPTIA® SYSTEM VERSUS THE CLOSED-LOOP THERAKOS™ CELLEX™ SYSTEM

E. J. Dann[1,], E. Sabbag[1], T. Mashiach[2], T. Stravets[1], A. Badian[1], S. Finkelbaum[1], K. Smarkatin[1], M. Hayun[3], Y. Ofran[3]*
[1]Blood Bank and Apheresis Unit, [2]Quality Assurance Unit, [3]Department of Hematology and Bone Marrow Transplantation, Rambam Health Care Campus, Haifa, Israel

Introduction: Extracorporeal photopheresis (ECP) is a useful second-line therapy for patients with chronic and acute graft-versus-host disease (GVHD). While in the USA only the closed-loop system is used, in other countries both closed-loop and two-step open-loop procedures are employed. This study is aimed to prospectively compare the efficacy of the two ECP procedures, with each patient being treated with both treatment modalities.
Materials (or patients) and Methods: Patients were scheduled to undergo the open-loop procedure (modality A) for 2 consecutive days followed by the closed-loop procedure (modality B) to be performed for 2 consecutive days two weeks later. Data on levels of hematocrit (*Hct*), WBC, monocytes (Mon), lymphocytes (Lym), neutrophils (Neu), product bag volume and collection duration were planned to be analyzed. Additionally, early and late apoptosis of product cells was to be evaluated. Samples were collected from the product bag prior to adding 8-MOP and following UVA irradiation. Mononuclear cells (MNC) were separated using Lymphoprep (Axis-Shield), counted and suspended in cell culture flasks at a concentration of $4x10^6$/ml in the RPMI-1640 medium supplemented with 10% fetal bovine serum. The cells were incubated for 48 hours at 37°C. MNC subsets (Lym, Mon) were identified by flow cytometry. Early apoptosis was evaluated using Annexin V and late apoptosis was assessed using Propidium Iodid. Wilcoxon signed paired test was performed to evaluate significance of results.
Results: Twelve patients with chronic GVHD (4 females, 8 males; median age 44, range 20-67 years) underwent the procedures; 79 open-loop procedures and 35 closed-loop paired procedures were analyzed. Cell collection data are presented in Table 1. In collections performed using Spectra Optia and Cellex systems, similar median *Hct* levels (2.2 and 2.1, respectively) were obtained, while *Hct* was significantly higher when measured using the COBE Spectra system (3.3; *P*<0.05). In the open-loop modality, the number of early apoptotic Lym and Mon before UVA treatment was 16.8±24.4 and 75.7±14.8 $x10^7$, respectively (*P*<0.05); in the closed-loop procedure, these parameters amounted to 1.3±1.7 and 12.1±11.3 $x10^7$ (*P*<0.05). After UVA exposure, the number of early apoptotic Lym and Mon observed in the open-loop modality was 6±3.5 and 127.7±41.8 $x10^7$, respectively; in the closed-loop modality the corresponding numbers were 0.3±0.4 and 10.9±9.2 $x10^7$ (*P*<0.05).
Discussion: The current study has shown marked differences between the 2 ECP modalities. While duration of the open-loop procedure was longer, it yielded a significantly higher mean total MNC amount. Similarly, this modality provided a significantly greater number of early apoptotic cells in Lym and Mon gates both prior to and after UVA irradiation exposure, a finding that may be explained by difference in device design employed in the 2 modalities. These results, albeit obtained in a small cohort of patients, demonstrate the superiority of the open-loop procedure, suggesting that it could be efficiently used in patients with GVHD.
Disclosure of Interest: None Declared.

Collection modality	Collection time (min)	Processed volume (L)	WBC/ bag $x10^9$	MNCs $x10^9$	Neu $x10^9$	Mon $x10^9$	Lym $x10^9$
Open-loop	226	6.09±1.8	8.9±2.2	5.9±2	2.8±2	2.2±1	3.8±1.7
Closed-loop	114	1.52±0.3	2.6±1	2.1±0.8	0.4±0.3	0.8±0.3	1.3±0.8
P-value	<0.01	<0.01	<0.02	<0.02	<0.02	<0.02	<0.01

PH-P382

HUMAN CHORIONIC GONADOTROPIN HORMONE INDUCES INDOLEAMINE 2,3-DIOXYGENASE AND INTERLEUKIN-10 EXPRESSION IN PATIENTS WITH GRAFT-VERSUS-HOST-DISEASE

A. Elmaagacli[1,], M. Ditschkowski[2], N. Steckel[3], T. Gromke[2], H. Ottinger[2], U. Hillen[2], R. Trenschel[2], D. Beelen[2], M. Koldehoff[2]*
[1]Asklepios Klinik Hamburg Altona, Hamburg, [2]University Hospital of Essen, [3]University Hospital Essen, Essen, Germany

Introduction: Chronic graft-versus-host disease (GVHD) is a major complication following allogeneic hematopoietic stem cell transplantation and is associated with a substantial morbidity and mortality. It is a systemic inflammatory disorder that reflects the lack of immune tolerance between donor-derived immune competent cells and host organs. Human chorionic gonadotropin hormone (hCG) is a natural occurring hormone during pregnancy secreted by syncytiotrophoblasts of the placenta. We had previously observed (Koldehoff *et al*; *J Leukoc Biol* 2011) that the rejection of transplanted skin was significantly delayed by hCG in a mouse skin transplant model and had also demonstrated that tryptophan-catabolizing enzyme, indoleamine-2,3-dioxygenase (IDO), interleukin-10 (IL 10) and T-regulatory cells (Tregs) increased significantly in females treated with hCG as preconditioning therapy for *in-vitro*-fertilization. Since all these factors are known to induce tolerance and given the low rate of adverse effects, we off-label used low dose of hCG to treat 20 patients as forth- or fifth-line therapy with steroid-refractory or intolerant severe-grade chronic GVHD.
Materials (or patients) and Methods: Because all of these factors are known to induce tolerance and given the low rate of adverse effects in preconditioning therapy, we off-label used low dose of hCG (187 IU) to treat 8 male and 12 female patients (median age 48, *r*. 28-68) with moderate or severe grade of chronic GVHD according to the NIH criteria; all patients had been informed of the experimental state of this treatment and provided written consent
Results: The median number of sites of chronic GVHD involvement per patient was 3 (range, 1-6). hCG therapy was started as 4 or 5th line-therapy together with preexisting medication with prednisone and a calcineurin inhibitor. Twelve of 20 patients (60%) had an objective partial response during 8 weeks of hCG treatment with at least 30% improvement according to the TSS score. Responses included softened skin and subcutaneous tissue; decreased erythema and extent of sclerodermatous, hidebound skin; improved joint mobility and gait; gastrointestinal improvements; and resolution of neuropathy. Nine patients had stable disease (6 with minor responses). Only one patient with previous ATG treatment showed progression of her liver GVHD (histologically proven) and died from GHVD. All other patients were well and alive. Daily low-dose hCG was well tolerated. Adverse events that were possibly related to hCG included reversible and asymptomatic CTCAE grade 4 hypertriglyceridemia (n=1), grade 2 constitutional symptoms (fever, malaise, fatigue; flush, breast enlargement). IDO expression increased up to 7 times and IL10-serum level up to 2 times after 3 weeks of hCG therapy (P<0.003 and P<0.04). T-regulatory cell expansion was documented in one patients.
Discussion: This successful use of hCG in an immune disorder warrants further studies to assess its role as an immunosuppressant in GVHD and potentially other autoimmune disorders.
Disclosure of Interest: None Declared.

PH-P383

PHARMACODYNAMIC PREDICTION OF GVHD RISK IN REDUCED-INTENSITY CONDITIONING REGIMEN ALLOGENEIC STEM CELL TRANSPLANTATION (RIC ALLOSCT) USING MYCOPHENOLATE MOFETIL (MMF) PLUS CYCLOSPORINE A (CSA) AS GVHD PROPHYLAXIS

C. Martinez[1,], O. Millán[2], M. Rovira[3], F. Fernández-Avilés[3], M. Suárez-Lledó[3], Á. Urbano-Ispízua[4], M. Brunet[5]*
[1]Hematology, Institute of Hematology and Oncology, IDIBAPS, University of Barcelona, Hospital Clinic, [2]Pharmacology and Toxicology Department, Biomedical Diagnostic Center, CIBERehd, IDIBAPS, University of Bracelona, Hospital Clinic, [3]Hematology, Institute of Hematology and Oncology, IDIBAPS, University of Barcelona, Hospital Clinic, [4]Hematology, Institute of Hematology and Oncology, IDIBAPS, Institute Josep Carreras, University of Barcelona, Hospital Clinic, [5]Pharmacology and Toxicology Department, Biomedical Diagnostic Center, CIBERehd, IDIBAPS, University of Barcelona, Hospital Clinic, Barcelona, Spain

Introduction: Several reports have shown that the measurement of CSA and/or MMF blood concentrations does not sufficiently reflect the effects of the applied drugs on immune cells; in fact, despite having blood concentrations within therapeutic range, some patients still experience acute GVHD. Pharmacodynamic (PD) biomarker monitoring may reflect more accurately individual response to immunosuppressive drugs and clinical outcome.
Materials (or patients) and Methods: We selected a battery of biomarkers for prospective PD monitoring in 37 RIC alloSCT patients: T cell activation measured by intracellular ATP production in $CD4^+$-T lymphocytes as a reflection of the overall immunosuppressive status achieved with CsA+MMF; and specific biomarkers based on the mechanism of action of these drugs: soluble production (ELISA) and intracellular expression (Flow cytometry) of IL-2, IFNγ, and TNFα in $CD4^+$ and $CD8^+$-T cells such as effector T-cell response quantification. We also evaluated the frequency of $CD4^+CD25^{high}CD127^{low}FOXP3^+$ T-cell (Tregs).
Results: ATP levels at 0.5 months after alloSCT were significantly higher in those patients with grade 0-I GVHD (260 ng/ml, 6.6-876) in comparison to grade II-IV GVHD (144 ng/ml, 21.2-691.7) (P=0.028); however, at 1 month after transplantation a trend for higher levels was observed in patients developing grade II-IV GVHD in comparison to grade 0-I GVHD (357.1 ng/ml vs. 233 ng/ml, respectively) (P=0.26). ATP measured at GVHD diagnoses identified patients with severe (grade III-IV) GVHD with a ROC-AUC of 0.76. Patients with grade II-IV GVHD showed higher $\%CD3^+CD69^+CD4^+$ and $\%CD3^+CD69^+CD8^+$ lymphocytes expressing IL-2, IFNg and TNFa than those with grade 0-I GVHD along the period of study. These differences were statistically significant for $CD3^+CD69^+CD8^+IL\text{-}2^+$ levels at 0.5 and 1 month after transplantation (P=0.032 and P=0.016, respectively). No statistically significant differences were observed in soluble IFNγ and TNFα levels between grade 0-I and II-IV GVHD patients. The median %Tregs lymphocytes was higher in 0-I GVHD during the whole period of the study, particularly at 2 months after alloSCT (P<0.0001). The ratio $\%CD3^+CD69^+CD8^+IL2^+$/Tregs was higher in patients with grade II-IV GVHD than in those with 0-I GVHD, being significant at 1 (1.3, 0-12.06 vs. 0.07, 0-1.84; P=0.025) and 2 (1.96, 0-81.3 vs. 0.09,0-6.75; P=0.002) months after transplantation.
Discussion: In conclusion, ATP production in $CD4^+$ lymphocytes, $\%CD3^+CD69^+CD8^+IL2^+$ and $\%CD3^+CD69^+CD8^+IL2^+$/Tregs rate monitoring after transplantation may help physicians to identify individual response to CsA+MMF and patients at high risk of GVHD.
Disclosure of Interest: None Declared.

PH-P384

UNDER-EXPOSURE TO MYCOPHENOLATE ACID (MPA) AFTER REDUCED-INTENSITY CONDITIONING REGIMEN ALLOGENEIC STEM CELL TRANSPLANTATION (RIC ALLOSCT) USING MYCOPHENOLATE MOFETIL (MMF) PLUS CYCLOSPORINE A (CSA) AS GVHD PROPHYLAXIS IS ASSOCIATED TO HIGH RISK OF GRADE

C. Martinez[1,], O. Millán[2], M. Rovira[1], F. Fernández-Avilés[1], M. Suárez-Lledó[1], Á. Urbano-Ispízua[3], M. Brunet[2]*

[1]Hematology Department, Institute of Hematology and Oncology, IDIBAPS, [2]Pharmacology and Toxicology Department, Biomedical Diagnostic Center, CIBERehd, IDIBAPS, [3]Hematology Department, Institute of Hematology and Oncology, IDIBAPS, Institute Josep Carreras, University of Barcelona, Hospital Clinic, Barcelona, Spain

Introduction: The combination of CsA and MMF is widely used for prophylaxis of GVHD in the context of RIC alloSCT. CsA and MMF show large inter- and intraindividual pharmacokinetic variability and conflicting findings have been reported when studying the association between their blood levels and the risk of GVHD.

Materials (or patients) and Methods: Measures of through levels (C_{min}) (37 patients) and area under the curve ($AUC_{[0-8h]}$) (12 patients) of CsA and MPA were prospectively performed along the first 2 months after RIC alloSCT. CsA (5 mg/kg/d) and MMF (15 mg/kg/8h) were started IV and then switched to oral administration when tolerated by the patients.

Results: CsA C_{min} at 0.5 months was significantly higher than C_{min} at day 0 (P=0.001) and C_{min} at 1 month post-transplantation (P=0.039). Higher MPA C_{min} levels were achieved after oral MMF administration in comparison to IV (P<0.005); however, only a small proportion of subjects (3% with IV and 24% with oral MMF) achieved through concentration targets >2 mg/ml. There was no correlation between MPA C_{min} and AUC. Median $AU_{C[0-8h]}$ of MPA at d+1 during IV MMF administration was 52.9 mg*h/ml (range 16.3-93.9). MPA concentrations significantly declined after initiation of oral MMF (MPA $AUC_{[0-6h]}$ 27.6 mg*h/ml, 13.4-67.8; P=0.01) with only 36.4% of patients achieving levels > 30 mg*h/ml. No differences in CsA C_{min} on day 0 were observed between grades 0-I and II-IV GVHD patients. Patients with II-IV GVHD had lower C_{min} of CsA at 0.5 months than those with 0-I GVHD (P=0.018); however, no correlation was observed between CSA $AUC_{[0-8h]}$ and GVHD. No differences were observed between 0-I and II-IV GVHD patients in MPA C_{min} measured at day +1, 48-72h after MMF oral initiation, and 3-4 weeks after alloSCT. All patients with grade 0-I GVHD had MPA $AUC_{[0-8h]}$ on day +1 and 48-72h after MMF oral administration higher than 30 mg*h/ml; however, most patients with grade II-IV GVHD (63% on day +1 and 57% 48-72h after oral MMF, respectively) did not achieve this value (P=0.08) (Figure 1).

Discussion: In conclusion, our results show that trough MPA concentrations post-transplant do not accurately describe MPA $AUC_{[0-8h]}$ nor GVHD risk; and that high MPA exposure is associated with more efficacy in GVHD prevention. Individualized MMF dosing according to $AUC_{[0-8h]}$ levels during the first month after RIC alloSCT may be important for GVHD prophylaxis after RIC alloSCT.

Disclosure of Interest: None Declared.

PH-P385

TREATMENT OF STEROID-REFRACTORY GRAFT VERSUS HOST DISEASE (SR-GVHD) AFTER BONE MARROW TRANSPLANT (BMT) FOR PRIMARY IMMUNE DEFICIENCY–SINGLE CENTRE EXPERIENCE

T. Altmann[1,], M. Slatter[1], Z. Nademi[1], M. Abinun[1], A. Gennery[1]*

[1]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction: Graft versus host disease (GvHD) is a common complication following haematopoietic stem cell transplantation (HSCT). 1st line therapies are corticosteroids (appropriate dose/route for grade) and topical treatment if required [1]. Many cases resolve but some develop steroid-resistant (SR)-GvHD. 2nd line therapy should commence if no response after 5 days, or after 3 days if symptoms progress [1], including additional immunosuppressive agents, monoclonal antibodies, serotherapy, mesenchymal stromal cells and extracorporeal photopheresis. We compared and evaluated practice at our unit in the last 5 years to recently published guidelines providing 1st and 2nd line treatment of SR-GvHD.

Materials (or patients) and Methods: A retrospective review of patient records and the CBMTU database between 31/01/08–20/12/12. Patient Cohort: Patients developing SR-GvHD following HSCT for primary immunodeficiency. Data collected: Diagnosis, HSCT date, GvHD: prophylaxis, occurrence date, grade, treatment, treatment start date.

Results: Of 152 patients, 18 (12%) developed SR-GvHD, 5 (28%) of whom died. 8 (44%) developed Grade II GvHD (6 skin, 2 mixed), 5 (28%) Grade III (1 gut, 4 mixed), 5 (28%) Grade IV (1 skin, 4 mixed). Median day of GvHD occurrence: 24 (Range: 0-125). Some patients received a boost infusion for falling chimerism that appeared to induce more severe GvHD. All patients received 1st line treatment in accordance with the guideline. Median time to 2nd line treatment: 24.5 days. 2nd line: 33% of patients received 1 monoclonal antibody, 11% received 2 monoclonal antibodies - none received serotherapy or other therapy on/by the 5th day of onset of symptoms [1].

Discussion: 1st line therapy was extremely well implemented but there was significant variation in time between GvHD diagnosis and 2nd line treatment for SR-GvHD patients. Reasons why 2nd line therapy was not given according to the guideline were potentially: Fluctuating symptoms, varying timeline/severity and presentation of GvHD in many patients. Potential cautious prescribing by clinicians as there are worries due to possible serious side effects of 2nd line treatments [1]. Outside referral was required for MSC therapy, which delayed treatment. Limitations of the study include: Progressive symptoms could not be assessed, as often records were unclear whether symptoms were progressive and of the exact timeframe of events. Guidelines are useful, but there is no clinical consensus for application of 2nd line treatment as this is an ever developing field [1].Our cohort includes patients treated before these guidelines were published [1]. SR-GvHD is a life-threatening diagnosis with poor outcomes. Increasing access of patients to new treatment options and reducing the time taken to implement these therapies may help to improve outcomes.

Disclosure of Interest: None Declared.

PH-P386

THE ANALYSIS OF HEMATOGONES (HGS) AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-HSCT)

N. Doki[1,], K. Haraguchi[2], K. Kakihana[1], S. Ishida[1], G. Oshikawa[1], T. Kobayashi[1], Y. Okuyama[2], K. Ohashi[1], H. Sakamaki[1]*

[1]Hematology Division, [2]Division of Transfusion and Cell Therapy, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan

Introduction: Increased normal B-cell precursors, termed hematogones (HGs), could be observed in regenerating bone marrow (BM) after chemotherapy or hematopoietic stem cell transplantation (HSCT). Recent reports suggested that emergence of HGs would be associated with better outcomes.

Materials (or patients) and Methods: Patients: We have analyzed the clinical features of the patients who developed HGs in BM (HGs+) after allo-HSCT, and which were also compared with that of the patients without HGs in BM (HGs-). Between July 2007 and January 2013, a total of 262 patients with hematological malignancy underwent allo-HSCT in our institution. Of them, 41 patients were excluded from our analysis because of early relapse before day 30 or engraftment failure. Thus, we reviewed in detail the clinical features of 221 patients (147 male, 80 female; median age 47, range 16-73). Types of HSCT were 164 bone marrow transplantation (BMT): 15 related, 149 unrelated; 34 related peripheral blood stem cell transplantation (PBSCT):33 related, 1 unrelated; 23 unrelated cord blood stem cell transplantation (CBT). Preparative regimens were 176 myeloablative and 45 reduced intensity

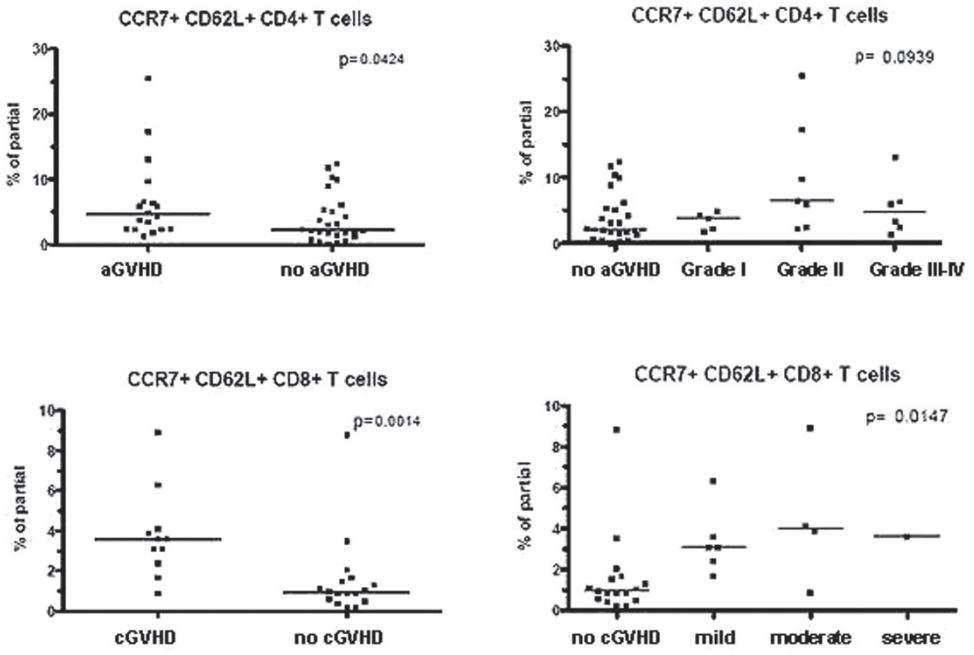
conditioning. Underlying diseases were chronic myeloid leukemia (*n*=11), acute nonlymphoid leukemia (AML, *n*=107), acute lymphoid leukemia (ALL, *n*=52), myelodysplastic syndrome (*n*=35), severe aplastic anemia (*n*=4), non-Hodgkin's lymphoma (*n*=10), and others (*n*=2).
Methods: The emergence of HGs was regularly checked by flow cytometry (FCM) using BM samples obtained at day 30. Besides, 110 patients, who maintained complete remission at day100 after allo HSCT, received the additional FCM analysis at day 100. HGs are defined by very low side scatter, intermediate expression of CD45, and bright expression of CD19, CD10, and CD38.
Results: The emergence of HGs was confirmed in 105 patients (47%) at day30 and 67 patients (61%) at day 100, respectively. There were no significant difference between HGs+ and HGs- patients in terms of sex, age, primary disease, disease status, or conditioning regimen. While, patients receiving CBT or PBSCT were more likely developed HGs at day 30 compared to BMT (73.9%, 76.4% vs. 37.8%; *P*<0.001). Moreover, patients with HGs at day 30 had better 2 years overall survival (OS) compared to HGs- patients (64.5% vs. 51.2%, *P*=0.001). In univariate analysis, the emergence of HGs at day 30 was a significant factor of better 2 years OS in patients with AML in remission (69.9% vs. 44.5%; *P*=0.014), in patients with ALL (77% vs. 46%; *P*=0.04) and in patients receiving CBT (75.5% vs. 20.8%; *P*=0.01). HG- patients were more likely to develop grade II to IV acute graft-versus-host-disease (GVHD) compared to HG+ patients (47.8% vs. 26.7%, *P*=0.0016, at day30, 68.4% vs. 26.7%, *P*<0.001, at day100).
Discussion: The emergence of HGs at day30 may be a useful indicators of subsequent survival outcomes or acute GVHD.
Disclosure of Interest: None Declared.

PH-P387
CYCLOSPORINE A IN COMBINATION WITH MYCOPHENOLATE MOFETIL OR METHOTREXATE FOR PROPHYLAXIS OF GVHD
M. Dübbers[1], K. Borchert[1], D. Glaeser[1], M. Schmitt[1,2], C. Junghanss[1], I. Hilgendorf[1,]*
[1]Hematology, Oncology, Palliative Care, Universityhospital Rostock, Rostock, [2]Cellular Immunotherapy, Department of Internal Medicine V, University Clinic Heidelberg, Heidelberg, Germany

Introduction: The prevention of severe acute Graft-versus-Host-Disease (GvHD) and yet preserving an adequate Graft-versus-Leukemia-Effect are still challenging tasks for the patient's outcome after allogeneic HSCT (alloHSCT). We retrospectively compared the standard GVHD-prophylaxis consisting of Cyclosporine A (CSA) and Methotrexate (MTX) with the more tolerable combination of CSA and Mycophenolate-Mofetil (MMF) in a ten year single-center study.
Materials (or patients) and Methods: We retrospectively analysed the outcome of 201 patients who underwent alloHSCT between 2000 and 2010 at our institution. The median age of patients was 48 years (15-71 years). GVHD prophylaxis consisted of CSA/MTX (*n* = 125) or CSA/MMF (*n* = 76). In case of mismatched or unrelated donor grafts ATG (*n* = 110) was additionally given. The analysed groups did not differ significantly in terms of age, conditioning regimen, gender, HLA-matching and CMV-serostatus. The median follow up of survivors was 47 months (4-124 months).
Results: The overall rate of acute GvHD was 53.7% and occurred in median 29 days (9–187) after alloHSCT. After CSA/MTX acute GvHD occurred in median 9.5 days earlier than after CSA/MMF (26 vs. 35.5 days, *P* = 0.041), whereas the overall severity of acute GvHD was comparable in both groups. There was a trend for a higher frequency of aGVHD °IV of the liver in patients receiving CSA/MMF when compared to patients receiving CSA/MTX (54.5% vs. 15%, *P* = 0.071). This reached statistical significance in the subgroup of patients without ATG (0% vs. 55.6%, *P* = 0.023). In addition the incidence of steroid-refractory aGVHD was higher in the subgroup without ATG after prophylaxis with CSA/MMF when compared with CSA/MTX (46.2 vs. 20.7%, *P*= 0.083). Of note, the incidence of intestinal acute GvHD was higher in the CSA/MTX/ATG subgroup (54.1% vs. 12.5%, *P* = 0.006). 29.6% of patients experienced chronic GvHD after a median time of 192 days (33-1454) after alloHSCT. However, neither in rate nor in severity of chronic GvHD statistically significant differences were found between the groups. The incidence of CMV infections was 29.6%, without significant differences in the analysed groups. The estimated survival after 5 years for the CSA/MTX vs. CSA/MMF group was: overall survival 47.0 vs. 50.7% (*P* = 0.91), relapse-free-survival 31.3% vs. 29.4% (*P* = 0.588), event-free-survival 41.8 vs. 41.9 (*P* = 0.725) and the non-relapse-mortality 38.9 % vs. 47.2% (*P* = 0.588), respectively. Cox-regression-method identified involvement of intestinal and liver acute GvHD, severity of acute GvHD and steroid-refractory acute GvHD as negative influence on OS. Presence of chronic GvHD had a positive influence on OS.
Discussion: GVHD prophylaxis with CSA/MMF is a feasible alternative to CSA/MTX with respect to frequency and severity of acute and chronic GvHD, incidence of CMV infection, as well as survival after alloHSCT.
Disclosure of Interest: None Declared.

PH-P388
T CELLS EXPRESSING THE HOMING RECEPTORS CCR7 AND CD62L MEDIATE THE PATHOGENESIS OF GRAFT-VERSUS-HOST DISEASE
V. Gomez Garcia De Soria[1,], I. Portero[2], M. Royg[1], A. Kreutzman[2], C. Fernandez Arandojo[1], A. Ramirez[2], C. Muñoz Calleja[2]*
[1]Hematology, [2]Immunology, Hospital De La Princesa, Madrid, Spain

Introduction: The GVHD pathogenesis involves migration of the donor naïve T-cells into the secondary lymphoid organs in the recipient, which is mainly steered by CD62L and CCR7. Therefore, we aimed to study whether the expression of CD62L and CCR7 in T cells is associated with GVHD, both acute and chronic.
Materials (or patients) and Methods: This single center study included 52 donor-recipient pairs. Samples were collected prospectively from the apheresis product and phenotyped by flow cytometry, and CD62L and CCR7 expression in CD4+ and CD8+ T-cells were compared between patients who developed acute or chronic GVHD (*n*=26) and those who did not (*n*=21).
Results: Interestingly, in the case of the acute form, the GVHD group received a significantly higher percentage of either CCR7+CD4+ (*P*=0.03) and CCR7+CD62L+CD4+ T-cells (*P*=0.042) compared to the no GVHD group. These results were confirmed when the patients with acute GVHD were divided in degrees according to the severity of the disease. Regarding chronic GVHD, significantly higher percentage of CCR7+ CD8+ (*P*=0.011), CD62L+ CD8+ T cells (*P*=0.015), CD62L+CCR7+ CD8+ T cells (*P*=0.001) was observed in those patients who developed chronic GVHD in comparison to those who did not. These data were also confirmed when patients were subdivided in degrees of the disease severity. The multivariable analysis confirmed that these T cell subpopulations had no association with the classical clinical GVHD predictors studied.
Discussion: Our results indicate that CCR7+ and CD62L+ expressing T-cells are the alloreactive cells in the graft, so they would mediate the pathogenesis of GVHD and are potential therapeutical targets for the disease.
Disclosure of Interest: None Declared.



PH-P389

EXTRACORPOREAL PHOTOPHERESIS IN ACUTE GRAFT-VERSUS-HOST DISEASE: CLINICAL OUTCOME, SURVIVAL AND STEROID SPARING EFFECT IN 20 PATIENTS TREATED OVER A 6-YEAR PERIOD

C. Savignano[1,], C. Rinaldi[1], M. Isola[2], F. Patriarca[3], M. E. Zannier[3], R. Fanin[3], V. De Angelis[1]*

[1]Medicina Trasfusionale Udine, Azienda Ospedaliero Universitaria di Udine, [2]Istituto Di Statistica, Dipartimento di Ricerche Mediche e Biologiche, [3]Clinica Di Ematologia, Dipartimento di Scienze Sperimentali e Cliniche, Università di Udine, Udine, Italy

Introduction: The aim of our retrospective analysis was to evaluate the clinical effect of extracorporeal photopheresis (ECP) and its impact on immunosuppressive therapy in patients treated for acute graft-versus-host disease (aGVHD) after allogeneic hematopoietic stem cell transplantation.

Materials (or patients) and Methods: Over a 6-year period (2007-2013), 167 procedures were performed in 20 patients (pts) with steroid refractory aGVHD (10 male/10 female, median age 51, range 22-71). ECP was performed as third or fourth line of therapy in 10/20 pts. 15/20pts were in grade III/IV aGVHD at the beginning of the treatment. ECP was performed using an off-line technique (Cobe Spectra cell separator and PIT System UV-A devices) with the following schedule: 2 procedures a week for 1 month then 2 procedures every other week for 1 month. Stopping ECP was decided on the clinical basis. Venous access was provided by a CVC in 12/20 pts. The response was assessed according the 1994 Consensus Conference criteria: complete remission (CR), partial remission (PR) no response or progression (NR).

Results: ECP was started at a median of 18 days after aGVHD onset (range 5-34). Pts underwent 3 to 15 procedures (median 8) with a mean treatment period of 26d (range 14-127).No relevant treatment related complications were observed. At one month, a clinical response (CR+PR) was obtained in 12 of 20 (60%) pts: 5/5 with grade II and 6/15 (40%) with grade III/IV aGVHD.The highest percentage of response was observed in patients with muco-cutaneous involvement (87% RC+PR) while only 40% of pts with prevalent liver involvement were responsive to ECP. The treatment was continued in 8 /20 pts (1 in CR, 5 in PR, 2 in NR): the patient in CR stay in remission, 3 of 5 pts in PR obtained a CR and 1of 2 pt in NR gained a PR. Finally, at the end of treatment 9 pts were in CR, 3 pts were in PR e 8 pts in NR. In responders patients, the prednisone equivalent dose was 1 mg/kg/die at the onset of ECP (range 0,5-2,7) and 0,33 mg/Kg/die at the end of treatment (range 0,06-0,6). Only two of the responders pts with grade III aGVHD received new additional immunosuppressive therapy (1pts infliximab, 1 pts etanercept) started during ECP. The median follow up in responders group was 140 days (range 63-1022), 5 pts survived and 8 pts died (4pts for relapsing disease, 2pts for infections, 1pt for PT-PTT). In the NR group, the ECP was stopped after 3 procedures in 3/8 pts due to clinical reasons (post transplant PTT, hepatic grade IV aGVHD) and after 8 procedures in the remaining 5 pts. All the 8 non responders patients died (6pts for aGVHD e 2 pts for pulmonary infections). The Kaplan Mayer probability of overall survival was significantly higher among responders pts compared with those with NR (72% vs 25% at three months and 46% vs 0% at six months) (P=0.001).

Discussion: In our experience ECP proved to be a safe and effective treatment in patients with corticosteroid-resistant aGVHD. The low reported toxicity of ECP allowed the procedure to be performed also in pts with poor performance status. In our analysis better response rates were obtained in patients with cutaneous involvement and the reported steroid-sparing effect of ECP could be confirmed. Finally a significant association between the clinical response and the OS could be shown, which is in line with previous published results.

Disclosure of Interest: None Declared.

PH-P390

CIRCULATING ENDOTHELIAL CELLS COUNT CHANGES ARE A DYNAMIC BIOMARKER OF ACUTE GVHD IN PATIENTS UNDERGOING ALLOGENEIC HSCT

C. Almici[1,], C. Skert[2], R. Verardi[1], A. Di Palma[2], A. Bianchetti[1], A. Neva[1], S. Braga[1], M. Malagola[2], D. Russo[2], M. Marini[3]*

[1]Transfusion Medicine, Laboratory for Manipulation and Cryopreservation of Stem Cells, A.O. Spedali Civili di Brescia, [2]Chair of Hematology, Unit of Blood Diseases and Stem Cell Transplantation, University of Brescia, A.O. Spedali Civili di Brescia, [3]Transfusion Medicine, Blood Bank, A.O. Spedali Civili di Brescia, Brescia, Italy

Introduction: Allo-HSCT can be burdened by life-threatening complications, being GVHD the major cause of morbility and mortality. Clinical and physio-pathological evidences showed that vascular endothelium could be a target of GVHD in very early phase; therefore markers of endothelial damage are warranted as valuable support in GVHD diagnosis. We conducted a study with primary endpoint to identify and count circulating endothelial cells (CEC) in peripheral blood of patients undergoing alloBMT as a function of endothelial damage.

Materials (or patients) and Methods: The CellSearch System® is used to capture and enumerate CEC. Enriched and stained cells are dispensed into a MagNest® cartridge that is scanned and individual images of cells are scored as CEC, based on CD146+, CD105+, DAPI+ and CD45- phenotype. Patients undergoing allo-HSCT were tested before (T1), after the conditioning regimen (T2), at engraftment (T3), at GVHD onset (T4) and at 1-2 weeks after steroids treatment (T5). Ten healthy subjects served as controls.

Results: We enrolled 40 patients with hematologic neoplastic diseases (7 HD, 13 AML, 5 ALL, 8 MM, 3 CLL, 1 NHL, 1 CML, 2 SAA) undergoing allo-HSCT from either HLA-matched familial (*n*=12) or unrelated donor (*n*=28). aGVHD (grade I-IV) manifested in 19/39 patients. No clinical and transplant characteristics differences were present between patients with and without GVHD. The median CEC/ml pre-alloBMT was 20 (*n*=40, range 4-718), in comparison to a value of 2 (range 1-14) in the 10 healthy subjects. At time of engraftment CEC/ml were 47 (range 16-148) in patients with GVHD and 92 (range 23-276) in patients without GVHD (*P*=0.006). This difference remained significant in multivariate analysis by logistic regression model (OR 0.97, 95% C.I. 0.96-0.99; *P*=0.02). At GVHD onset, the relative increase of CEC counts (T4 vs T3) was 44% (range, -43 - 569%) in GVHD patients versus 0% (range, -49 – 2%) in patients without GVHD (*P*=0.003), being confirmed in multivariate analysis (OR 1.04, 95% C.I. 1.0-1.08; *P*=0.04).

Discussion: Circulating endothelial cells can represent a promising marker to monitor endothelial damage in patients undergoing allo-HSCT. The confirmation of the clinical utility of CEC counts in a larger series of patients, together with the use of a semi-automatic, standardized and reproducible technology, will allow a valuable help in the diagnostic definition of GVHD in early phase, and moreover could be a valid complement in the prognostic stratification of patients candidates to allo-HSCT.

Disclosure of Interest: None Declared.

PH-P391

SINGLE NUCLEOTIDE POLYMORPHISMS WITHIN THE THROMBOMODULIN GENE (THBD) PREDICT RISK OF NON-RELAPSE MORTALITY IN PATIENTS WITH GRAFT-VERSUS-HOST DISEASE

T. Luft[1,], S. P. Rachakonda[2], O. Penack[3], O. Blau[3], S. Dietrich[1], P. Dreger[1], R. Kumar[2], B. Isermann[4]*

[1]University Hospital Heidelberg, [2]Department Epidemiology, German Cancer Research Centre DKFZ, Heidelberg, [3]Charité - Universitätsmedizin Berlin, ßInterdisziplinäre Klinik und Poliklinik für Stammzelltransplantation, Berlin, [4]Institute for Clinical Chemistry and Pathobiochemistry, Otto-von-Guericke University Magdeburg, Magdeburg, Germany

Introduction: Steroid-refractory graft-versus-host disease (GVHD) is a major and often fatal complication after allogeneic stem cell transplantation (alloSCT). Although the pathophysiology of steroid-refractoriness is not fully understood, evidence is accumulating that endothelial cell stress is involved, and endothelial thrombomodulin (TM) plays a role in this process. Here we assess if single nucleotide polymorphisms (SNPs) within the TM gene (THBD) predict outcome after alloSCT.

Materials (or patients) and Methods: Seven SNPs within the THBD gene were studied (rs1962.TC, rs1042579.TC, rs1042580.AG, rs3176123.TG, rs3176124GA, rs3176126.GA and rs3176134.CT) in a training cohort of 465 allografted patients. The relevant genotypes were then re-assessed in an independent validation cohort (*n*=386).

Results: In the training cohort, an increased risk of non-relapse mortality (NRM) was associated with three SNPs out of seven tested: rs1962.CC, rs1042579.TT (455V, linked with rs3176123GG) and rs1042580.GG. When patients were divided into risk groups (one vs. no high-risk SNP), a strong correlation with NRM was observed (HR 2.32 95% CI 1.36-3.95; *P*=0.002). More specifically, NRM was predicted by THBD SNPs in patients who later developed GVHD (HR 3.03 95% CI 1.61-5.68, *P*=0.0006), but not in patients without GVHD. In contrast, THBD SNPs did not predict incidence of acute GVHD. Multivariate analyses adjusting for clinical variables confirmed the independent effect of THBD SNPs on NRM. All findings could be reproduced in the validation cohort.

Discussion: THBD SNPs predict mortality of manifest GVHD but not the risk of acquiring GVHD, supporting the hypothesis that endothelial vulnerability contributes to GVHD refractoriness

Disclosure of Interest: None Declared.

PH-P392

COMBINED HLA AND SEX-MISMATCHED HSCT IS ASSOCIATED WITH INCREASED CD4+ AND CD8+ T CELL NUMBERS IN ACUTE GVHD CUTANEOUS INFILTRATES

K. Thus[1,], A. G.S. van Halteren[2], T. de Hond[1], M. van Dijk[1], R. Q.H. Kloos[1], A. C. Lankester[2], M. B. Bierings[1], E. Spierings[1]*

[1]UMC Utrecht, Utrecht, [2]Leiden University Medical Center, Leiden, Netherlands

Introduction: Graft-versus-host disease (GVHD) remains a major limiting factor for hematopoietic stem-cell transplantation (HSCT). Risk factors for GVHD include, but are not limited to, HLA mismatches, a female donor for a male recipient, and stem-cell source. Especially sex mismatching combined with HLA mismatching seems to lead to an increased risk of alloreactive T-cell responses. The pathophysiological mechanisms via which these factors affect the GVHD risk, are poorly understood. GVHD evidently involves T-cell recognition of non-self antigens expressed by recipient cells, as depletion of T cells from the graft minimizes the GVHD incidence. We earlier reported significant differences in skin-infiltrating T cells in male pediatric patients who developed GVHD after receiving a graft derived from an unrelated donor. This previous study suggested that CD4/CD8 ratios among skin-infiltrating T cells were affected by donor sex. In the current study, we retrospectively analyzed if HLA and sex mismatching quantitatively affects the composition of GVHD-induced cutaneous T-cell infiltrates in a pediatric HSCT cohort.

Materials (or patients) and Methods: We analyzed tissue sections from steroid-naïve pediatric HSCT-patients (*n*=15 males and *n*=8 females) with histologically and clinically confirmed acute GVHD. The absolute numbers of CD3+CD4+ and CD3+CD8+ T cells were quantified in tissue sections prepared from archived formalin-fixed paraffin embedded skin biopsies. These sections were subjected to a routine immunofluorescent staining protocol using a validated set of CD3, CD4 and CD8-specific antibodies, where after the T-cell infiltrates were quantified by fluorescent microscopy in a blinded manner.

Results: Graft source (HLA-identical related donor (IRD, *n*=7), matched-unrelated donor (MUD, *n*=12) or cord blood (CB, *n*=4)) had no significant impact on the numbers of skin-infiltrating CD8+ T cells. HSCT with CB seemed to result in the highest number of CD4+ T cells in the skin (compared to MUD *P*=0.02). Patients receiving an HSCT from a sex-mismatched donor displayed an increased number of CD4+ T cells when compared to

recipients of a graft from a sex-matched unrelated donor when the donor was unrelated (either MUD or CB) (P=0.01). This sex-mismatching effect was not observed in IRD transplant pairs. Furthermore, unrelated patient-donor pairs in which the donor can recognize T-cell epitopes derived from the recipient mismatched HLA, so called predicted indirectly recognizable HLA epitopes (PIRCHES), showed an increased number of skin-infiltrating CD8+ T cells when compared to patients who did not express PIRCHES. The combined expression of HLA class I- or II-presented PIRCHES with a sex mismatch resulted in the highest number of skin-infiltrating T cells.
Discussion: Our results provide the first indication that an increasing number of major (i.e. HLA) and minor (i.e. HY) histocompatibility antigen-derived T cell epitopes expressed by recipient skin cells leads to increased accumulation of CD4+ and CD8+ T cells in the skin. These observations may explain the pronounced effect of sex mismatching in HLA-mismatched transplants as evidenced by registry-based epidemiological studies.
Disclosure of Interest: None Declared.

PH-P393
HIGH-DOSE RITUXIMAB IN THE CONDITIONING REGIMEN BEFORE ALLOGENEIC STEM CELL TRANSPLANTATION FOR RELAPSED B-CELL LYMPHOMAS IS ASSOCIATED WITH REDUCED GVHD-RELATED DEATHS, BUT DID NOT IMPROVE DISEASE CONTROL

A. Dodero[1,], F. Spina[1], B. Sarina[2], C. Carniti[1], F. Patriarca[3], A. M. Carella[4], A. Levis[5], A. Bosi[6], M. Falda[7], F. Di Raimondo[8], P. Di Bartolomeo[9], S. Dalto[1], A. Bacigalupo[10], A. Rambaldi[11], P. Corradini[1] and GITMO*
[1]Ematologia, Fondazione IRCCS Istituto Nazionale dei Tumori, milano, [2]Ematologia, Humanitas Cancer Center, Rozzano, [3]University of Udine, Udine, [4]IRCCS Casa Solievo della Sofferenza, San Giovanni Rotondo, [5]Ematologia, Ospedale di Alessandria, Alessandria, [6]Ematologia, Azienda Ospedaliera-Universitaria Carreggi, Firenze, [7]Ematologia, Ospedale San Giovanni Battista, Torino, [8]Ematologia, Osepedale Ferrarotto, Catania, [9]Ematologia, Ospedale di Pescara, Pescara, [10]Ematologia, Ospedale San Martino, Genova, [11]Ematologia, Ospedaliera Papa Giovanni XXIII, Bergamo, Italy

Introduction: The best conditioning regimen before allogeneic stem cell transplantation (alloSCT) for relapsed B-cell lymphomas remains to be identified. Several studies have shown that Rituximab (R) is implicated in the pathophysiology of acute and chronic graft-versus-host disease (GVHD).
Materials (or patients) and Methods: We analyzed 153 adult patients (pts) who received alloSCT for chemosensitive relapsed/refractory B-cell lymphomas. All pts received the same preparative conditioning regimen consisting of thiotepa/cyclophosphamide± fludarabine, and GVHD prophylaxis consisting in cyclosporine and mini-methotrexate. ATG was added to pts allografted from class I antigen mismatched sibling or unrelated donors (MUD). Eighty-two pts (group A, study group) received high-dose R (500 mg/m^2 on day -6) and were enrolled in a prospective multicenter study, whereas seventy-one consecutive pts (group B, control group) were transplanted without R in the conditioning. The two groups were not significantly different in terms of diagnosis (group A: n=48 indolent, n=34 aggressive; group B: n=32 indolent, n=39 aggressive; P=0.10), donor types (group A: n=48 sibling, n=34 MUD; group B: n=39 sibling, n=32 MUD; P=0.74), rate of complete remission at alloSCT (group A: 32/82 versus group B: 32/71, P=0.51). Median follow-up was 31 versus 48 months in A versus B group, respectively.
Results: The crude cumulative incidence (CCI) of non-relapse mortality (NRM) at 2-years was 17% in A group and 18% in B group, respectively (P=0.77). Main cause of death were infections without GVHD in A group (6/14) and extensive GVHD in B group (6/14). Deaths associated to acute or chronic GVHD were significantly lower in A (6 of 14) as compared to B group (13 of 14) (P=0.01). The CCI of grade II-IV and III-IV acute GVHD was 24% versus 35% (P=0.16) and 7% versus 14% (P=0.11) in A versus B group, respectively. Pts allotransplanted with R from matched related sibling or MUD donors had a trend for reduced acute GVHD as compared to the control group [23% versus 35% (P=0.12); 23% versus 31% (P=0.61)]. Group A and B had a similar CCI of chronic GVHD [46% versus 47% (P=0.81)] and a trend for reduced GVHD in those receiving transplant from MUD donors with R as compared to control group [33% versus 40%, (P=0.88)]. A delayed immune reconstitution of B-cells was still evident at 2 years after alloSCT in A versus B group [median value CD19+: 126/µL versus 300/µL with reduced immunoglobulin production: 502 versus 778 mg/dL of IgG (P=0.04)]. Progression free survival (PFS) and overall survival (OS) at 3-years were similar in A and B groups in indolent [PFS: 68% versus 75% (P=0.21); OS: 78% versus 75% (P=0.47)] and aggressive lymphomas [PFS: 42% versus 43% (P=0.85); OS: 53% versus 54% (P=0.83)], respectively. In multivariate analysis, acute GVHD and aggressive hystotype were associated to a lower OS [HR=2.23, P=0.009; HR=2.1, P=0.009] and PFS [HR=1.73, P=0.05; HR=2.3, P=0.001].
Discussion: Rituximab administration in the setting of alloSCT is associated with reduced GVHD-related deaths and increased infection-related deaths, likely due to delayed B-cell immune-reconstitution. Thus, as compared to historical controls, pre-transplant Rituximab fails to improve the outcome of alloSCT.
Disclosure of Interest: None Declared.

PH-P394
THE USE OF AUTOLOGOUS SERUM EYEDROPS IN SEVERE OCULAR GRAFT VERSUS HOST DISEASE

A. Laing[1,], A. Yasmin[1], K. Wong[2], G. McQuaker[1], A. Parker[1], M. Copland[1], D. Anijeet[2], R. Kanna[2], A. Clark[1]*
[1]Blood and Marrow Transplant Unit, West of Scotland Cancer Centre, [2]Ophthalmology Department, Gartnavel General Hospital, Glasgow, United Kingdom

Introduction: Ocular manifestations occur in 25-45% of patients with chronic graft versus host disease (cGvHD) following allogeneic BMT. Treatment options are limited and often ineffective. We describe a series of multiply treated patients, with severe, debilitating, refractory disease. All patients responded to treatment with autologous serum eye (ASE) drops. In some cases there was a remarkable restoration of vision related function and a dramatic improvement in Q.O.L.
Materials (or patients) and Methods: Five patients were diagnosed as having severe ocular cGvHD following ophthalmic review which included schirmer's testing and slit lamp examination after fluorescein staining to examine the tear meniscus and assess the degree of corneal surface ulceration. The validated ocular surface disease index (OSDI) questionnaire was used to assess functional debility. After processing, an autologous unit of blood provided a 3 month supply of individual daily ASE for each eye.
Results: Patient characteristics and treatments are shown in table 1. All patients had already 'failed' systemic immunosuppression (IS) and multiple lines of ophthamological treatments. OSDI scores were uniformly high at the start of treatment and improved for all patients. Two patients noted improvement and discontinued ASE to be maintained on acetylcysteine drops. Three patients had dramatic functional improvement that significantly improved their quality of life and remain well on ASE. This subjective improvement was confirmed by ophthalmology assessment with marked improvement in the appearance of the ocular surface. No adverse reactions were described.
Discussion: Ocular cGvHD manifests as dry, gritty, painful eyes and if severe can lead to corneal ulceration, perforation and visual loss. ASE have been found to have epitheliotrophic and antimicrobial, as well as lubricating properties, due to the presence of growth factors, fibronectin and vitamins. It is these properties that may explain the benefits seen in patients who have had no significant response to systemic IS, lubricants and ophthamological procedures. In our experience few treatments have made such an immense difference to patients quality of life and functional capabilities.
Disclosure of Interest: None Declared.

[PH-P394]

Age	Diagnosis	Transplant Conditioning & Source	Organs affected by GvHD	Systemic IS	Ophthalmological treatments	OSDI pre-ASE	OSDI post-ASE
50	AML	Treo/Cyclo PBSC (sib)	Skin, liver	P, CSA, MMF, ECP	Lubricants: A, SH, C, LP Bandage CL Punctal plugs Steroid: Pr CSA eye drops Lacrimal gland injections (dex) Amniotic membrane graft	100 (Severe)	4.55 (Normal)
50	AML	Cyclo/TBI PBSC (sib)	Skin, liver, lung	P, T, MMF, ECP	Lubricants: A, CS Topical steroid: Pr	60.41 (Mod/Severe)	14.58 (Mild/Normal)
55	AML	Cyclo/TBI/Camp PBSC (VUD)	Skin, liver	MP, CSA, MMF, T	Lubricants: A, C, CS, LP Lacrimal gland injections (dex) Steroids: Pr, FMO	68.18 (Severe)	52.27 (Moderate)
53	ALL	Etoposide/TBI PBSC (sib)	Skin, mouth, liver	P, CSA, MMF, T	Lubricants: A, LP, PA, C, SH Steroids: FMO, B Punctual plugs Botox injection Bandage CL	85.41 (Severe)	16.67 (Mild)
52	Biphenotypic leukaemia	Cyclo/TBI PBSC (sib)	Skin, mouth, liver	CSA, MMF, T	Lubricants: LP, CS Steroids: Pr	57.5 (Mod/Severe)	25 (Mild)

P= prednisolone, CSA=ciclosporin, MMF= mycophenolate mofetil, extracorporeal photopheresis, T=tacrolimus, MP=methylprednisolone, A= acetylcysteine, SH= sodium hyaluronate, C=carbomers, LP = liquid paraffin, CL= contact lensesPr= predsol 0.5% eye drops, CS= carmellose sodium, FMO= flurometholone, PA= polyvinyl alcohol, B= betnesol

PH-P395

EXTRACORPOREAL PHOTOAPHERESIS FOR THE TREATMENT OF ACUTE GRAFT VERSUS HOST DISEASE (AGVHD). A SINGLE CENTER EXPERIENCE

M. Malagola[1,], P. Ferremi Leali[2], C. Skert[1], A. Turra[1], E. Ferrari[2], M. L. Sala[2], A. Tiburzi[2], C. Bergonzi[1], C. Fili[1], V. Cancelli[1], F. Cattina[1], R. Ribolla[1], M. Marini[2], D. Russo[1]*

[1]Chair of Hematology, Bone Marrow Transplant Unit, University of Brescia, AO Spedali Civili Brescia, [2]Transfusion Medicine and Immunohematology Service, Brescia, Italy

Introduction: Graft versus Host Disease (GVHD) represent one of the most important life-threatening complications of allogeneic stem cell transplantation (allo-SCT). The conventional treatment of acute GHVD (aGVHD) includes calcineurin inhibitors (namely cyclosporine A) associated to high dose methyl-prednisolone (namely 2 mg/Kg/day). Unresponsive patients may be salvaged with second-line therapy including immunosuppressive drugs (e.g. mycophenolate), polyclonal antibodies, monoclonal antibodies and anti-cytokine antibodies. Extracorporeal photoapheresis (ECP) is an immunomodulatory treatment for steroid-refractory acute and chronic graft versus host disease (a/cGVHD) and it has been proven to be effective both in acute and chronic GVHD.
Materials (or patients) and Methods: From March 2007 to March 2013, 138 adult patients were addressed to allo-SCT at our Institution for haematological malignancies. Forty-three out of 138 patients (31%) developed aGVHD grade ≥2 according to Glucksberg grading and were treated with conventional approach (6-methyl prednisolone 2 mg/Kg/day associated with calcineurin inhibitor). Nineteen out of 43 patients (44%) achieved a complete response and 24/43 (56%) did not, according to the standard criteria. This report focus on this 24 patients with steroid-refractory grade II – IV aGVHD, 15 (62%) of whom were treated with ECP and 9 (38%) with other second-line immunosuppressive agents.
Results: The clinical and the transplant characteristics of these two groups of patients were comparable. Interestingly, 93% and 67% of the patients had skin involvement in the ECP and non ECP group, respectively. The liver and the gut were involved in 20%, 13% and 56%, 88% in the ECP and non ECP group, respectively. The median time from aGVHD onset to ECP initiation was 20 days (range 11-41). Overall, 13/15 patients (87%) addressed to ECP and 6/9 (67%) of those treated without ECP showed a complete response according to the standard criteria. The median time to achieve a response with ECP was 51 days (range 3-117) and the median of ECP cycles was 20 (range 8-50) for a median number of 117 days (22-271) under ECP. At last follow up 7/15 (47%) patients treated with ECP are alive (two cases with limited cGVHD) and 2/9 (22%) patients not addressed to ECP are alive (both with extended cGVHD). The projected EFS at 3 years is 30% (95% CI 0-61) for patients who received ECP and 22% (95% CI 0-49) for patients who received treatments other than ECP (*P*=0.28).
Discussion: Our preliminary results show that the response rate in patients treated with ECP is higher, although not significantly, than that of patients not addressed to ECP (87% vs 67%). This translate into longer EFS in the ECP group (30% vs 22%; *P*=0.28). Prospective studies are warranted for establishing the exact role of ECP in the early treatment of aGVHD.
Acknowledgments: this work was supported in part by Lions Club Bassa Bresciana, Banca di Credito Cooperativo di Pompiano e Franciacorta, fondi 60% Prof Domenico Russo, fondi 60% Dr Michele Malagola.
Disclosure of Interest: None Declared.

PH-P396

ASSOCIATION BETWEEN URIC ACID LEVELS AND ACUTE GRAFT VERSUS HOST DISEASE

B. Nils Ostendorf[1,], O. Blau[1], L. Uharek[1], I. Blau[1], O. Penack[1]*

[1]Hematology, Oncology and Tumor Immunology, Charité, Berlin, Germany

Introduction: Endogenous danger signals are increasingly recognized in the pathogenesis of graft-versus-host disease (GVHD). Uric acid is a known danger signal and is released from injured cells during conditioning for allogeneic hematopoietic stem cell transplantation (HSCT). Recently, pre-clinical data have implicated uric acid in the development of GVHD (Jankovic *et al*., JEM, 2013) and depletion of uric acid prior to HSCT using rasburicase

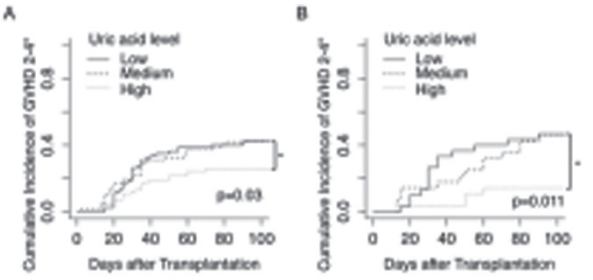


Figure 1 | Low uric acid levels at transplantation are significantly associated with acute GVHD grades 2-4.
Retrospective analysis of 232 consecutive patients revealed a significant association between low uric acid levels at the time of allogeneic HSCT with the development of acute GVHD grades 2-4 (A). This impact was more pronounced when restricting analysis to patients without in vivo T-cell depletion using anti-thymocyte globulin (B).

reduced the incidence of GVHD in a small pilot trial (Brunner *et al*., ASH abstract 3063, 2012). Clinical data studying the association between uric acid levels and GVHD in larger cohorts are lacking.
Materials (or patients) and Methods: Here, we retrospectively review 232 patients undergoing HSCT between 2005 and 2011 at Charité Campus Benjamin Franklin to evaluate the impact of uric acid levels at the time of HSCT on the development of acute GVHD. All patients were transplanted from 10/10-HLA matched related (*n*=67) or unrelated donors (*n*=165) and most patients were transplanted for acute leukemia (68 %).
Results: Unexpectedly, low acid levels were significantly associated with acute GVHD grades 2-4 in univariate (HR 0.849, 95% CI 0.743–0.97, *P*=0.016) and multivariate analyses (HR 0.856, 95% CI 0.741–0.989, *P*=0.035). This was even more pronounced in patients without *in vivo* T-cell depletion by anti-thymocyte globulin (HR 0.452, 95% CI 0.223-0.913, *P*=0.027). Uric acid levels did not have a significant impact on overall survival (HR 1.020, 95% CI 0.909-1.146, *P*=0.733), non-relapse mortality (HR 1.08, 95% CI 0.895-1.3, *P*=0.42) and relapse (HR 1.1, 95% CI 0.921-1.32, *P*=0.29).
Discussion: Our data underline the clinical significance of danger signals for HSCT. Surprisingly, we found that low uric acid levels at the time of HSCT were associated with increased incidence of acute GVHD. This association has to be confirmed in independent cohorts and mechanisms behind it remain speculative at this point. Currently, the EBMT GVHD working party is planning a retrospective survey on the association of uric acid and GVHD using the EBMT database. Hopefully this study will add more detailed information on the association between uric acid and GVHD.
Disclosure of Interest: None Declared.

PH-P397
THE IMPACT OF ENTEROCOCCAL FLORA ON INTESTINAL GVHD AND OVERALL SURVIVAL
D. Sporrer[1,], J. Koestler[2], A. Gessner[2], P. Oefner[3], J. Hahn[1], E. Holler[1]*
[1]Internal Medicine III, [2]Microbiology, [3]Functional Genomics, University Hospital Regensburg, Regensburg, Germany

Introduction: Intestinal GvHD as a severe complication in patients undergoing allogenic stem cell transplantation (ASCT) seems to be affected by gastrointestinal (GI) microbiota.
Analysis of stool microbiomes in patients after ASCT revealed al loss of bacterial diversity with predominance of enterococcal flora, which was associated with prophylactic and therapeutic use of antibiotics as well as GvHD.
We now extended our pilot study on a larger number of patients and asked, if enterococcal positivity influences GvHD and overall survival of patients with ASCT.
Materials (or patients) and Methods: In a prospective cohort study of 77 patients undergoing ASCT between 1/2011 and 6/2013, stool specimens were collected weekly starting before conditioning until 4 weeks after ASCT. Presence of Enterococcus (E) faecium and/or E faecalis was monitored by PCR-analysis. During this period, we switched our strategy of antibiotic prophylaxis due to increasing microbial resistance: the first 32 patients received the standard prophylaxis with ciprofloxacin and metronidazol, whereas in the subsequent 45 patients rifaximin, a synthetic derivate of rifamycin with <1% GI absorption, was used at a dose of 200 mg twice daily. A clinical analysis of intestinal GvHD and documented infections as well as overall survival was performed, and risk factors for enterococcal positivity and gastrointestinal GvHD were analyzed.
Results: On day 28, 24 patients (31%) remained negative for both enterococci, whereas 39 (50%) and 14 (19%) patients, respectively, tested positive for either or both enterococci. Enterococcal positivity was associated with severe GI GvHD (stage III-IV): 27.3% of patients positive for E faecalis developed severe GvHD as compared to 5.5% of patients negative for E faecalis (*P*=0.007). In those testing positive for both germs, E faecalis and E faecium, this relation was 35.7% to 4.2% (*P*=0.008). One-year TRM analyses showed a poor outcome for patients with positivity for E faecalis (*P*=0.019) and both germs (*P*=0.007), respectively. Enterococcal positivity seemed to be affected by the type of antibiotic prophylaxis. Patients on rifaximin showed less frequently a shift to positivity for both germs compared to systemic decontamination (11.1% to 28.1%, *P*=0.02). For E faecalis only, a minor insignificant effect was observed (22.2% to 37.5%, *P*=0.14). This association was confirmed in multivariate analysis, as merely the type of antibiotic prophylaxis (p 0.034), but neither systemic use of antibiotics nor other clinical risk factors correlated with enterococcal positivity. Between rifaximin and the standard prophylaxis we found no significant differences regarding the incidence of fever of unknown origin (25% to 18.2%, *P*=0.47), bacteremia (9.1% to 8.3%, *P*=0.91) or sepsis (21.2% to 16.7%, *P*=0.61). Finally, rifaximin (*P*=0.015) and early stage of underlying disease (*P*=0.04) seemed to exert a protective effect against severe GvHD according to a multivariate evaluation.
Discussion: In conclusion, positivity for E. faecalis alone or both, E faecalis and E faecium in stool specimens increases the risk of severe GI GvHD and poor overall survival after ASCT. The choice of antibiotic prophylaxis might contribute to enterococcal overgrowth. Enterococcal positivity at day 28 might be an indicator of loss of bacterial diversity or even directly contribute to GvHD associated damage; larger prospective clinical and preclinical studies are needed to clarify the exact interactions.
Disclosure of Interest: None Declared.

PH-P398
INCREASED REG3A SERUM LEVELS CORRELATE WITH SEVERE GASTROINTESTINAL DAMAGE FROM EARLY ACUTE GI GVHD, LATE ACUTE GI GVHD AND NEUTROPENIC ENTEROCOLITIS
E. Holler[1,], M. Kreutz[1], J. Ferrara[2], K. Schmid[1], K. Peter[1], D. Sporrer[1], S. Haffner[1], D. Wolff[1], W. Herr[1], J. Levine[2]*
[1]University Medical Centre, Regensburg, Germany, [2]University of Michigan, Ann Arbor, United States

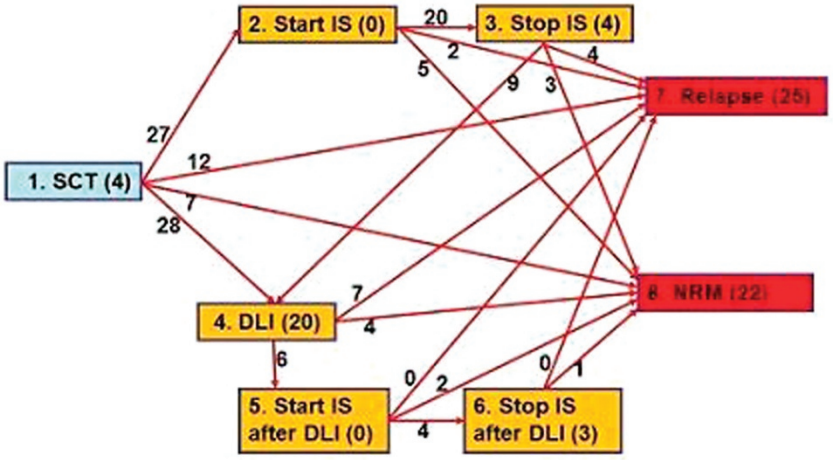
Introduction: Reg3a is a GI GVHD specific biomarker that is not elevated in patients with non-GVHD enteritis developing after engraftment. Plasma levels of Reg3a at the onset of GvHD have been shown to be highly prognostic. However, the kinetics of Reg3a post-transplant, are not known. To answer this question we serially measured Reg3a levels after SCT and correlated the results with GI GVHD and neutropenic enterocolitis.
Materials (or patients) and Methods: Serum was cryopreserved weekly from admission until d42 and thereafter according to outpatient intervals (up to d560). A total of 86 patients were included. Reg3a serum levels were assessed by ELISA. Admission and peak levels between day 0-14, 21-42 and 90-360 were correlated with the development of GI GVHD and/or neutropenic enterocolitis. Diagnosis of intestinal GvHD was confirmed by histology in all symptomatic patients.
Results: Mean Reg3a levels were lowest at admission (62.0 + 12.6 ng/ml) and did not differ according to later GVHD or neutropenic colitis. The mean peak Reg3a level between d0-14 was not statistically different between patients who developed severe GI GvHD

(stage 2 or higher) or not (272.9 ± 101 vs 126.7 ± 31.3 ng/ml, ns). However, neutropenic enterocolitis may have confounded this result. Mean peak Reg3a levels between d0-14 were highest in patients who developed both GI GvHD and neutropenic colitis (329.9±73.9 ng/ml) and lowest in patients who developed neither (63.2 ± 4.8 ng/ml), this difference was highly statistically significant (*P*=0.000). Reg3a levels were similar in patients who developed enterocolitis but remained GI GVHD free or who developed GI GVHD without prior enterocolitis (187.8 ± 32.6 vs 145.8 ± 20.5 ng/ml, ns, but in both cases were significantly higher than for patients with neither complication. Mean peak Reg3a levels between d21-42 were significantly higher in patients who developed GI GVHD (546.9 + 121.7 vs 239.8 + 65 ng/ml, *P*<0.001). Likewise, mean peak Reg3a levels after day 90 (measured in 14 patients who received DLI or after taper of immunosuppression) who developed late acute GVHD compared to those who did not (239.8 + 65 vs 59.7 + 25.1 ng/ml, *P*=0.03).
Discussion: Our study demonstrates Reg3a release is associated with severe GI epithelial damage from both GVHD but also from neutropenic enterocolitis. We were able to confirm that Reg3a as a biomarker of early GI GVHD as well as GI GVHD that occurs late post transplant after DLI. Our new finding shows that in contrast to non-GVHD enteritis developing after engraftment, during the early posttransplant period, neutropenic enterocolits causes additional and independent Reg3a release.
Disclosure of Interest: None Declared.

PH-P399
MULTI-STATE MODELS ENHANCE INSIGHT IN OUTCOMES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR AML/MDS FOLLOWED BY DONOR LYMPHOCYTE INFUSIONS
L. de Wreede[1,], M. Eefting[2], C. Halkes[2], P. von dem Borne[2], S. Kersting[2], E. Marijt[2], H. Veelken[2], H. Putter[1], J. Schetelig[3], J. Falkenburg[2]*
[1]Medical Statistics and Bioinformatics, [2]Hematology, LUMC, Leiden, Netherlands, [3]Medical Department I, University Hospital Carl Gustav Carus, Dresden, Germany

Introduction: Outcome analyses of studies in the field of SCT are mainly focused on time of relapse and death, measured from the moment of transplantation. This approach does not take into account the application of post-SCT interventions, such as DLI. To assess the dynamic impact of post-transplant interventions and clinical events on eventual failures or successes, the methodology of multi-state models has been developed. In our current study we demonstrate how a multi-state model helps to gain insight into the events after SCT in a cohort of AML and MDS patients who were transplanted using a strategy including scheduled DLI.
Materials (or patients) and Methods: 78 patients who underwent T-cell depleted allogeneic SCT for AML (*n*=69, 83% (very) poor risk) or MDS (*n*=9) in CR (85% CR1, 15% CR2) after myeloablative conditioning at the Leiden University Medical Center between January 2002 and June 2011 were included in this analysis. Patients were eligible for DLI in the absence of GvHD from 3 months after the transplantation onward. We constructed a multi-state model to analyse (1) the impact of DLI and GvHD on the failure probabilities of relapse and non-relapse mortality over time, and (2) the probability of treatment success, which we defined as the absence of disease and of GvHD requiring immuno-suppressive treatment (IS), over time (see Figure 1; states 1, 3, 4 and 6). To assess the impact of IS and DLI on subsequent failure, a dynamic prediction approach was chosen, which can be considered as an extension of landmark modelling, in which both starting and end time, and starting and end state are variable. For accurate and objective measurement of duration of severe GvHD, date of start of IS for GvHD treatment until cessation of IS was taken.
Results: From ca. 8 months after SCT, a substantial proportion of patients (ca. 30%) remained in the DLI state, meaning they had received DLI, were still alive without relapse and did not require IS for GvHD after DLI. In general, only few patients required IS for GvHD after DLI. Both relapse and NRM were considerable forces of failure of comparable magnitude. Yet while relapse is the dominant cause of failure from the SCT state, its impact is reduced after GvHD and especially after DLI.
The long-term estimated probability of treatment success from start was around 40%; if a patient survived the first months after SCT without subsequent events, this substantially improved the expected long-term outcome. A comparison between these patients and those having received DLI suggests that the latter's probability of treatment success is larger: approximately 80% at 60 months after SCT for a patient in the DLI state at 9 months.
Discussion: Our multi-state model helps to get insight into the dynamics of post-SCT interventions and clinical events and gives summary measures of treatment success. This model helps to extract more information from observational data. When data of different centers are analyzed by our model, a comparison of treatment strategies is possible.
Disclosure of Interest: None Declared.

Multi-state model. Each arrow indicates a transition in the model. The numbers next to them give the observed transitions in our dataset. The number between brackets within each state indicates the observed number of patients in that state at the end of their follow-up.



PH-P400

OUTCOME OF CHILDREN DEVELOPING GRADE III-IV ACUTE GRAFT-VERSUS-HOST-DISEASE AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

A. Bertaina[1,], F. Locatelli[1,2], A. Lawitschka[3], J. Cornish[4], P. Bader[5], J. H. Dalle[6], P. Sedlacek[7], I. Yaniv[8], C. Peters[3]on behalf of EBMT Paediatric Diseases Working Party*

[1]Hemato-Oncology and Stem Cell Transplantation, Bambino Gesù Children's Hospital, Rome, [2]University of Pavia, Pavia, Italy, [3]Stem Cell Transplantation Unit, St. Anna Children's Hospital, Vienna, Austria, [4]Stem Cell Transplantation Unit, Bristol Royal Hospital for Children, Bristol, United Kingdom, [5]Stem Cell Transplantation and Immunology, Hospital for children and Adolescents, Frankfurt, Germany, [6]Hemato-Oncology, Robert Debré Hospital, Paris, France, [7]Hemato-Oncology, Teaching Hospital Motol, Prague, Czech Republic, [8]Pediatric Hematology Oncology and Stem Cell Transplantation, Schneider Children's Medical Center of Israel, Petach Tikva, Israel

Introduction: Acute graft-versus-host disease (aGvHD) still remains one of the major causes of procedure-related morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Information on the outcome of paediatric patients experiencing this complication is limited. We, therefore, conducted a retrospective registry-based analysis on 4193 children who developed grade III-IV aGVHD and were reported to the EBMT registry.
Materials (or patients) and Methods: Included in the study were children below age of 18 years transplanted between 1972 and 2012. Median year of HSCT was 2002. Patients were transplanted in 262 centers. Of these children, 1232 had a non-malignant disorder, while 2956 had malignancies. Information on the donor used was available for 3753 patients. It was an HLA-identical sibling in 1680 cases and an unrelated donor in 1879 cases. Umbilical cord blood was employed as stem cell source in 408 cases, while a relative other than a compatible sibling was utilized in 194 cases. Overall, 2831 patients were given bone marrow, while 893 received peripheral blood stem cells.
Results: Grade III aGVHD occurred in 2525 patients (60%), while grade IV aGVHD was diagnosed in 1668 (40%). These children represent 11.6% of the 36261 paediatric patients reported to the EBMT registry in the same time period. The median time to occurrence of grade III-IV aGVHD was 17 days from HSCT (range 9-93 days). Chronic GVHD (cGVHD) occurred in 1240 patients (29.6% of the overall number of children who developed grade III-IV aGVHD). Data on cGVHD severity were available in 1167 children (94% of the whole population); it was extensive in 679 (16.2%) and of limited severity in 488 (11.6%). Relapse occurred in 478 children (11.4% of the whole population). At time of last follow-up, 1585 patients were alive (37.8%), while 2504 were dead (59.7%); 89 patients were lost to follow-up. The 3-year Kaplan-Meier probability of overall survival (OS) of this cohort of patients was 40+1%, while the 3-year cumulative incidence of transplantation-related mortality (TRM) was 47+1%. The probability of 3-year OS increased over time, being 32+1% for children transplanted before 2000, 39% for those transplanted between 2000 and 2005 and 47% for children transplanted in the time period comprised between 2005 and 2010 (*P*=0.001). Conversely, the cumulative incidence of TRM decreased over time (see also table). Transplant-related causes were responsible for the death of 1979 patients (47.2%), relapse or progression of the original disease for 355 patients (8.5%), while 18 patients (0.4%) developed a secondary malignancy. In children with malignancies, in comparison to patients who did not developed cGHVD, the extensive form of the disease had a detrimental effect on OS, the hazard ratio (HR) being 1.213 (1.034-1.424, *P*=0.01). By contrast, limited cGVHD was associated with better OS (HR 0.754, range 0.612-0.929, *P*=0.008).
Discussion: These data indicate that the occurrence of grade III-IV aGVHD is associated with a dismal outcome also in paediatric patients. The main cause of fatality is represented by TRM, while leukemia recurrence affected outcome of a lower number of children. Although the outcome of children experiencing grade III-IV aGVHD is improving over time, strategies aimed at preventing this immune-mediated complication and at optimizing its treatment are desirable.
Disclosure of Interest: None Declared.

PH-P401

PROSPECTIVE STUDY OF EXTRACORPOREAL PHOTOCHEMOTHERAPY USING A REDUCED INTENSITY REGIMEN IN GRAFT VERSUS HOST DISEASE

M. Luisa Lozano[1,], V. Lopez[1], M. Fernandez[1], I. Heras[1], C. Castilla-Llorente[1], V. Vicente[1]*

[1]Hematology, Hospital JM Morales Meseguer, Murcia, Spain

Introduction: Extracorporeal photochemotherapy (ECP) has become a recognized treatment for graft versus host disease (GVHD), but the optimal frequency and duration of treatment are yet to be established. For either acute (aGVHD) or chronic (cGVHD) disease, in the absence of controlled trials the most frequently applied schedule is two ECP sessions per week until maximum response, followed by tapering, tailored to the individual patient. The aims of this study were (1) to compare MNC collections performed with the off-line procedure with historical data from in-line procedure in patients with GVHD to generate products for ECP, and (2) to evaluate the efficacy of a reduced-intensity ECP regimen in a pilot study respect to that adopted by most centers.
Materials (or patients) and Methods: In all, 15 patients with refractory GVHD (12 cGVHD [83% severe disease], 3 aGVHD [66% grade 2, 33% grade 3]) were enrolled on this prospective study. The schedule was as follows: one procedure every week for 6 weeks, one procedure every 2 weeks for 1.5 months, and then one procedure every month for 6 months or more until maximal response was achieved. Written informed consent was obtained from all patients and our institution's ethics committee approved the study. Apheresis procedures were performed with either the COBE Spectra (Terumo BCT, Lakewood, CO, USA; version 7.0) or Spectra Optia v9 (Terumo BCT) by processing two times the patient´s blood volume. The product was transferred to a UVA-permeable bag (UVA

[PH-P400]

		TRM			
	Patients	**n**	**100-day CI**	**1-year CI**	**3-years CI**
Total	**4193**	**1934**	**0.26±0.01**	**0.43±0.01**	**0.47±0.01**
< 2000	1741	972	0.34±0.01	0.51±0.01	0.54±0.01
2000 - < 2005	858	388	0.24±0.01	0.43±0.02	0.46±0.02
2005 - < 2010	1132	413	0.18±0.01	0.34±0.01	0.38±0.02
2010 or later	462	161	0.18±0.02	0.35±0.02	0.45±0.03
P-value				<0.001	

PIT, Med Tech Solutions Gmbh, Fürt, Germany), added 5 mL (0.1 mg) of 8-methoxypsoralen (8-MOP) aqueous solution (S.A.L.F., Cenate Sotto, Italy), exposed to UVA irradiation (UVA PIT System), and then reinfused.
Results: We analyzed 216 consecutive procedures (Cobe Spectra 100, Spectra Optia 116), having a mean white blood cell (WBC) and MNC dose of 128.2 and 122.1 x 10^6/Kg, respectively. Compared with historical data obtained from adults using the in-line devices (Denney H, *et al*. 38th Annual Meeting of the European Group for Blood and Marrow Transplantation, 2012), in our hands one treatment collection resulted in twice the number of WBCs, and most important, four times the number of MNCs. The median duration of treatment was 241 days, and the median number of ECP cycles was 14. The response rates were recorded according to the involved organ. The overall response rate in cGVHD was 100% (25% complete response [CR]; 75% partial response [PR]). The mean response rate in patients with cutaneous, mucosal and joint involvement was of 100%, and that of other organs (gastrointestinal, lung, liver) was of 50%. We observed excellent response rates in the three patients with aGVHD with an overall response rate of 100% CR (skin, gut and liver involvement).
Discussion: Prospective clinical trials with ECP will enable recommendations to be made with regard to the number of cycles and cells to be infused. In our hands, one treatment collection with the off-line procedure was at least as effective as two treatment collections with the in-line system, in terms of MNC dose. This reduced-intensity ECP schedule with the off-line procedure is feasible and effective in the management of GVHD, results in a decrease in at least 50% the number of treatments that are usually performed, allows for substantial cost reduction, and is more convenient for the patient.
Disclosure of Interest: M. Lozano Conflict with: Received research support from Terumo BCT, V. Lopez: None Declared, M. Fernandez: None Declared, I. Heras: None Declared, C. Castilla-Llorente: None Declared, V. Vicente Conflict with: Received research support from Terumo BCT.

PH-P402
EXTRACORPOREAL PHOTOPHERESIS AND RITUXIMAB FOR THE TREATMENT OF STEROID REFRACTORY GRAFT VERSUS HOST DISEASE

R. Cekauskiene[1,], L. Kryzauskaite[1], A. Bertasiute[1], I. Trociukas[1], A. Griskevicius[1], R. Matuzeviciene[2], K. Sinkevic[2], T. Zvirblis[1], V. Ivanauskaite[1], L. Griskevicius[1]*
[1]Hematology, Oncology and Transfusion medicine Center, [2]Center of Laboratory medicine, Vilnius University Hospital Santariskiu Clinics, Vilnius, Lithuania

Introduction: Treatment options for steroid refractory Graft versus host disease (SR-GVHD) after allogeneic stem cell transplantation are unsatisfactory. Extracorporeal photopheresis (ECP) has immunomodulating effect mainly on T cells and has been used for the treatment of SR-GVHD with encouraging results. Rituximab (Rtx) targeting B cells showed efficacy in SR-GVHD. We present an analysis of ECP and Rtx combination targeting two pathogenetic GVHD treads T and B cells used in our center to treat SR-GVHD.
The aim of study was o determine the safety and clinical benefit of ECP and Rtx combination in SR-GVHD in terms of clinical response rate (RR) and site using the National Institutes of Health consensus criteria. To evaluate lymphocyte subpopulations as prognostic markers of response.
Materials (or patients) and Methods: Patients with acute or chronic SR-GVHD, received ECP plus Rtx (off label use) as add-on to steroids ± Cyclosporin A. 12 ECP cycles (each consisting of 2 procedures on 2 consecutive days) were given by weekly schedule followed by monthly ECP for up to 16 cycles or more. 1 g of Rtx was given after 1st and optionally 3rd ECP cycles. Evaluations were performed after 6, 12, 14 and 16 ECP cycles.
Results: ECP+Rtx treatment was initiated for 60 patients with SR-GVHD. 48 patients who survived at least for 6 weeks were further analysed for clinical response. 25 patients had grade III-IV acute GVHD (aGVHD). 13 patients with chronic GVHD (cGVHD) and 10 with overlap syndrome (oGVHD) (10 – moderate, 13 – severe) were analysed together. The median observation time was 10 months (1-61).
Among 25 aGVHD patients overall clinical response rate (RR) was 80%, 44% were complete responders (CR), 36% – partial responders (PR) and 20% did not respond (NR). aGVHD response by site was: skin (17 patients) CR 82.4%, PR 11.8%, NR 5.8%; gastrointestinal (20 patients) CR 45%, PR 25%, NR 30%; liver (5 patients) CR 20%, PR 60%, NR 20%.
RR was 95.6% in c/oGVHD. 9 (39.1%) c/oGVHD patients achieved CR, 13 (56.5%) – PR and one (4.4%) did not respond. The best response was observed in skin (20 patients) – RR 100% (CR 85%, PR 15%); mouth (12 patients) – RR 100% (CR 75%, PR 25%). RR in other sites was lower: gastrointestinal (13 patients) RR 84.6% (CR 53.8%, PR 30.8%, NR 15,5%); liver (9 patients), RR 100%, (CR 33.3%, PR 66.7%); 2 patients with eye,1 with genital and 1 with lung involvement achieved PR.
The dose of glucocorticosteroids (GCS) was reduced by 50% or more in 7 c/oGVHD and 6 aGVHD patients and discontinued in 6 c/o GVHD and 6 aGVHD patients on the first evaluation (after 6 weeks). 23 of 48 patients are alive, 5 of them have very mild cGVHD without treatment or solely on MMF. 2 have reoccurrence of cGVHD and ongoing systemic treatment. 2 year overall survival (OS) of CR and PR patients was 68% and 48% respectively and was significantly higher than OS of NR patients, who all died within first 2 months (P<0.0001). The causes of death were: infection in 15, disease relapse in 4 and other causes not related to infection, disease or GVHD in 6 patients. Lymphocyte subpopulations (CD3+ CD4+, CD3+ CD8+, NK cells, Treg) did not predict response to treatment, however lower initial Treg counts in responders were observed. ECP treatment was well tolerated with no procedure related serious adverse events.
Discussion: ECP and Rtx treatment is feasible and effective in SR-GVHD. Prospective, randomized trials are warranted.
Disclosure of Interest: None Declared.

PH-P403
MONOCLONAL GAMMOPATHY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT AS A POSSIBLE MARKER FOR GVHD ONSET

F. Monaco[1,], F. Zallio[1], G. Catania[1], M. T. Corsetti[1], L. Mele[2], F. Dallavalle[2], S. Tamiazzo[1], A. Baraldi[1], F. Salvi[1], M. Pini[1]*
[1]Hematology, [2]Transfusion Medicine, Azienda Ospedaliera SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Alessandria, Italy

Introduction: Transient monoclonal gammopathy is common alteration of the laboratory test after allogeneic stem cells transplantation (alloBMT); until now only scattered work have been published about it. The main paper reported on PubMed was presented by the Dana Farber group's at the end of eighty's. The author of that paper showed an apparently strong correlation between development of graft versus host disease (GvHD) and appearance of a monoclonal gammopathy. Starting from that observation we decided to evaluate among our allogeneic transplanted patients the incidence of M-component e its possible relationship with GvHD.
Materials (or patients) and Methods: 71 patients undergoing alloBMT at the Hematology Unit of Alessandria (Italy) between 2006 and 2013 were evaluated. 52% of patients were male and 48% were females. Pretransplantation diagnosis included: 37 acute myeloid leukaemia (52.1%), 8 acute lymphoblastic leukaemia (11.3%), 7 non-Hodgkin Lymphoma (9.9%), 5 chronic leukaemia (7%), 5 myelodysplastic syndrome (7%), 2 Hodgkin lymphoma (2.8%) and 7 other less common malignancies (9.9%). All patients had, at least, two pre-transplantation serum electrophoresis with no evidence of pre-existing monoclonal component. From the analysis we haven't considered patients submitted to allo-BMT for Myeloma.
In all patients serum electrophoresis was performed at 90, 180 and 360 days after transplantation. In our survey, 21 patients relapsed after alloBMT, 31 (43%) patients developed cGvHD and 26 (36%) patients died. Post-transplantation follow up ranged from 81 to 2695 days with a median of 496 days.

Results: As a whole, 36/72 (50.7%) of the patients developed monoclonal gammopathy after transplantation. A decreased relapse incidence (19% vs 40% at median +436 days) and an increased GvHD development (54% vs 34% at median +436 days) was observed in patients with an appearance of monoclonal gammopathy; no difference was observed regarding overall survival and post-transplantation mortality (not statistical significance was reached). Comparing patients with or without monoclonal gammopathy at +90 and +180 days post transplant respectively, we have not detected any statistical difference in overall survival, GvHD development, relapse incidence and post-transplantation mortality. Vice versa, after 360 days from the transplant, among patients with a monoclonal gammopathy, was observed an increased rate of GvHD (50% vs 32%, $P = 0.07$). In those patients GvHD frequently seemed aspects of cGVHD.
Discussion: Monoclonal gammopathy, also in our experience, is common after allo-BMT. The few papers published in the past, found this laboratory alteration more frequently associated with GVHD but without any long term adverse effect. Our data would seem to confirm these conclusions. In the past the explanation for the evidence of a monoclonal gammopathy was correlated to an aberrant immune reconstitution after allo-BMT. Recently many evidence showed that B cells are involved in the pathogenesis of chronic GVHD (cGVHD) and anti-B-cell therapy has been proposed for the therapy mainly of cGVHD. We speculate that the presence of a monoclonal gammopathy after allogeneic transplant is expression of the activation of the B- 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 Interest: None Declared.

PH-P404
EXTRACORPOREAL PHOTOPHERESIS IN TREATMENT OF PATIENTS WITH STEROID REFRACTORY OR RELAPSED CHRONIC GVHD: A SINGLE CENTER RETROSPECTIVE ANALYSIS OF A ONCE WEEKLY INITIAL SCHEDULE

F. Ayuk[1,], M. Guellstorf[1], T. Wolbers[1], T. Zabelina[1], C. Wolschke[1], H. Alchalby[1], T. Stuebig[1], H. Lellek[1], A. Zander[1], N. Kroeger[1]*
[1]Clinic for Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: Extracorporeal photopheresis is increasingly used to treat chronic GVHD (cGVHD). While its limited toxicity is well established and accepted, data on efficacy are still rare und most reports so far included less than 50 patients. While the optimal schedule for ECP is still unknown, most centers use a back-to-back treatment, initially repeated weekly or every two weeks.
Materials (or patients) and Methods: In this single center analysis we retrospectively evaluated treatment success in 61 adult patients who received ECP for steroid refractory or relapsed cGVHD at our center. Treatment success was defined as "patient being alive at 6 months after initiation of ECP, having achieved a partial (PR) or complete response (CR) of cGVHD according to NIH criteria and having no relapse of underlying disease". ECP was performed once weekly for the first 4 weeks, thereafter once every two weeks for responding patients. Patients not responding and with no progression of cGVHD continued once weekly until 15 treatments. Patients progressing after 4 weeks or not responding after 15 weeks were considered treatment failure. ECP was performed using the Therakos in-line technique with UVAR XTS or Cellex systems.
Results: All patients received at least 4 ECP treatments. 50 Patients had concomitant systemic immunosuppression while 11 patients received ECP as sole systemic treatment, mainly due to intolerance of systemic immunosuppression. In all the rate of treatment success was 60.7%, with 3.3% CR ($n = 2$) and 57.4% PR ($n = 35$), while 39,3% ($n=24$) had treatment failure. Reasons for treatment failure were: non response to ECP ($n = 19$), death before 6 months ($n = 3$) and relapse of underlying malignancy ($n = 2$). Of 11 patients who received ECP as only systemic therapy, rate of treatment success was 91% (all PR, $n = 10$), with only one patient not responding to treatment.
Discussion: In this relatively large retrospective analysis, we found that once weekly ECP treatment is an effective and well tolerated schedule for patients with steroid refractory or relapsed cGVHD. Further investigation is essential to determine the optimal treatment schedule.
Disclosure of Interest: None Declared.

PH-P405
ANTI-CD20 THERAPY IN COMBINATION WITH NILOTINIB FOR THE TREATMENT OF CHRONIC GRAFT VERSUS HOST DISEASE

L. Van Der Wagen[1,2,], L. te Boome[3], E. Meijer[4], J. Kuball[3]*
[1]Hematology, UMC Utrecht, [2]Immunology, [3]Hematology, UMCU, Utrecht, [4]Hematology, VUMC, Amsterdam, Netherlands

Introduction: About 60% of all patients receiving an allo-SCT and surviving beyond day 100 develop cGvHD, assuring high morbidity within this patient group. For this reason, chronic GvHD is associated with a substantial impairment in quality of life and loss of employment in long-term allo-SCT survivors. For physicians, cGvHD poses a big challenge to treat adequately and satisfactory. The current treatment relies heavily on corticosteroids, however refractoriness is a big problem as well as the unwanted effects of longterm corticosteroid use. Therefore, new therapies are urgently needed. Earlier studies by others and us have demonstrated amelioration of chronic GvHD symptoms by B-cell depletion. In addition inhibition of the PDGF pathway by tyrosine kinase inhibitors also provided provocative data. However, complete resolution of cGVHD is usually not reported with single drug therapies and cGvHD with ulcera has so far been refractory to most experimental therapies.
Materials (or patients) and Methods: Hypothesis: We aim to test whether the sequential therapy of the anti-CD20 antibody Rituximab followed by a 6 month period of treatment with the tyrosine kinase inhibitor Nilotinib can further improve response rates.
Methods: 30 patients are treated with a combination of 4 weekly infusions of the anti-CD20 antibody Rituximab followed by a 6 month period of treatment with the tyrosine kinase inhibitor Nilotinib. Patients have been evaluated monthly.
Results: 4 patients have completed the study period whilst 7 patients are still being treated. Three out of four patients who completed the study showed a partial response. Two patients showed complete resolution of ulcerative skin lesions. Response rates for the remaining patients will be available shortly.
Discussion: The sequential therapy of B-cell depletion and tyrosine kinase inhibition might show for the first time a complete resolution of ulcerative cGvHD and provides a new and interesting alternative treatment option for this difficult patient category.
Disclosure of Interest: None Declared.

PH-P406
GRAFT-VERSUS-HOST DISEASE AFTER DONOR LYMPHOCYTES INFUSIONS IN PATIENTS WITH RELAPSED HEMATOLOGICAL MALIGNANCIES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

R. Bogdanov[1,], L. Mendeleeva[1], I. Galtseva[1], L. Kuzmina[1], O. Pokrovskaya[1], E. Parovichnikova[1], V. Savchenko[1]*
[1]Bone Marrow Transplant Department, National Research Center for Hematology, Moscow, Russian Federation

Introduction: Graft-versus-host disease (GVHD) is a serious complication after donor lymphocytes infusions (DLI), as well as part of an immunological response flowing parallel effect-graft-versus leukemia. The presence of acute or chronic GVHD is associated with a lower risk of relapse after allogeneic stem cell transplantation (allo-SCT).
Aim of this study was to investigate the frequency of GVHD depending on the scheme DLI, and the impact of GVHD on the results adoptive immunotherapy in patients with relapsed hematological malignancies after allo-SCT.

Materials (or patients) and Methods: The study comprised 44 patients (pts) (AML – 28, ALL - 4, CML - 8, MDS - 4) with relapsed hematological malignancies after allo-SCT from HLA-matched related (n = 42) and unrelated (n = 2) donors. The median age was 30 years (16 - 60 years), male - 29, female - 15. Relapse was diagnosed in 9 months (range from 2 to 51 months) after allo-BMT.
DLI were performed in two ways. The first was DLI alone (n = 15) and the second -DLI after chemotherapy (during aplasia or after reconstitution of hematopoiesis) (n = 29). The number of DLI range from 1 to 8 (median 3 DLI) per patient. Total amount of the CD3+ cells varied from 1 to 76x10^7 CD3+cells/kg (median 20,5 x10^7 CD3+cells/kg). All pts received 2 - 6 MUE Interleukin-2 (IL-2) subsequently after DLI. Chimerism was monitored by PCR analysis (VTTR and STR), and by FISH - analysis for centromers of X and Y – chromosomes.
Results: Remission with full donor chimerism was achieved in 25 (57%) of 44 pts. The median duration of remission with full donor chimerism was 8 months (2 to 162 months). Acute GVHD after DLI was diagnosed in 16 pts: I-II grade - 5 (31%); III-IV grade - 11 (69%). Chronic GVHD after DLI was diagnosed in 13 pts (30%): limited - 8 pts (62%), extensive - 5 pts (38%). Acute and chronic GVHD were associated with full donor chimerism (88% and 92%, respectively).
Chronic GVHD after DLI significantly influenced on the overall survival. The 5-year overall survival in pts after DLI with chronic GVHD was 45% and without chronic GVHD - 17% (P= 0,01).
The effectiveness of treatment is two times higher after DLI with chemotherapy (33% and 69%, respectively) (P = 0.03). The frequency of acute and chronic GVHD was 2 and in 1.5 times higher, respectively.
Discussion: DLI after chemotherapy is effective in patients with relapsed hematological malignancies after allo-SCT. GVHD after DLI is associated with longer overall survival (P = 0.01).
Disclosure of Interest: None Declared.

PH-P407

A LIMITED SAMPLING STRATEGY FOR THERAPEUTIC DRUG MONITORING OF MYCOPHENOLATE MOFETIL FOR PROPHYLAXIS OF ACUTE GRAFT VERSUS HOST DISEASE IN ALLOGENEIC STEM CELL TRANSPLANTATION

V. Gota[1,], K. Pathak[1], S. Mittal[2], M. Gurjar[1], A. Patil[1], B. Bagal[2], N. Khattry[2]*
[1]Department of Clinical Pharmacology, [2]Bone Marrow Transplant Unit, Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Navi Mumbai, India

Introduction: Mycophenolate mofetil (MMF) is used for prevention of acute graft versus host disease (aGVHD) in allogenic hematopoietic stem cell transplantation (alloHSCT). We explored the feasibility of limited sampling strategy (LSS) for therapeutic drug monitoring (TDM) of MMF in alloHSCT setting.
Materials (or patients) and Methods: All patients received GVHD prophylaxis with oral MMF 600 mg/m^2 q12h along with cyclosporine as per institutional practice. Intensive blood sampling was done on day 7 of MMF treatment prior to (0 hr) and at 0.5, 1, 2, 4, 6, 8 and 12 after the morning dose. Plasma mycophenolic acid (MPA) concentrations were measured by HPLC assay. Area Under Concentration-time Curve (AUC) was calculated by linear trapezoid rule. Acute GVHD (aGVHD) was defined as GVHD occurring within 100 days of transplant. The study was conducted in two phases. Phase 1 (Feasibility): MMF monitoring was deemed necessary if at least six out of first 20 patients failed to achieve total MPA exposure (defined by MPA $AUC_{0\text{-}12}$) of 30 mg.h/L. This cut-off was based on recommended MPA exposure in solid organ transplant. Phase 2a: To determine the discriminatory potential of MPA $AUC_{0\text{-}12}$ to differentiate between those who developed aGVHD and those who did not. Discriminatory potential of MPA $AUC_{0\text{-}12}$ was determined by Receiver Operating Characteristic (ROC) curve. Phase 2b: To develop a limited sampling strategy (LSS) that could accurately predict $AUC_{0\text{-}12}$ from smaller number of sampling points.
Results: Fifteen out of 20 patients did not achieve the AUC recommended for solid organ transplant (30 mg.h/L). Additional 12 patients were enrolled for phase 2 of the study. Thirteen out of 32 patients developed aGVHD. The average MPA exposure was 23.99 mg.h/L. An AUC of 25 mg.h/L could discriminate between responders (no GVHD) and non-responders with highest sensitivity and specificity. Patients with $AUC_{0\text{-}12}$ ≤25 mg.h/L had a higher risk of developing aGVHD (RR-1.98, 95% CI-0.73, 3.14; P=0.12) compared to those who achieved higher exposure. $AUC_{1\text{-}4}$ had highest correlation with $AUC_{0\text{-}12}$ (r=0.75, P<0.001) compared to other limited sample AUCs including $AUC_{0.5\text{-}2}$ (r=0.61, P<0.01) and $AUC_{2\text{-}6}$ (r=0.69, P<0.001). These strategies require only 3 sampling time points each. $AUC_{0\text{-}12}$ can be estimated from $AUC_{1\text{-}4}$ using the equation $AUC_{0\text{-}12} = 1.18(AUC_{1\text{-}4}) + 9.81$.
Discussion: Although TDM is practiced routinely for solid organ transplants with recommendation to achieve MPA AUC between 30-60 mg.h/L, no such consensus exists in HSCT. Several investigators in the past have been unsuccessful in evolving a strategy for TDM of MMF in HSCT. Our data clearly demonstrate that patients with MPA exposure ≤25 mg.h/L tend to have a two-fold higher risk of developing GVHD compared to those with higher exposure. Moreover, we could demonstrate that total AUC can be estimated with reasonable accuracy from just three point sampling. The average MPA exposure was found to be significantly lower in our setting compared to that reported in solid organ transplant. This could be attributed to concomitant use of cyclosporine in this setting which limits the entero-hepatic recirculation if MPA. The three point sampling can be done for a total cost of Rs. 1500 (Approximately 19 euros). Thus we have developed a cost-effective LSS for TDM of oral MMF prophylaxis against aGVHD in alloHSCT.
Disclosure of Interest: None Declared.

PH-P408

GENETIC BACKGROUND OF IMMUNE REACTIONS AFTER PEDIATRIC STEM CELL TRANSPLANTATION-WHOLE GENOME EXPRESSION STUDY

S. Skoczen[1,2,], J. Gozdzik[1,2], M. Bik-Multanowski[3], A. Krasowska-Kwiecien[1], A. Dluzniewska[1], O. Wiecha[1], W. Czogala[1], A. Grabowska[3], J. Jozef Pietrzyk[3], M. Siedlar[2], K. Fijorek[4]*
[1]Transplantation Centre, University Children's Hospital, [2]Department of Immunology, Chair of Clinical Immunology and Transplantation, Polish-American Institute of Pediatrics, [3]Chair of Pediatrics, Polish-American Institute of Pediatrics, Jagiellonian University Medical College, [4]Department of Statistics, University of Economics, Krakow, Poland

Introduction: Immune reactions are common complications of allogeneic hematopoietic stem cell transplantation (HSCT) and include: graft-versus-host disease (GvHD, acute-50%, chronic 40-70%), graft rejection-12%. To explore the pathogenesis of immune complications after allogeneic HSCT, we carried out genome wide transcriptional profiling of RNA extracted from peripheral blood mononuclear cells collected before and after transplantation followed by pathway enrichment analysis in an attempt to identify biological pathways that were preferentially associated with immune reactions induced by the procedure.
Materials (or patients) and Methods: Study group composed of 44 patients (pts.) referred to HSCT 1.5-19 (average 9.9) years old, 31 boys and 13 girls. Children were diagnosed with neoplastic-32 (73%) and non-neoplastic-12 (27%) diseases. Blood was collected before transplantation and 7 months in average after transplantation. The second collection was performed in 27 pts. 2.8-19.5 (average 10.4) years old, 20 boys and 7 girls. All patients with two collections were treated with allogeneic transplantations according to EBMT protocols including: match unrelated donor- 60%, match sibling donor-33% and match family donor-7% transplantations. The mRNA samples isolated from peripheral blood mononuclear cells were evaluated for gene expression with the use of GeneChip® Human Gene 1.0 ST microarrays. DAVID annotation tools (DAVID Bioinformatics Resources 6.7) were used to explore which predefined gene sets were significantly enriched before HSCT compared to after HSCT. The KEGG (Kyoto Encyclopedia

of Genes and Genomes; www.genome.jp/kegg) was selected for analysis. A list of 250 genes differentially expressed between before and after-HSCT samples with *P*-values adjusted for multiple testing below 0.01 and with fold change greater than 1.5 was used as input for pathway enrichment analysis in DAVID.
Results: There were no signs and symptoms of the primary disease in patients at the second collection. Graft versus host disease was diagnosed in 52% of pts.(acute-33%, chronic-15%, overlap syndrome-4%). Organ localizations were as follows: skin-50%, gut-21%, skin/gutt-7%, skin/liver-15%, skin-gut/liver-7%. No patients presented with graft rejection. Four genes pathways were detected: "Allograft rejection", "Graft-versus-host disease", "Autoimmune thyroid disease" and "Type I diabetes mellitus" all connected with immune reactions after transplantation. The relationships between genes in the pathways were significant ($P<0.05$).
Discussion: Allogeneic transplantations by definition induce immune reactions with varied intensity. As mononuclear cells are involved in the process, activation of gene pathways regulating immune response should be visible. Graft-versus-host disease is the leading cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). So far, there is no validated diagnostic or predictive blood biomarker for GvHD which could improve diagnosis and prognosis and help guide therapeutic interventions. Our study confirm activation of genes responsible for aggression of donor/recipient immune system against donor/recipient cells. Further study will explain if whole genome expression profile may serve as a biomarker for immune reactions observed after stem cell transplantation in children.
The study was sponsored by grants number NN 407 198737 and K/ZDS/003825.
Disclosure of Interest: None Declared.

PH-P409
PRE-TRANSPLANT CONDITIONING RESULTS IN OVERT DERMAL RECRUITMENT OF HLA CLASS II POSITIVE MACROPHAGE-LIKE CELLS AFTER TRANSPLANTATION

B. van der Zouwen[1], A. B. Kruisselbrink[1], P. van Balen[1], M. Eefting[1], K. Jordanova[1], J. Falkenburg[1], I. Jedema[1,]*
[1]Hematology, Leiden University Medical Center, Leiden, Netherlands

Introduction: Infusion of mature donor T cells with or early after allogeneic stem cell transplantation (alloSCT) is associated with a high risk for the induction of graft versus host disease (GvHD) in different organs, including the skin. Postponed application of donor T cells at 6 months after T cell depleted alloSCT significantly reduced the risk for coinciding acute GvHD. In this study we investigated the effect of pre-transplant conditioning on the numbers and phenotype of antigen presenting cells (APCs) and T cells in the skin.
Materials (or patients) and Methods: Skin biopsies were routinely taken after informed consent of the patients at 0, 3, 6, 12 and 26 weeks after alloSCT at the site of the bone marrow aspiration from patients who did not display signs of GvHD. Skin biopsies taken at similar time-points from patients who underwent an autologous transplantation (autoSCT) served as autologous controls and skin biopsies taken from healthy individuals served as normal controls. Diagnostic biopsies taken from affected skin (GvHD/dermatitis) were analyzed in parallel. Biopsies were cryopreserved and stored. Cryo-sections were stained with antibodies for HLA-class II, HLA-DP, HLA-DR, HLA-DQ, CD3, CD8, CD68, CD54, and CD40, counterstained with fluorescently labeled secondary antibodies and analyzed by fluorescent microscopy. In biopsies taken from sex-mismatched donor/patient pairs we assessed the origin of the specific cell types by combining the surface immune-staining with XY FISH.
Results: At the moment of transplantation directly following the conditioning regimen no significant changes were observed compared to normal skin. At 3 and 6 weeks after the transplantation we observed an increased number of HLA class II positive macrophage-like cells in the dermal region of the skin. In patients without GVHD, this was not associated with a significant increase in the number of local T cells. No overt differences associated with the type of pre-transplant conditioning (myelo ablative (MA) versus reduced intensity (RIC)) were observed. Both the HLA class II positive cells and the local T cells were found to be of patient origin at these time points. We observed a remarkable further increase in the number of HLA class II positive cells in the biopsies taken at 3 months after transplantation, which was still pronounced at 6 months after transplantation in both the alloSCT and autoSCT biopsies. This increase was most pronounced in patients who received MA conditioning, even in the absence of any sign of GvHD. The origin of the HLA-class II positive cells and the local T cells differed between patients. We observed complete patient and complete donor origin, as well as mixed origin. In contrast to the HLA class II positive cells recruited in affected skin from patients with GvHD or dermatitis, the HLA class II positive cells in the routine biopsies taken after alloSCT and autoSCT did not express dendritic cell like markers, including adhesion and costimulatory molecules.
Discussion: Pre-transplant conditioning results in overt recruitment of HLA class II expressing macrophage-like cells after transplantation. Despite their relatively unprofessional APC phenotype, these cells may amplify the GvHD response resulting in the local formation of a pro-inflammatory milieu. The origin of the class II positive cells and the patrolling T cells will dictate the chance of local GvHD induction.
Disclosure of Interest: None Declared.

PH-P410
EARLY OCCURRENCE OF SEVERE HYPOALBUMINEMIA IS ASSOCIATED WITH ACUTE GVHD ONSET AND GRADING AFTER ALLOGENEIC HCT

M. A Kharfan-Dabaja[1,], B. Miladinovic[2], T. Nishihori[1], E. Ayala[1], A. Tsalatsanis[2], J. Mathews[3], A. Mina[4], J. Pidala[1], T. Field[1], H. F. Fernandez[1], B. Betts[1], R. Hillgruber[3], D. Coyle[3], M. Tomblyn[1], A. Kumar[2], C. Anasetti[1]*
[1]Blood and Marrow Transplantation and Oncologic Sciences, H. Lee Moffitt Cancer Center and University of South Florida College of Medicine, [2]Center for Evidence-Based Medicine, University of South Florida College of Medicine, [3]Blood and Marrow Transplantation, H. Lee Moffitt Cancer Center, Tampa, United States, [4]Faculty of Medicine, American University of Beirut, Beirut, Lebanon

Introduction: Our group has shown that severe hypoalbuminemia (‹3.0 g/dL) at day +90 post allografting is an adverse prognostic factor for non-relapse mortality and overall survival in patients with AML and MDS. However, the chronological relationship between development of severe hypoalbuminemia and acute GVHD onset and grading is not clearly understood.
Materials (or patients) and Methods: We analyzed 1066 patients who received an allogeneic HCT at the H. Lee Moffitt Cancer Center between January 01, 2005 and March 31, 2013. Serum albumin levels were obtained for all subjects from day of stem cell infusion (day 0) until day +100 post-transplantation whenever available. The association between occurrence of severe hypoalbuminemia (defined as a serum albumin level ‹ 3.0 g/dL) and onset of acute GVHD and its severity grading was assessed using time-to-event analysis where time to follow up was defined as time from day 0 to the first serum albumin level below 3.0 g/dL. Differences in time-to-event according to the severity of acute GVHD were assessed using the Cox proportional hazard model. The significance level was set at ≤ 0.05. All analyses were performed using Stata statistical analysis software version 13.
Results: The median age for all subjects was 52 (18-76) years. The most common preparative regimen consisted of fludarabine and pharmacokinetically-targeted intravenous busulfan (FLU-BU) in 72% of cases. Acute GVHD prophylaxis consisted of a calcineurin-inhibitor in combination with methotrexate (53%) or sirolimus (25%), or mycophenolate mofetil (22%). These and other patient characteristics are shown (Table 1). Median time to ANC engraftment was 16 days. Time-to-event analysis demonstrated severe hypoalbuminemia by day +20 in 804 (75%) subjects.

Severe hypoalbuminemia was not associated with a difference in the cumulative incidence of acute GVHD (all grades) whether it occurred before day +20 [OR=0.93 (95%CI=0.77, 1.11), *P*=0.41] or ≥ day +20 [OR=1.23 (95%CI=0.76, 1.98), *P*=0.41]. However, occurrence of severe hypoalbuminemia before day +20 post allogeneic HCT was associated with an earlier median onset of acute GVHD (27 vs. 32 days, p ‹0.0001). In addition, an association was observed with acute GVHD grading when severe hypoalbuminemia occurred before day +20 [OR=1.12 (95%CI=1.02, 1.24), *P*=0.02] or ≥ day +20 [OR=1.33 (95%CI=1.09, 1.62), *P*=0.004].

Table 1. Patient, disease, and treatment related characteristics (*N*=1066)

Variables	Results
Recipient median age (range), years	52 (18-76)
Recipient gender (%)	F= 43%
	M= 57%
Donor recipient gender (%)	
F→M	22%
F→F	19%
M→M,F	59%
Donor source (%)	
MRD	37%
MUD	42%
MMD	16%
Cord blood	5%
Cell source (%)	
PBSC	94%
BM	1%
Cord blood	5%
Diagnoses (%)	
AML/MDS	47%
Lymphomas	16%
ALL	11%
Others	26%
Preparative regimen (%)	
FLU-BU*	72%
Others	28%
GVHD prophylaxis (%)	
MTX-based	53%
Sirolimus-based	25%
MMF-based	22%
Recipient/Donor CMV serologic status	
+/+	35%
+/-	32%
-/+,-	33%

*includes pharmacokinetically-targeted IV busulfan with a median daily AUC ranging from 3500 to 9000 µmoles min/L.

Discussion: We demonstrate a chronological association between occurrence of severe hypoalbuminemia before day +20 post-allogeneic HCT and time of acute GVHD onset. Moreover, patients who develop severe hypoalbuminemia have a higher likelihood of developing a higher acute GVHD grade.
Disclosure of Interest: None Declared.

PH-P411

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN INTERLEUKIN 1A AND 1B GENES ASSOCIATE WITH PATIENT SUSCEPTIBILITY TO COMPLICATIONS AFTER HLA-IDENTICAL SIBLING ALLO-SCT.

E. Buces[1,2], C. Martínez-Laperche[2,3], M. González-Rivera[2,3], A. Bosch-Vizcaya[4], B. Martín-Antonio[5], V. Guillem[6], J. Nieto[7], M. González[8], R. de la Cámara[9], S. Brunet[10], I. Espigado[11], C. Vallejo[12], A. Sampol[13], D. Serrano[2,3], M. Kwon[2,3], J. Gayoso[2,3], P. Balsalobre[2,3], C. Solano[14], A. Urbano-Izpizua[5], A. Jiménez-Velasco[15], D. Gallardo[16], J. Díez-Martín[2,3], I. Buno[2,3] and on behalf of the GvHD/Immunotherapy committee of the Spanish Group for Hematopoietic Transplantation (GETH)*
[1]Department of Hematology, HGU G.Marañón, [2]IIS G.Marañón, [3]HGU G.Marañón, Madrid, [4]ICO, H.J.Trueta, Girona, [5]H.Clinic, IDIBAPS, Barcelona, [6]H.Clínico, Valencia, [7]HU M.Meseguer, Murcia, [8]HU, Salamanca, [9]HU La Princesa, Madrid, [10]HSC Sant Pau, Barcelona, [11]HU V.Rocío, Sevilla, [12]HC Asturias, Oviedo, [13]HU S.Espases, Mallorca, [14]HCU, Valencia, [15]HRU, Málaga, [16]ICO, HJ Trueta, Girona, Spain

Introduction: The interleukin 1 (IL1) cytokine family includes two major pro-inflammatory molecules, IL1A and IL1B, which play a key regulatory role in initiating and modulating immune responses. Polymorphisms in IL1 genes have been shown to be associated with plasma levels of these cytokines and have been associated with the development of complications after SCT. However, reported data are inconsistent as a result of the heterogeneity of the transplant cohorts or the small number of subjects. The objective of the present study was to evaluate the impact of IL1 SNPs on the development of complications and survival in a large cohort of HLA-identical sibling allogeneic stem cell transplant patients.
Materials (or patients) and Methods: The study includes 509 patients with hematological malignancies and their donors (1018 individuals) from the DNA Bank of the Spanish Group for Haematopoietic Stem Cell Transplantation (GETH; Table 1). Four SNPs were genotyped, one in the promoter of IL1A gene (rs763780), 2 in the promoter of IL1B (rs16944, rs1143627) and one in IL1B exon 5 (rs1143634), by mass spectrometry (MALDI-TOF, Sequenom MassARRAY; CEGEN).
Results: The association between genotypes and complications after allo-SCT after univariate analysis (Table 2) are as follows. Acute GVHD: patients harbouring the minor allele C for rs1143627 showed a higher incidence of grade II-IV and III-IV aGVHD. Patients with genotype GG for the SNP rs16944 showed a lower incidence of grade II-IV and III-IV aGVHD. Chronic GVHD: Donors and recipients with genotype CC for rs1143634 or allele C for rs1800587 associate with a lower risk of cGVHD. In addition, donors with allele C for rs1143634 associate with a lower risk of extensive cGVHD. Relapse: A higher risk of relapse was observed in patients harbouring genotype CC for rs1800587 or genotype CC for rs1143634 as well as in patients transplanted from donors CC for rs1143627. On the other hand, donors with the major allele G for rs16944 associate with a lower incidence of relapse. All polymorphisms in the IL1 gene remained as independent risk factors in the multivariate analysis (Table 3), except for the association of patients with allele C for rs1800587 and the development of chronic GVHD and that of donors with allele C for rs1143634 and the risk of extensive chronic GVHD.
Discussion: This results further support the idea of a genetic predisposition to certain complications after allo-SCT. IL-1A and IL-1B SNP genotyping might be useful to anticipate complications after sibling HLA-identical allo-SCT and, therefore, to improve the clinical management of transplanted patients.
Disclosure of Interest: None Declared.

Table 1. Patient characteristics

	N (%)
N=509 SCT	
Median age (range)	45 (0-69)
Gender	
Male patients	308 (60.5)
Female don/male rec	130 (25.5)
Diagnosis	
AML/ALL/MDS	287 (56.4)
NHL/HD/MM	127 (24.9)
Disease status at SCT	
CR	239 (47)
Conditioning regimen	
Myeloablative	318 (62.5)
Graft vs host disease (GVHD)	
acute GVHD (aGVHD)	
Grades II-IV	137 (26.9)
Grades III-IV	51 (10)
chronic GVHD (cGVHD)	
Limited + Extensive	172 (33.8)
Extensive	99 (19.4)
Relapse	
Incidence	150 (29.5)
Deaths	221 (43.4)
Relapse	92 (18.1)
GVHD	33 (6.5)
Infection	23 (4.5)
Other	73 (14.3)

Table 2. Univariate

	Gene	SNP	Don/Rec	Genotype	HR/OR	95%CI	p-value
aGvHD grades II-IV	IL1B	rs1143627	Recipient	CT+CC	1.63	1.12-2.36	0.010
	IL1B	rs16944	Recipient	GG	0.57	0.39-0.84	0.005
aGvHD grades III-IV	IL1B	rs1143627	Recipient	CT+CC	2.4	1.22-4.73	0.011
	IL1B	rs16944	Recipient	GG	0.35	0.17-0.72	0.004
cGvHD	LI1B	rs1143634	Donor	CC	0.26	0.07-0.94	0.039
	LI1B	rs1143635	Recipient	CC	0.62	0.39-0.99	0.043
	LI1A	rs1800587	Donor	CT+CC	0.3	0.11-0.84	0.021
	LI1A	rs1800587	Recipient	CT+CC	0.37	0.15-0.90	0.028
Extensive cGvHD	IL1B	rs1143634	Donor	CT+CC	0.35	0.13-0.98	0.048
Relapse	IL1B	rs1143627	Donor	CC	1.63	1.07-2.49	0.022
	IL1B	rs1143634	Recipient	CC	1.52	1.05-2.20	0.028
	IL1B	rs16944	Donor	AG+GG	0.63	0.41-0.96	0.03
	IL1A	rs1800587	Recipient	CC	1.59	1.11-2.27	0.012

Table 3. Multivariate

	Variable	HR/OR	95%CI	p-value
aGvHD grades III-IV	Female Donor/Male Recipient	2.12	1.17-3.84	0.013*
	Myeloablative vs reduced intensity conditioning	0.69	0.38-1.24	0.214
	IL1B rs1143627 Recipient CT+CC	2.26	1.15-4.47	0.019*
	IL1B rs16944 Recipient GG	0.37	(0.18-0.78	0.009*
cGvHD	Patient age (years; median 45)	1.33	0.77-2.30	0.312
	Female Donor/Male Recipient	1.55	0.86-2.79	0.143
	Stem cell source (bone marrow *vs* peripheral blood)	2.21	1.24-3.96	0.008*
	Myeloablative *vs* reduced intensity conditioning	0.74	0.40-1.36	0.33
	IL1B rs1143634 Donor CC	0.55	0.34-0.90	0.018*
	IL1B rs1143634 Recipient CC	0.52	0.32-0.85	0.009*
	IL1A rs1800587 Donor CT+CC	0.29	0.10-0.86	0.025*
	IL1A rs1800587 Recipient CT+CC	0.43	0.17-1.10	0.077
Extensive cGvHD	Patient age (years; median 45)	1.32	0.74-2.36	0.355
	Female Donor/Male Recipient	2.37	1.32-4.25	0.004*
	Stem cell source (bone marrow *vs* peripheral blood)	4.69	2.24-9.81	<0.001*
	Myeloablative vs reduced intensity conditioning	1.05	0.57-1.93	0.868
	IL1B rs1143634 Donor CT+CC	0.35	0.11-1.12	0.076

PH-P412

VITAMIN D DEFICIENCY AND COMPLICATIONS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

L. Von Bahr[1,2,], J. Alm[1], K.-J. Malmberg[3], A. Björklund[3], M. Labopin[4], P. Ljungman[2], K. Le Blanc[1]*

[1]Laboratory medicine, Karolinska Insitutet, [2]Hematology Centre, Karolinska University Hospital Huddinge, [3]Centre of Infectious Medicine, Karolinska Insitutet, Stockholm, Sweden, [4]Universite Pierre et Marie Curie, Paris, France

Introduction: Vitamin D has emerged as a central player in the immune system, with deficiency being implicated in the pathogenesis of several autoimmune diseases, including chronic graft-versus-disease (cGvHD). This study aimed to evaluate possible associations of vitamin D deficiency prior to allogeneic stem cell transplantation (ASCT) with complications after ASCT, with cGvHD of grade moderate to severe as the main endpoint.

Materials (or patients) and Methods: This is a retrospective cohort analysis on 162 patients who have undergone ASCT at Karolinska University Hospital, Huddinge, between 2005 and 2011, with stored serum samples available. Children under the age of 12 and cord blood transplantations were excluded. A total of 137 patients were eligible for evaluation regarding cGvHD after excluding patients with graft rejection or with a survival of less than 100 days after transplantation.

Patient data were retrospectively collected from clinical patient files. For chronic GvHD diagnosis and scoring the NIH consensus criteria were used and both classical chronic GvHD and overlap syndrome were included, but not late onset acute GvHD.

Rejection was defined as lack of engraftment, engraftment with recipient cells or later developing full (>95%) recipient chimerism in the absence of relapse of the underlying disease.

Vitamin D was analyzed as 25-OH-cholecalciferol from cryopreserved serum samples using a chemiluminescence method (CLIA), performed by the laboratory for clinical chemistry at Karolinska University Hospital, Solna,

Results: Median level of vitamin D before transplantation was 42 nmol/l (range 10-118), hence below the level of insufficiency (50 nmol/l).

For chronic GvHD grade moderate to severe, the cumulative incidence was 56% when vitamin D level prior to transplantation was below 25 nmol/l, 34% when between 25 and 50 nmol/l and 26% when vitamin D was above 50 nmol/l. This was confirmed in a multivariate analysis including vitamin D level, age, conditioning, anti-T-treatment and sex mismatch (female donor to male recipient) as covariates with death as competing risk, identifying vitamin D level prior to transplant as a significant independent risk factor for development of cGvHD (P=0.007).

Incidence of acute GvHD grade II-IV showed no correlation to vitamin D level.

There was a trend towards lower overall survival in the vitamin D-deficient patients, which was significant at the median vitamin D level in the cohort (42 nmol/l, P=0.03).

Patients with graft rejection tended to have lower vitamin D-levels. As 11 out of 12 cases of rejection were in patients with reduced intensity (RIC) conditioning, we chose this subgroup for a post-hoc analysis. This showed a median vitamin D level of 34 nmol/l in patients with rejection as compared to 44 in the others (P =0.017).

Discussion: In this study, we confirm the previous findings by Glotzbecker *et al* (2012) of an association between low levels of vitamin D before transplantation and increased incidence of chronic GvHD.

An unexpected finding was the indication of an association between low levels of vitamin D and graft rejection. These results must be interpreted with caution as rejection was not a predefined endpoint and the effect was in the RIC subgroup, but are interesting.

These findings could be highly relevant for the care of ASCT patients worldwide, and prospective, randomized studies on the effect of vitamin D supplementation are therefore needed.

Disclosure of Interest: None Declared.

PH-P413

ORAL METRONIDAZOLE IN COMBINATION WITH QUINOLONES VS ORAL QUINOLONES ALONE FOR THE PREVENTION OF AGVHD

E. Garcia Torres[1,], D. Buenasmañanas[1], G. Rodriguez[1], C. Martin[1], J. Serrano[1], S.-G. Joaquin[1], C. Chic Acevedo[1], J. Casaño[1], R. Rojas[1]*
[1]Hematology, University Hospital Reina Sofia, Córdoba, Spain

Introduction: Previous studies have shown that germ-free or completely decontaminated rodents showed a decrease in aGVHD event in recipients of MHC mismatched blood marrow transplants. Oral metronidazole (MTZ) reduced the incidence of aGvHD in matched sibling donor in allo-HSCT. We analyze if the use of oral metronidazole with quinolones vs quinolones alone decreases the incidence of aGvHD in unrelated donor (UD) allo-HSCT.
Materials (or patients) and Methods: We collected a total of 123 consecutive UD Allo-HSCT from 2001 to 2012 submitted to a HSCT in our center. Our study compared two different regimens of intestinal bacterial decontamination in order to investigate the incidence of aGVHD and were classified into two groups; Arm A: oral metronidazole (500 mg tid) and quinolones (ciprofloxacin 500 mg td/levofloxacin 500mg od) (*n*=65) and Arm B: quinolones alone (*n* = 58). Both groups were similar in age, sex, underlying disease and aGVHD prophylaxis. There were differences in stem cell source and conditioning regimen. In group A (with MTZ), 22 patients received cord blood (cb), 19 bone marrow (bm) and 24 peripheral blood (pb). In group B, 11 patients received cb, 29 bm and 18 pb ($P = 0.044$). The primary objective was to evaluate the incidence of aGVHD (grades 0-I vs II-IV) between groups. Secondly we analyze overall survival and transplant-related mortality at +100 days (TRM100). The data analysis was performed with SPSS 17.1 software, using the Kaplan-Meier tests, LonRank test, T-student and Chi-squared test.
Results: the incidence of aGVHD was 51% (*n*=46) for group A and 49% (*n*=45) for group B with no statistical differences between groups. When we analyzed the incidence of aGVHD grades 0-I vs II-IV we didn´t find statistical differences (A=51% Vs B=36% and A=49% vs B=64%) (*P*=0.2). TRM +100 was 14% in the global serie (*n* = 14), with no statistical differences between groups (*P*=0.1). The most frequent causes of death at +100 days were infection (*n*=7) and aGVHD (*n*=2).
Discussion: In our experience the use of oral metronidazole do not decrease the incidence of aGVHD in unrelated donor Allo-SCT.
Disclosure of Interest: None Declared.

PH-P414

DONOR B CELLS POPULATIONS, IFNG-PRODUCING T CELLS, AND CD56BRIGHT NK CELLS CORRELATE WITH DEVELOPMENT OF CHRONIC GVHD IN G-CSF STIMULATED SIBLING DONOR PERIPHERAL BLOOD AND BONE MARROW: RESULTS FROM THE CANADIAN BMT GROUP 0601 RANDOMIZED, PHASE III TRIAL

K. Schultz[1,], A. Kariminia[2], S. Ivison[3], M. Levings[4], R. Broady[5], M. Aljurf[6], G. Devins[7], H. Kerr[8], S. J Lee[9], D. Szwajcer[10], C. Toze[5], J. Rozmus[11], P. Subrt[11], T. Panzarella[12], S. Couban[13], R. Foley[14]*
[1]Childhood Cancer and Blood Research, University of British Columbia, BC Children's Hospital, [2]Pediatrics, Child Cancer and Blood Research, University of British Columbia/CFRI, [3]Pediatrics, Childhood Cancer and Blood Research, [4]Surgery, University of British Columbia, [5]Leukemia BMT Program, BC Cancer Agency, Vancouver, Canada, [6]Oncology Center, King Faisal Specilaist Hospital & Research Center, Riyadh, Saudi Arabia, [7]Ontario Cancer Institute, Toronto General Hospital, Toronto, [8]Leukemia BMT Program, BC Cancer Center, Vancouver, Canada, [9]Fred Hutchinson Cancer Research Center, Seattle, United States, [10]Cancer Care Manitoba, Winnipeg, [11]Childhood Cancer and Blood Research, CFRI, University of British Columbia, BC Children's Hospital, Vancouver, [12]Dalla Lana School of Public Health, University of Toronto, Toronto, [13]Capital Health Blood and Marrow Transplant Program, Dalhousie University, Halifax, [14]McMaster University, Hamilton, Canada

Introduction: G-CSF stimulated peripheral blood (G-PB) as a donor source has a higher frequency of chronic GVHD (cGVHD). Recently the Canadian BMT Group (CBMTG) performed a prospective Phase III clinical trial entitled "A Randomized Multicentre Study Comparing G-CSF Mobilized Peripheral Blood and G-CSF Stimulated Bone Marrow in Patients Undergoing Matched Sibling Transplantation for Hematologic Malignancies (CBMTG 0601)". The donor grafts were evaluated for immune cell populations that may predict the onset of cGVHD.
Materials (or patients) and Methods: This study evaluated donors enrolled on CBMTG0601 for graft lymphocyte populations. Proportions of specific lymphocytes in donor grafts were associated with cGVHD in the recipient using logistic regression. Associations were significant if the corresponding *P*-value was less than 0.05. Recipients were considered cGVHD positive if diagnosed with extensive cGVHD and cGVHD negative if free of extensive cGVHD for a minimum of 12 months post transplant. Relapsed recipients and those who died without previous diagnosis of GVHD were excluded from analyses. Cell phenotypes and cytokine production of lymphocytes in the donor grafts were analyzed by flow cytometry in a set of panels that enabled identification of distinct NK, T and B cell subsets.
Results: Analyses of $cGVHD^{-}$ vs. $cGVHD^{+}$ showed the following associations: higher proportions of IFNg-producing T helper cells and $CD56^{bright}$ NK cells in donor grafts were associated with a lack of cGVHD ($P<0.005$ and $P<0.05$, respectively). On a multinomial logistic analysis, there was a higher graft proportion of immature B cells ($CD19^{+}IgD^{-}CD27^{-}$), a lower proportion of memory B cells ($CD19^{+}CD27^{+}$), and higher proportion of TLR9+ B cells ($P<0.01$) with development of de novo cGVHD. Evaluation for differences between G-BM and G-PB showed that there were significant difference between both memory B cells ($P = 0.002$) and immature B cells ($P<0.0001$).
Discussion: We have identified two population, IFNg-producing $CD4^{+}$ T cell and $CD56^{bright}$ NK cells that are associated with tolerance after both G-BM and G-PB transplantation. We found three donor B cell populations, including immature and memory B cell populations associated with development of de novo cGVHD in donor grafts that impact the development of cGVHD. These findings require confirmation in a larger population but suggest that donor B cell populations rather than T cell populations may have the strongest impact on the high frequency of cGVHD after G-PB transplantation.
Disclosure of Interest: None Declared.

PH-P415

SCLERO-CORNEAL LENSES SAFE AND EFFICIENT FOR THE TREATMENT OF KERATOCONJUNCTIVITIS SICCA IN PATIENTS WITH REFRACTORY OCULAR GVHD.

L. Magro[1,], M. Richet[2], V. Coiteux[1], F. Ernould[2], R. Dulery[1], T. Randon[2], P. Labalette[2], I. Yakoub-Agha[1]*
[1]maladies du sang, [2]Ophtalomologie, CHRU, LILLE, France

Introduction: Keratoconjunctivitis sicca syndrome (KCS) due to chronic GvHD (cGvHD) is responsible for major alteration in quality of life of patients undergoing allogeneic stem cell transplantation (allo-CST). The conjunctival fibrosis secondary to inflammation is often slow and subtle and is responsible for impaired corneal and conjunctival epithelial surfaces potentiated by tear quantitative and qualitative deficiency. Treatment of KCS remains disappointing; variable success in the correction of the conjunctival dryness has been obtained with variety of topical treatments with or without systemic immunosuppressive agents. These treatments, though, do not seem to have any effect on sicca symptoms and patients' quality of life. Sclero-corneal lenses bring a valid therapeutic option by creating a pre-corneal reservoir of tears allowing permanent lubrication of the epithelium, the protection of the corneal surface against the eyelid and ciliary mechanical friction and against environmental stresses and the creation of a uniform refractive surface to be taken into optimum optical load and stable visual acuity.
Materials (or patients) and Methods: We describe the safety and efficacy of Sclero-corneal lenses in a retrospective analysis of 7 patients with KCS due to cGvHD following allo-CST. Table 1

summarizes patients' and GvHD characteristics. All patients had superficial punctate keratitis refractory to standard treatments. Evaluation of patients was carried out by the same ophthalmologist and hematologist. cGVHD was recorded according standard criteria. Ocular surface disease index (OSDI) and Oxford score were used to evaluate ocular symptoms. The scale of "Monoyer" was used and converted into visual acuity "LOG MAR" for comparative purposes of the study.
Results: All patients but one agreed to hold the lenses. The 6 remaining patients were equipped with lenses type ICD (LCS Company). With a median follow-up of 6 months (4-13) all patients have experienced an improvement in their quality of life with a clear improvement of dry-eye symptoms. This quality of life is also improved by decreasing the frequency of eye-drop instillation and the attenuation of the discomfort and post-instillation visual fluctuation. We observed 100% improvement in OSDI score with an average improvement of 67.08 points, 100% improvement or stability of visual acuity with an average gain of 0.23 LOG MAR acuity and 100% improvement or stability of the Oxford score with a mean gain of 1,917 points.
Discussion: Despite its limited size, this cohort of patients treated with sclero-corneal lenses is promising. Whenever possible, this approach should be considered in patients experiencing KCS due to cGVHD.
Disclosure of Interest: None Declared.

PH-P416
IMPACT OF NIH GLOBAL SCORING SYSTEM OF CHRONIC GRAFT-VERSUS-HOST DISEASE ON THE OUTCOMES FOLLOWING STEM CELL TRANSPLANTATION

D. Stamatovic[1,], M. Elez[2], B. Balint[2], O. Tarabar[2], S. Marjanovic[2], A. Zivanovic Ivic[2], B. Todoric Zivanovic [2], M. Strnad[2], Z. Tatomirovic[2], O. Tasic [2], G. Ostojic[2], M. Malesevic[2], L. Tukic[2]*
[1]Clinic of hematology, [2]Military Medical Academy, Belgrade, Serbia

Introduction: Chronic graft-versus-host disease (cGvHD) as a major complication after allogeneic stem cell transplantation (SCT) is associated with late morbidity, mortality and inferior quality of life. In 2005. the National Institute of Health (NIH) proposed new criteria for the diagnosis and classification of cGvHD. Overall data suggested that the proposed NIH classification is prognostically useful, but the impact of both the syndrome and global severity grading on outcome of the SCT is still unknown. We evaluated the association of the proposed NIH classification (syndrome classification and the global severity) with transplantation outcomes.
Materials (or patients) and Methods: We retrospectively analyzed 174 adult patients (pts), average age 29 years, F/M ratio 68/106, with different hematological malignancies who survived beyond 100 days following allo-SCT. All pts have HLA identical sibling donor. Peripheral blood was source of stem cells (SC) in 99 pts, main conditioning regimen was myeloablative and GvHD prophylaxis was combination of Cyclosporine A with Methotrexate. cGvHD was retrospectively classified with NIH proposed classification and was correlated with overall survival (OS) and non relapse mortality (NRM) after SCT.
Results: From our cohort of pts, 114 (65%) have cGvHD with late onset in 12 (10,5%), classic in 46 (40,3%) and overlap syndrome in 56 (49,2%). According to onset, 16 (19,6%) pts have progressive, 28 (29,6%) de novo and 58 (59,8%) quiscent type of cGvHD. Median time of onset of cGvHD was 158 days (range 100-473). Skin, mouth, eyes, gastrointestinal tract and liver were affected mostly. Considering of global severity scoring system, cGvHD was mild in 27 (23,8%), moderate 49 (42,9%) or severe in 38 (33,3%) pts. Pts with progressive type of onset and with severe form of cGvHD had significantly worst OS in comparison to other subgroups (log rank $P < 0.0001$, $P = 0.009$, respectively). Pts with classic cGvHD had significantly better OS in comparison to pts with late acute and overlap syndrome (log rank $P = 0.009$). NRM was significantly higher in pts with progressive and severe cGvHD (log rank $P < 0.0001$, $P<0.0001$, respectively).
Discussion: cGvHD have impact on global outcomes following SCT. NIH proposed classification of cGvHD (syndrome classification and the global severity) is useful and predictive, but further investigations in homologous subgroups of pts (according to type of the diseases, SC sources, conditioning regimen and donor) are needed.
Disclosure of Interest: None Declared.

PH-P417
MIRSNPS ARE ASSOCIATED WITH AGVHD AFTER UNRELATED ALLO-HSCT IN CHINESE HAN POPULATION

C. Zhang[1,], Y. Hu[1], Y. Luo[1], Y. Tan[1], H. Huang[1]*
[1]The 1st Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

Introduction: Recent studies demonstrate that polymorphisms in non-HLA genes, including cytokine genes, innate immunity genes, are significantly associated with the risk of graft versus host disease (GVHD). Now microRNAs have been demonstrated to play important roles in GVHD pathogenesis. So we hypothesize that microRNA gene polymorphisms may contribute to transplantation outcome by controlling different essential gene regulatory modules in posttransplantation immune reconstruction.
Materials (or patients) and Methods: The cohort was consisted of 74 pairs of recipients and their unrelated donors who underwent allo-HSCT in our single center from January 2008 to December 2010. Recipient genomic DNA was extracted from bone marrow of recipients before transplantation. Donor genetic DNA was extracted from recipient bone marrow 100% donor chimerism by short tandem repeat(STR) after transplantation. Eleven Single-nucleotide polymorphism were detected by multiplex SNaPshot technology,including mir-101(rs2549855), mir-21(rs17539020,rs9684042), mir-92a(rs2716830), mir-164a(rs2910164), mir-142(rs11934028), mir-125b(rs10503344), mir-142(rs11934028), mir-19b(rs67308836). Microrna genotypes were analyzed by the Fisher exact test. The cumulative incidence of aGVHD, II-IVaGVHD, cGVHD, relapse, transplantation related mortality(TRM), and overall survival(OS) were estimated using the Kaplan-Meier method. The Cox proportional hazard model was applied to multivariate analysis of outcomes of transplantation. All probability values were two-sided and P values less than 0.05 were considered statistically significant. P values between 0.05 and 0.1 were considered to be indicative of a trend.
Results: Our results show that presence of rs11934028AA genotype in mir-142-3p of the recipient (AA genotype 100% vs. AC/CC genotypes 55.3.P=0.012) and the donor (AA genotype 100% vs. AC/CC genotypes 55.3%, P=0.001) were associated with aGVHD than other genotypes. Besides, the presence of recipent rs11934028 AA genotype was associated with higher incidence of II-IV aGVHD(AA genotype 100%versus AC/CC genotypes 44.9% P=0.08). Interestingly, the presence of donor rs10503344TT genotype in mir-125b reduce the incidence of aGVHD and II-IV aGVHD statistically significant in the study. Multivariate Cox regression analysis was carried out to sort out risk factors of aGVHD and II-IVaGVHD (Table.1). The results confirmed that donor11934028 AA genotype was an independent risk factor for aGVHD and donor rs10503344 genotype was a protective risk factor (Table 1). No association was observed between mirSNPs and cGVHD, TRM, relapse and OS.
Discussion: The present study first demonstrate that mirSNPs have an influence on the development of aGVHD and higher grades aGVHD. It suggests that mirSNPS are potential to be the novel biomarker for prevention and trentment of aGVHD. This is instructive and meaningful to the choose of donor and early prevention of aGVHD. Studies remains to be carried out *in vitro* and *in vivo* to further explore the molecular mechanism how mirSNPs influence the outcome in the posttransplantation immunity reconstruction.
Disclosure of Interest: None Declared.

Table.1 Multivariate Analysis of Risk Factors for aGVHD and Grades II-IV aGVHD

		aGVHD		II-IVaGVHD	
		RR(95%CI)	P value	RR(95%CI)	P value
Recipent-11934028	AA allele	2.995(0.983-9.123)	0.054	3.682(0.997-13.595)	0.050
	Other genotypes				
Donor-11934028	AA allele	7.847(2.525-24.388)	0.000	6.333(1.339-29.959)	0.020
Other genotypes					
Donor -rs10503344	TT allele	0.457(0.235-0.888)	0.021	0.438(0.201-0.954)	0.038
Other genotypes					

PH-P418

IMPAIRED NK CELLS CYTOTOXICITY AGAINST ACTIVATED, ALLOREACTIVE DONOR T CELLS IN GVHD CAN BE PARTLY RESTORED BY IL-15 *EX VIVO*

L. Sheng[1,], H. Fu[1], Y. Hu[1], Y. Luo[1], J. Shi[1], Y. Tan[1], Y. He[1], X. Lai[1], H. Huang[1]*

[1]The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Introduction: In murine models, donor natural killer cells(NK) exhibit immunoregulatory functions and can lyse donor alloreactive T cells during the initiation of graft versus host disease(GVHD). The mechanism and immunoregulatory role of NK cells in human GVHD remains unclear. This study was designed to investigate the immunoregulatory and cytotoxicity functions of donor CD56+NK cells against activated, alloreactive donor T cells in human GVHD.
Materials (or patients) and Methods: We comprehensively evaluated the subgroup, receptors expression and immunoregulatory functions of NK cells in 57 patients receiving allogeneic hematopoietic stem cell transplantation(allo-HSCT) and their donors. CFSE labeled donor T cells were stimulated by allogeneic dendritic cells(Allo-DCs). The immunoregulation of donor T cells proliferation and cytotoxicity against activated donor T cells by autologous NK cells(auto-NK) were determined by flow cytometry. In blocking experiments, NK cells were pretreated with anti-NKG2D, anti-CD226, anti-LFA-1, anti-FAS-L, anti-NKG-2A or anti-TIM-3 mAbs.
Results: Donor T cell proliferation in response to Allo-DCs were suppressed by auto-NK cells. Donor NK cells killed proliferating T cells($CFSE^{low}$) more efficiently than nonproliferating T cells ($CFSE^{high}$) at all E:T ratios tested. Donor NK cells degranulated in response to activated, but not resting T cells. $CD56^{dim}$ degranulated more than $CD56^{bright}$ NKcells, and NKG2A- degranulated more than NKG2A+ NK cells. Activated T cells express high levels of the NKG2D ligand s(MIC-A, MIC-B and ULBP-1) and C226 ligand PVR. Blocking of LFA-1,NKG2D or DNAM-1 led to significant reduction of NK cell cytotoxicity, whereas blocking of NKG2A and TIM-3 resulted in an increase in NK cytotoxicity. In the early period after HSCT, reconstituted NK cells were mainly $CD56^{bright}$ and $NKG2A^{+}$ subgroup. NK cells in GVHD patients expressed lower level of NKG2D and CD226 when compared with NK cells in non-GVHD recipients or their corresponding donors. In GVHD patients, both degranulation and cytotoxicity of NK cells towards activated auto-T cells were depressed. NK cells in GVHD up-regulated the expression of CD226 and NKG2D when stimulated *ex vivo* with IL-15, but not IL-2. Both IL-2 and IL-15 enhanced the degranulation and cytotoxicity of NK cells towards activated T cells.
Discussion: This study provides new insight into the role of NK cells in the regulation of GVHD, demonstrates for the first time that unlike in mice models, the ability of donor NK cells to inhibit and lyse autologous activated T cells is impaired during human GVHD, possibly due to altered subgroup and down regulated activated receptors. When treated with IL-15 *ex vivo*, this phenotype and function impairment can be partly reversed, which potentially may provide an opportunity for therapeutic treatment of GVHD.
Disclosure of Interest: None Declared.

PH-P419

IMPLEMENTATION AND DISSEMINATION OF CHRONIC GRAFT-VERSUS-HOST DISEASE NIH CRITERIA AND RECOMMENDATIONS - FORMATION OF A MULTIDISCIPLINARY TEAM AND PROGRAM AT THE UNIVERSITY HOSPITAL CENTER ZAGREB, CROATIA

L. Grkovic[1,], D. Pulanic[1], R. Serventi-Seiwerth[1], N. Matic[1], M. Mravak-Stipetic[2], E. Bilic[3], R. Ceovic[4], D. Ljubas[5], L. Rajic[6], E. Bilic[6], N. Durakovic[1], Z. Peric[1], T. Klepac Pulanic[7], K. Kovac[8], D. Dusek[9], I. Bojanic[10], M. Grce[11], D. Batinic[12], R. Vrhovac[1], S. Z Pavletic[13], D. Nemet[1]*

[1]Hematology, [2]Dental Medicine, [3]Neurology, [4]Dermatology, [5]Nutrition, [6]Pediatric Hematology, [7]Gynecology, [8]Rehabilitation Medicine, UHC Zagreb, [9]Infectious Disease, University Hospital for Infectious Diseases "Dr. Fran Mihaljević", [10]Transfusiology, UHC Zagreb, [11]Ruder Boskovic Institute, [12]Immunology, UHC Zagreb, Zagreb, Croatia, [13]Transplantation & Immunology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, United States

Introduction: Due to increasing safety of allogeneic hematopoietic stem cell transplantation (alloHSCT), the number of survivors is increasing and more patients are at risk of developing chronic Graft-versus-Host disease (cGVHD), a leading cause of non-relapse mortality and morbidity after alloHSCT. Approximately 50% of alloHSCT recipients develop cGVHD. CGVHD is a disorder affecting many organ systems in highly variable fashion and requires multidisciplinary medical management. Division of Hematology at the University Hospital Center Zagreb (UHC Zagreb), Croatia, has 30-year long experience with alloHSCT. Recognizing cGVHD as one of the most serious challenges after alloHSCT and wishing to achieve better clinical care for long-term survivors, a Center for cGVHD and long-term follow-up was recently established at UHC Zagreb. Multidisciplinary clinic infrastructure was set up using established cGVHD-related grading scales and measurements in collaboration with the National Cancer Institute, USA.
Materials (or patients) and Methods: From 1983, a total of 753 patients received alloHSCT at UHC Zagreb. From 2009 to July 2013, 119 transplanted patients were analyzed, of which 102 patients are alive and 33 (32%) developed cGVHD. Since the establishment of a comprehensive cGVHD Center, patients are first seen by a hematologist, with history and physical exam, focusing on prior acute GVHD (aGVHD) history, current symptoms and onset of cGVHD, performed as part of an organ-system oriented exam. Standard cGVHD evaluation and scoring forms are filled according to NIH Consensus recommendations. Selected nurse is engaged in visit organization. Comprehensive laboratory workup is done, and patients are seen and evaluated by sub-specialists (Dental,

Dermatology, Rehabilitation, Neurology, and other) with further workup as needed. Quality of life questionnaires (SF 36, EORTC QLQ-C30, Lee symptom scale) are filled during the visit. All data are stored in a database and weekly team meetings are organized.
Results: Using comprehensive cGVHD approach, 19 patients (1 pediatric) were assessed, median age 36.5 years. Three patients were ineligible, as they did not meet criteria for cGVHD diagnosis. Twelve transplants were from matched related donor, 11 using myeloablative conditioning, and 11 using PBSC as stem source. Eleven patients had previous aGVHD. Seven patients had *de novo* cGVHD, 7 quiescent and 2 progressive onset. Fifteen were classified as classic and 1 as overlap. Ten patients had score 3 according to NIH global scoring, and 6 had score 2. Most involved organs were skin, joint/fascia, eyes, and lungs. Challenges included organizing timely access to sub-specialists and liberating dedicated research time for team members. An internationally peer reviewed grant through a World Bank program (UKF Fund) awarded in 2013 will help with strengthening research infrastructure and staff support.
Discussion: Establishment of multidisciplinary team for cGVHD and long-term follow-up after HSCT in a new environment proves feasible. It enhances the quality of medical documentation and management of these complex patients. Such comprehensive clinical and research infrastructure will allow further collaboration and integration with other European and international cGVHD centers.
Disclosure of Interest: None Declared.

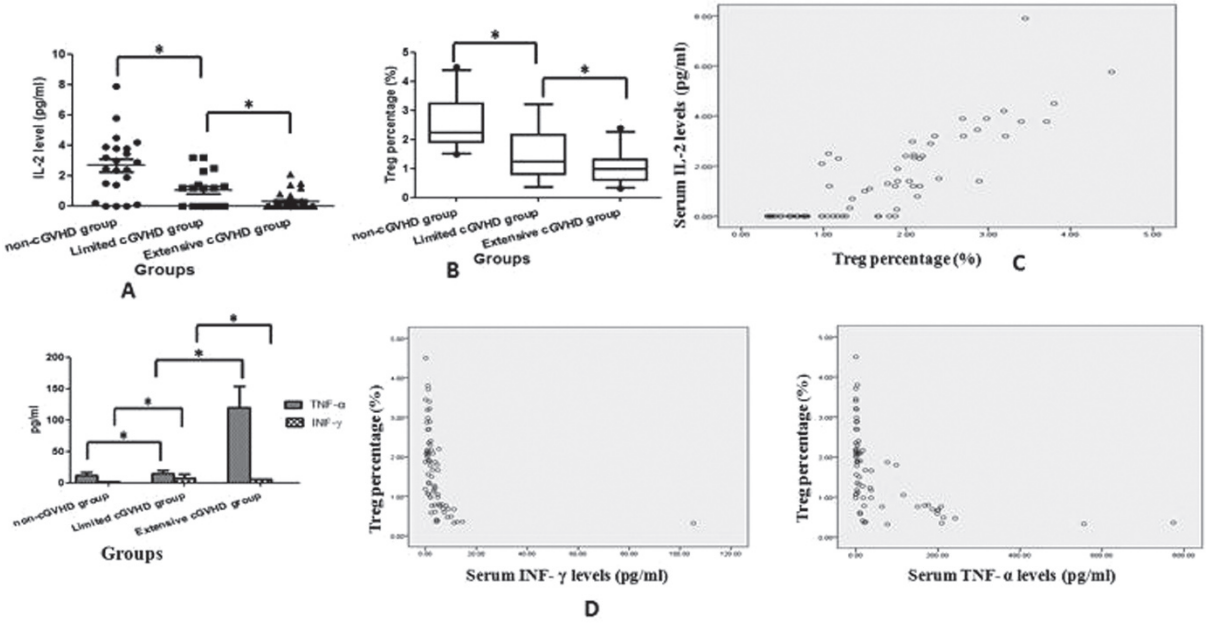
PH-P420

SERUM IL-2 LEVELS REGULATE CHRONIC GRAFT VERSUS HOST DISEASE SEVERITY VIA CD4+ FOXP3+ REGULATORY T CELL HOMEOSTASIS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Y. Hu[1,], Y. Luo[1], Y. Tan[1], Y. Gu[1], H. Huang[1]*
[1]The 1st Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

Introduction: CD4+ Foxp3+ regulatory T cells (Tregs) have been shown to play important roles in the maintenance of tolerance after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and patients with chronic graft versus host disease (cGVHD) have a relative deficiency of Tregs partly due to the abnormalities in Treg homeostasis. IL-2 is a critical homeostatic cytokine for Tregs. Low-dose IL-2 therapy have been reported to restore Treg homeostasis in patients with cGVHD and have a potential therapeutic efficacy on cGVHD in some patients (John Koreth *et al*, *New England Journal of Medicine*, 2011;365:2055-66). So we analyzed the relationship among serum IL-2 levels, Treg frequency and cGVHD severity after allo-HSCT.
Materials (or patients) and Methods: Patients undergoing allo-HSCT in our center from January 2000 to July 2012 were selected. They were divided into 3 groups according to cGVHD criteria including non-cGVHD, limited cGVHD and extensive cGVHD group. 10ml peripheral blood was drawn from all the selected patients for Tregs analysis by flow cytometry. Serum cytokine levels of TNF-α, INF-μ and IL-2 were evaluated by ELISA. Data were processed and analyzed using SPSS 17.0. Statistical significance in different groups was assessed by two-sample t-test. Spearman's correlation was used to test the correlation between two continuous variables.
Results: 22, 18 and 30 patients were selected in non-cGVHD, limited cGVHD and extensive cGVHD group respectively. As shown in Figure 1A, we found that serum IL-2 level was the highest in non-cGVHD group (2.67±2.02pg/ml), higher in limited cGVHD group (1.04±1.14pg/ml) while the lest in extensive cGVHD group (0.28±0.55pg/ml) (P<0.05). IL-2 can facilitate the homeostasis of Tregs. We further found the percentage of Tregs was significantly increased in non-cGVHD group(2.58±0.81%) compared to limited cGVHD (1.51±0.83%) and extensive cGVHD group (1.04±0.55%) (P<0.05) (Figure 1B). Spearman's correlation analysis revealed that the increased level of IL-2 was positively associated with increased Tregs (r=0.856, P<0.01), (Figure 1C). Moreover, we demonstrated increased Tregs were associated with less severity of cGVHD including reduced serum levels of INF-γ (r=-0.744, P<0.01) and TNF-α(r=-0.744, P<0.01) (Figure 1D).
Discussion: For the first time our result implied that serum IL-2 levels regulated cGVHD severity via increased Tregs after allo-HSCT and provided novel pathogenesis of cGVHD. It has been reported that low dose IL-2 administration was associated with preferential, sustained Tregs expansion *in vivo* and amelioration of the manifestations of chronic GVHD in a substantial proportion of patients. So our results might provide a prognostic factor associated with the efficacy of IL-2 therapy for cGVHD after allo-HSCT.
Disclosure of Interest: None Declared.

[PH-P420]



PH-P421

LARGE SCALE CLINICAL CHARACTERIZATION OF AUTOANTIBODIES IN PATIENTS WITH CHRONIC GVHD

Z. Kuzmina[1,], F. Hakim[1], V. Gounden[2], J. Rose[1], E. W Cowen[3], H. B Naik[3], S. A Hasni[4], J. Mays[5], L. Curtis[1], K. Cole[1], D. Avila[1], T. Taylor[1], S. Mitchell[6], J. Baruffaldi[1], K. Baird[7], S. Steinberg[8], S. Z Pavletic[1]*

[1]Graft-versus-Host and Autoimmunity Unit, Experimental Transplantation and Immunology Branch, National Institutes of Health, National Cancer Institute NCI, Bethesda, MD, [2]Clinical Chemistry, Department of Laboratory Medicine, [3]Dermatology Branch National Cancer Institute NCI, [4]National Institute of Arthritis and Musculoskeletal and Skin Diseases, [5]National Institute of Dental and Craniofacial Research, [6]Research and Practice Development Service, [7]Pediatric Oncology Branch NCI, [8]Biostatistics and Data Management Section, Center for Cancer Research, Bethesda, United States

Introduction: In chronic graft-versus-host disease (cGVHD) a pathogenic role for B cells has been suggested by the presence of anti-HY antibodies in female-into-male transplants, by elevated plasma levels of B cell activation factor and by the therapeutic efficacy of B-depletion. In systemic autoimmune disorders, such as SLE, Sjogren's syndrome, and scleroderma, pathogenic autoantibodies can be found in serum, and immune complex deposits may be identified in affected tissues, and have been useful as markers of disease activity, severity or organ specificity.

Materials (or patients) and Methods: The focus of this study was to determine the incidence and associations of autoantibodies with cGVHD severity, activity and organ damage in a large patient cohort. 20 antibodies were characterized.

Results: 280 cGVHD patients (29% moderate, 70% severe), with median duration of cGVHD 2 years, who received a median of four therapy lines, were consecutively enrolled in a cross-sectional natural history study protocol(NCT00092235). Circulating autoantibodies were present in 62% of cGVHD patients (*n*=174), and multiple antibodies were detected in 35% of patients (*n*=61) (Table). Patients with circulating autoantibodies had significantly higher levels of IgM (<.0001), IgG (<.0001) and IgA (.001), elevated uric acid (0.008) and total protein (.0004), and higher numbers of CD3+ (.002), CD4+ (.001), CD8+ (.02) T cells and CD19+ B cells (<.0001). Prior rituximab therapy (*n*=66) reduced the incidence of autoantibodies (48 vs. 66% *P*=.01). Patients with moderate cGVHD severity by NIH criteria had higher numbers of CD19+ B cells (542 vs. 248 x10^6 *P*=.008) than those with severe cGVHD. Patients with moderate cGVHD had a higher total incidence of autoantibodies (67.5%) and of multiple antibodies (27.5%) than those with severe cGVHD (60% and 19.4% respectively). Moderate cGVHD was associated with significantly higher incidence of cardiolipin M (.007) and higher titers of both RF (.001) and cardiolipin M (.002). Patients with active cGVHD (*n*=169) were not significantly different from non-active, however the frequency of autoantibodies accounted for more than 75% of ds-DNA, smooth muscle, Smith, and centromere in seropositive patients. Among organ systems affected by cGVHD, only oral cGVHD was associated with circulating antibodies, (67% vs. 53% *P*=.02), high ANA (32% vs. 18%, *P*=.002 and ANA titer 0.7 vs. 1.3 *P*=.008).

Discussion: Circulating autoantibodies are common in patients with advanced cGVHD, its presence is associated with better quantitative immunologic reconstitution and do not reflect the disease activity, severity or organ specificity. Antibody detection has limited role in clinical assessment of cGVHD, however, the biological significance of these autoantibodies remains to be determined.

Disclosure of Interest: None Declared.

PH-P422

TOTAL GAMMA-GLUTAMYLTRANSFERASE ACTIVITY AND ITS FRACTIONS IN ALLOGENIC AND AUTOLOGUS STEM CELL TRANSPLNTATION: CORRELATION WITH ACUTE GRAFT VERSUS HOST DISEASE AND TRANSPLANT RELATED MORTALITY

E. Benedetti[1,], I. Bertaggia[2], M. Franzini[3], A. Paolocchi[3], E. Bramanti[4], R. Passera[5], B. Bruno[5], B. Bruno[5], A. Bacigalupo[6], I. Petrini[7], M. Petrini[8]*

[1]Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, [2]Oncology, Hematology Unit Ospedale S. Chiara Pisa, [3]Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Pathology University of Pisa, [4]ICCOM, CNR, Pisa, [5]University of Turin, Torino, [6]Ospedale San Martino, Genova, [7]Oncology, Ospedale S. Chiara Pisa, [8]Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, University of Pisa, Pisa, Italy

Introduction: acute graft versus host disease (aGVHD) is one of the major complications after allogenic hematopoietic stem cell transplant (HSCT). Diagnosis is made with histology. Biomarkers as a diagnostic and possibly prognostic tools are under continuous development. Total serum gamma-glutamyltransferase (TGGT) activity can be fractionated by size exclusion chromatography in 4 fractions: "big", "medium", "small" and "free" GGT (b-, m-, s-, and f-GGT, respectively). These fractions are involved in cardiovascular, metabolic, nephrologic and neurologic diseases. Aim: we investigated the role of TGGT and its fractions in patients undergoing allogeneic (AlloTx) and autologous transplantation (AutoTx). We addressed if TGGT and its fractions increase due to conditioning, to aGVHD, if the increase is due to a predominant fraction, and if there is a correlation with transplant related mortality (TRM).

Materials (or patients) and Methods: we prospectively enrolled in the study 65 patients (pts): 51 underwent an AlloTx and 23/51 (45%) developed aGVHD (study group). Twenty-eight pts without aGVHD and 14 AutoTx were considered our control groups. Conditioning in the AlloTx group were myeloablative (MyC) in 36 pts and Reduced Intensity (RIC) in 15 pts. Acute GVHD involved skin (*N*=10), lower intestinal (*N*=8), stomach (*N*=1) and hepatic+skin (*N*=2), lower intestine+skin (*N*=2). TGGT and its fractions were assessed before conditioning, at day+7,+14,+30,+60,+90, +180, +360 and any time GVHD occurred.

[PH-P421]

Table. Frequency of circulating antibodies in chronic GVHD patients after HSCT

Autoantibody	Patients tested	Number of positive (%)	Normal range	Median	Range	Unit
ANA	280	82 (29)	<1	2.6	1.2-12	EU
ENA*	280	23 (8)	<20	32	21-79	EU
AMA	280	22 (7)	<0.3	1	0.4-1.1	U
RF	280	35 (13)	<20	28	21-288	IU/mL
Liver-Kidney	280	3 (1)	<25	35	22-84	U
Cardiolipin M	280	23 (8)	<16	27	18-150	MPL
Cardiolipin G	280	23 (9)	<12	20	13-75	GPL
CCP	280	21 (7)	<20	37	22-110	Units
Ds-DNA	280	7 (2)	<30	49	36-145	IU/mL

*Positive ENA antibodies were further analyzed for SmRNP (*n*=15/65%), Smith (*n*=4/17%), SSA (*n*=9/39%) and SSB (*n*=1/4%)

Results: all patients showed an increase in TGGT at day+7 post-transplant. However, it was higher in MyC (median 205 U/L; range 27-874), intermediate in RIC (median 90 U/L;range 19-209), and modest in AutoTx (median 61 U/L; range 1-257;*P*=0.037 as compared with AlloTx). sGGT (median 59,55 U/L; range 6,53- 192,43) and bGGT (median 39,45 U/L; range 2,31- 163,45) but not fGGT or mGGT were the fractions involved after an allograft whereas, though to a lesser extent, all fractions were elevated after an autograft. Regardless of the conditioning, in patients developing acute GVHD, there was a second spike in TGGT (median 215,45 U/L; range 22.2-1660) due to sGGT (median 115,51 U/L; range 4,06- 1231,58) and bGGT (median 60,50 U/L; range 2,44- 259,41) primarily. By contrast, patients who did not develop GVHD did not show any further increase after day +7. Of note, all treatment-responsive pts showed a gradual reduction until normalization of TGGT. Moreover, we found a statistical significant increase in bGGT at day +7 (*P*=0.039) in pts dying of TRM as compared with surviving pts.
Discussion: TGGT and its fractions may be important biomarkers of incipient acute GVHD and treatment response. Larger prospective studies are warranted to confirm our observations and possibly correlate severity and clinical manifestations of GVHD with given GGT fractions.
Disclosure of Interest: None Declared.

PH-P423
IMPACT OF CD3/TREGS RATIO IN DONOR GRAFT ON SURVIVAL RATES IN ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

M. Delia[1,], D. Pastore[1], A. Mestice[1], P. Carluccio[1], T. Perrone[1], F. Gaudio[1], P. Curci[1], A. Ricco[1], A. Russo Rossi[1], A. Maria Giordano[1], G. Specchia[1]*
[1]Hematology - University of Bari, Bari, Italy

Introduction: The therapeutic efficacy of allogeneic stem cell transplantation (alloSCT) for hematological malignancies relies largely on the graft versus leukemia (GvL) effect exerted by the donor CD3 cells, but an uncontrolled graft-versus-host-disease (GvHD) bears a risk of complications. On the other hand, T regs cells (CD4+CD25high Foxp3+) are believed to maintain tolerance and to inhibit GvHD after alloSCT; also, the Foxp3 gene encodes a transcription factor that is a key for thymic development, so T regs cells could preserve an optimal microenvironment for the reconstitution of functional immunity after alloSCT. Moreover, when looking at post allotransplant patients' outcomes, there is no evidence that donor graft CD3/T regs ratio may determine an effect in terms of OS, NRM and relapse free survival rates so far. In this study we analyzed the graft CD3+/Tregs ratio (gCD3/Tregs R) and determined its impact on acute GVHD (aGVHD) and survival rates (OS, NRM and Relapse) after myeloablative alloPBSCT.
Materials (or patients) and Methods: We analyzed 102 consecutive patients (median age 39 yy) transplanted with unmanipulated peripheral blood stem cells from an HLA identical related donor (*n*=78) or an HLA identical unrelated donor (*n*=32); diagnoses were acute myeloid leukaemia (*n*=82), acute lymphoblastic leukaemia (*n*=20).
Results: The median CD3+ and Tregs dose administered was 240 (range (r): 67-550) and $13x10^6$/Kg (r: 2-21), respectively; the median gCD3/Tregs R was 22 (r: 8-250). Patients were subdivided into a high gCD3/Tregs R(>36) group(*n*=46) and a low gCD3/Tregs R(<36) group (*n*=56). The incidence of aGVHD (grade II-IV) in the low gCD3/Tregs R (LR) group was lower than in the high gCD3/Tregs R (HR) group (10/56 or 18% vs 35/46 or 77%, *P*<.001). The OS, NRM and relapse rate at 3 years was 54, 29 and 34%, respectively. Comparing LR with HR group a statistically significant difference is demonstrated for OS and NRM rates at 3 years (65 vs 31%,*P*<.004; 3 vs 71%, *P*<.001, table 1), respectively, but not for the R one (35 vs 30%, *P*=ns). Comparing aGVHD+ with aGVHD- group OS, NRM and relapse were always statistically significant different at 3 years (39 vs 65%, *P*<.005; 61 vs 7%, *P*<.001; 9 vs 53%, *P*<.002).
Discussion: Taken together, our data may suggest that Tregs content is able to mediate protective effects against aGVHD, while preserving GvL effects as demonstrated by relapse rate comparison between H and LR groups. However, larger studies are needed to understand the real contribution of gCD3/Tregs R on survival rates.
Disclosure of Interest: None Declared.

PH-P424
MEASUREMENT OF SERUM IP-10 LEVELS AND PB DENDRITIC CELL (DC) NUMBERS AT 3 MONTHS AFTER ALLOGENEIC HSC TRANSPLANTATION AS POTENTIAL BIOMARKERS OF CHRONIC GVHD

G. Chirumbolo[1], G. Tolomelli[1,], F. Ulbar[1], M. Stanzani[1], M. Arpinati[1]*
[1]University Hospital S.Orsola Bologna, Bologna, Italy

Introduction: Chronic GVHD (cGVHD) is the major cause of long term morbidity after allogeneic HSC transplantation. No biomarkers are currently known that can consistently predict its occurrence. We have previously observed that patients with cGVHD have increased numbers of circulating activated monocytes (Arpinati Transplantation 85:1826; 2008). Also, the serum concentration of inflammatory chemokines has been associated with acute GVHD (aGVHD). We thus aimed to evaluate whether PB numbers of antigen-presenting cell (APC) subsets or serum chemokine concentrations are biomarkers of chronic GVHD occurrence.
Materials (or patients) and Methods: PB samples were collected at 3 months after transplant. 102 consecutive patients undergoing transplant between 2007 and 2011 were included in the study, provided they were evaluable for chronic GVHD. Multicolor flow cytometry was employed to determine the number of PB myeloid (mDC) and plasmacytoid DC (pDC), CD16+ DC, and CD16+ and – monocytes, as well as CD4+ and CD8+ T, CD56+ NK and CD19+ B cells. The serum concentration of IL-8, IP-10, MCP1, MIP-1alpha and –beta and RANTES was measured by cytometry bead array (CBA) assay.
Results: After a median of 72 days (IQ 27-174) following enrollment, 40 patients had developed cGVHD (25 extensive) with an actuarial probability of 51%. Patients with and without cGVHD had comparable clinical characteristics. However, more patients with cGVHD had had previous aGVHD (24/40, 14 grade II-IV, vs 18/62, 8 II-IV) (*P*<0.01). Patients with cGVHD had lower PB mDC (*P*=0.027) and pDC (*P*=0.004) while the other cell subsets were comparable. Moreover, patients with cGVHD had higher serum levels of IL-8 (*P*=0.02), IP-10 (*P*=0.001) and MIP-1alpha (*P*=0.01) while MCP, MIP-1beta and RANTES were similar. In univariate analysis, the risk of chronic GVHD was increased in patients with >0.41 ng/ml IP-10 (hazard ratio, HR, 2.9, *P*=0.0017), $<9.1x10^3$ mDC/ml (HR 2.5, *P*=0.0054) and $<3.9x10^3$ pDC/ml (HR 2.1, *P*=0.017). Patients who had had aGVHD were then evaluated separately. Lower PB mDC and pDC increased the risk of cGVHD both in patients with (HR 2.4 and 2.3 respectively, *P*=0.04) and without previous aGVHD (HR 3 and 3.1 respectively, *P*=0.03). A higher IP-10 level increased the risk of cGVHD in patients without previous aGVHD (HR 4.5, *P*=0.003).
Discussion: Serum IP-10 levels and PB mDC and pDC numbers at 3 months after allogeneic HSCT are potential biomarkers of the occurrence of cGVHD independently of previous aGVHD. A multiparameter score derived from these markers is currently being tested in a validation cohort.
Disclosure of Interest: None Declared.

PH-P425
RAPAMYCIN-INDUCED COMPARTMENTALIZATION OF TREGS AFTER UNMANIPULATED HLA-HAPLOIDENTICAL HSCT PROTECTS FROM GVHD AND LIKELY DOES NOT INTERFERE WITH GVL EFFECTS

F. Gullotta[1,], J. Peccatori[2], A. Forcina[2], M. Noviello[2], V. Valtolina[2], N. Cieri[2], M. T. Lupo Stanghellini[2], S. Olek[3], M. Battaglia[2], F. Ciceri[2], C. Bonini[2], A. Bondanza[2]*
[1]Dhitech s.c.a.r.l., Lecce, [2]San Raffaele Hospital Scientific Institute, Milan, Italy, [3]EPIONTIS GMBH, Berlin, Germany

Introduction: At our center, we are investigating the use of post-transplant rapamycin (sirolimus) in different protocols of

unmanipulated hematopoietc stem cell transplantation (HSCT) from HLA-haploidentical donors with the aim of inducing T regulatory cells (Tregs) *in vivo* and prevent graft-versus-host disease (GVHD). In one of these protocols (TrRaMM, Eudract 2007-5477-54), rapamycin administration to 113 pts with high-risk hematological malignancies associated with a low risk of grade III-IV acute GVHD (21%) and chronic extensive GVHD (35%). Importantly, three-years relapse incidence was 48%, indicating benefit from substantial graft-versus-leukemia (GVL) effects.
Aim: A long-standing question is whether Tregs-based strategies may interfere with the antitumor effects of HSCT. We therefore investigated if differential homing capacities of *in vivo* rapamycin-induced Tregs might explain the dissociation of GVHD from GVL effects.
Results: In pts receiving rapamycin post-transplant, we documented the accumulation of circulating CD4+/CD25+/FoxP3+/IL-7Ralpha- Tregs (at day 30, median 6.5% range 0.2-37.2, $P<0.01$ compared with controls), which were suppressive *ex vivo* in CFSE-dilution assays and were selectively demethylated at the FoxP3 locus. Tregs also expressed high levels of CD45RO, suggesting extensive post-thymic proliferation and acquisition of a memory phenotype. Interestingly, Tregs frequencies inversely correlated with the risk of developing subsequent acute GVHD ($P<0.05$). These effects were rapamycin-specific, since 5 pts receiving cyclosporine A post-transplant had low to undetectable Tregs levels and all developed grade IV GVHD. Surprisingly, the bone marrow (BM) of pts receiving rapamycin was depleted of Tregs (at day 30, BM Tregs frequencies: median 0.3% range 0.0-2.2, $P<0.01$ compared with PB Tregs), but enriched with $CD45RA^-/CD62L^-$ effector memory CD8+ T cells. Compared with effector T cells, circulating Tregs had significantly lower levels of the BM addressin CXCR4 ($P<005$). Tregs generated *in vitro* after CD3/CD28-beads stimulation and rapamycin culture also showed CXCR4 downregulation and displayed lower migratory capacities across SDF-1 gradients. These effects were specific for rapamycin-induced Tregs, as naturally occurring Tregs readily migrated *ex vivo* and could be inhibited by the CXCR4 antagonist plerixafor.
Discussion: Our results hint to a yet unrecognized compartmentalization effect of rapamycin on Tregs. Rapamycin-induced Tregs appear to re-circulate in the peripheral tissues and protect from GVHD, but are excluded from the BM and likely do not interfere with GVL effects locally.
Disclosure of Interest: None Declared.

PH-P426
URINARY PROTEOMIC PATTERN ANALYSIS OUTPERFORMS PLASMA BIOMARKERS FOR THE PREDICTION OF ACUTE GRAFT-VERSUS-HOST DISEASE IN HEMATOPOIETIC STEM CELL TRANSPLANTED PATIENTS
C. Human[1,], S. Borchers[1], L. Hambach[1], J. Metzger[2], P. Schweier[1], H. Diedrich[1], M. Stadler[1], M. Eder[1], J. Krauter[3], A. Ganser[1], E. Mischak-Weissinger[1]*
[1]Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, [2]Mosaiques Diagnostics and Therapeutics AG, Hannover, [3]Hematology and Oncology, Städtisches Klinikum Braunschweig Medizinische Klinik III, Braunschweig, Germany

Introduction: Severe graft-versus-host disease (GvHD) is one of the major complications after hematopoietic stem cell transplantation (HSCT). Early prediction and diagnosis of GvHD are important for an early intervention and better survival of the patients. We aim to predict GvHD without invasive techniques and at an early stage using urine and plasma biomarkers.
Materials (or patients) and Methods: 100 patients transplanted at Hannover Medical School were included in this study. Most patients were transplanted for acute leukaemia ($n=69$) and from HLA-matched donors ($n=82$). Reduced intensity conditioning was used for 42 pts and 93 pts received T-cell depleting antibodies prior to HSCT. Patients were divided into a test set ($n=69$, 80 samples) and a blinded validation set ($n=31$, 263 samples). For the test set plasma sampling was done close to time of diagnosis of GvHD and controls without GvHD were chosen on time-matched time points after HSCT. For the validation set plasma and urine samples were collected after transplantation weekly up to day 35 and biweekly thereafter. Here we analysed β2microglobulin (β2m) and CD99 and other GvHD-related biomarkers, such as CD25, Elafin, REG3α, ST2 and sTNF-RI as reported by Paczesny *et al*. in 2010 and 2013, and Vander Lugt *et al*. 2013 in all plasma samples collected after allogeneic HSCT via ELISA. In addition we analysed the simultaneously taken urine samples from validation set patients with CE-MS in order to compare both methods.
Results: As a follow-up to the test set data presented last year we measured seven biomarkers (β2m, CD99, CD25, Elafin, REG3α, ST2 and sTNF-RI) in plasma samples in a validation cohort for a time-period of up to one year after HSCT. After unblinding of the samples a receiver operating characteristic (ROC) analysis was performed revealing a mean of areas under the curve (AUC) of 0.57 ± 0.05 over all seven plasma biomarkers. Based on the cut-off values calculated within the test set results of the plasma biomarkers the validation cohort showed moderate conformity with aGvHD-diagnosis represented by sensitivity and specificity values of 0.60 ± 0.38 and 0.41 ± 0.25, respectively. We observed five cases of non-relapse mortality (NRM) within the validation patients. In four of these five patients all biomarker concentrations were highly increased. Especially β2m was up to 100-fold increased compared to the aGvHD-cut-off. Analysis of the urine samples of the same patients showed sensitivity and specificity for correct classification of patients with aGvHD of 0.75 and 0.50, respectively. Patients diagnosed with aGvHD and with positive urine proteomic patterns showed pattern positivity before clinical diagnosis (median 25.5 days before, interquartile range of 49 days).
Discussion: We confirmed published data that plasma biomarkers can predict poor overall survival and higher NRM-rates in patients after HSCT by a massive increase of marker concentration in plasma of those patients. The newly found β2m could be integrated into the list of markers predicting NRM. However, plasma markers did not allow for diagnosis of GvHD in our small cohort. In contrast, urine analysis showed higher sensitivity values for aGvHD even before clinical manifestation. Early intervention and pre-emptive therapy with steroids upon proteomic urine pattern analysis are currently under investigation in a clinical trial.
Disclosure of Interest: C. Human: None Declared, S. Borchers: None Declared, L. Hambach: None Declared, J. Metzger Conflict with: Mosaiques Diagnostics and Therapeutics AG, P. Schweier: None Declared, H. Diedrich: None Declared, M. Stadler: None Declared, M. Eder: None Declared, J. Krauter: None Declared, A. Ganser: None Declared, E. Mischak-Weissinger: None Declared.

PH-P427
SAFETY AND EFFICACY OF TACROLIMUS AND MINI DOSE METHOTREXATE IN CHILDREN
R. Raj[1,], D. Munirathinam[1], V. Kumar[1], H. Doss[1], K. Sri[1]*
[1]Paediatric Blood and Marrow Transplantation, Apollo Speciality Hospital, Chennai, India

Introduction: Tacrolimus use has been well documented in adult patients undergoing haematopoietic stem cell transplantation. The pharmacokinetics and bioavailability data is readily available from these patients. However, very little paediatric data has been published on the safety and efficacy of tacrolimus in children.
Materials (or patients) and Methods: We present our data on the use of tacrolimus and mini methotrexate as graft versus host disease prophylaxis in all of our paediatric patients. Between 2003 and 2013, we have studied the use of tacrolimus in 200 children undergoing haematopoietic stem cell transplantation at our unit. The children received human leukocyte antigen (HLA)-matched related donor or HLA-matched, or partially mismatched unrelated donor transplants. They were aged between 3 months and 18 years of age.
Results: The incidence of acute graft versus host disease in the related and unrelated donor groups was 22.6% and 54%, respectively. Children less than 5 years of age required a much higher dose of tacrolimus than older children. The drug could be safely administered to achieve therapeutic range sublingually in all our patients. The adverse events commonly associated with

tacrolimus included hypomagnesemia (100 %), renal dysfunction (5%), hypertension (8%), seizures (14%) and hyperglycemia (5%).
Discussion: An initial dose of 0.03 mg/kg/day either oral or sublingual was started on all children. To assess if target whole blood concentrations of tacrolimus in children undergoing haematopoietic stem cell transplantation can be achieved reproducibly with this dose or not was assessed by checking trough drug levels 3 days a week for the first two weeks. We reviewed the tacrolimus blood levels and calculated clearances for 55 children (aged 6 months to 18 years, median 9 years) using tacrolimus after allogeneic marrow, blood stem cell or cord blood transplantation. In children less than 5 years old undergoing haematopoietic stem cell transplantation we found tacrolimus clearance faster than older children and adults. Hence, careful therapeutic monitoring is needed in the first 2 weeks after transplantation to avoid prolonged subtherapeutic dosing for this age group.
Tacrolimus was well tolerated and effective in graft versus host disease prophylaxis in paediatric patients undergoing allogeneic stem cell transplantation. The less than 5 year old children undergoing transplantation required increased dosing and careful monitoring of drug levels. Hypomagesaemia and hypertension were the commonest side effects seen with the use of this drug.
Disclosure of Interest: None Declared.

PH-P428
ECP FOR ACUTE GVHD WITH DIFFERENT METHODS IN PEDIATRIC PATIENTS

V. Witt[1,], H. Pichler[2], C. Peters[2], S. Matthes[2], A. Lawitschka[2], W. Holter[3]*
[1]Pediatric Apheresis Unit, [2]Pediatric Transplantation Unit, [3]St. Anna Kinderspital / MUW, Vienna, Austria

Introduction: Extacoproreal photopheresis (ECP) is currently used as second and / or third line therapy for acute and chronic GVHD containing of the collection of MNC/Leukocytes, irradiation with UVA light after adding 8-MOP and then reinfusion to the patient. In our institution we use all three available methods for ECP: 1. Inline method (all 3 steps in one system, UVARXTS™, CELLIX™), offline method (Leukocyte collection by Leukapheresissystem (AMICUS™, OPTIA™,COBEspectra™) and separate irradiation system (Macogenic™)), and MINI method (manual blood drawing, and all steps are manually performed under GMP conditions). Whether there is a difference between the methods no data are available. To elucidate the question, we performed a retrospective analysis of our patients treated for acute GVHD refractory standard immunosuppressive therapy (SIT).
Materials (or patients) and Methods: From 2002 until 2013 23 patients with > grade 2 acute GVHD refractory to SIT were treated with ECP (median age 6.9y (1.5 – 12), median bw 31 kg (7 – 60), 11 f, 12 m). Patients chart were analysed for GVHD, SIT, GVHD treatment, side effects, number of ECP and outcome of acute GVHD. The schedule was performed to our in-house standards. ECP started by a frequency of 2 to 3 reinfusions/week, with individualized tapering due to response of the GVHD and the ability to reduce the immunosuppressive therapy (IT).
Results: In total 338 procedures in 23 patients were enroled (123 inline in 4 patients (bw median 40 kg, median 32ECP(10-49)/patient); 174 offline in 13 patients (bw median 24 kg, median 10 ECP(3-55)/patient); 38 MINI in 6 patients (bw median 10 kg, median 7 ECP (3-9)/patient). No severe side effects were observed in either method. 21/23 patients improved (20 CR, 1 PR (gut), 1 SD (skin+liver), 1 PD (skin, liver, gut)). Log-rank-sum-test showed no statistically significant difference ($P = 0.98$) between the used methods for outcome and survival. The groups were not comparable to age and bodyweight, therefore a bias has to be claimed. In 22/23 IT could have been reduced, especially corticoid dosage could be tapered.
Discussion: ECP is in our hands an effective second line therapy in acute GVHD. Neither the method used nor the individualized schedule applied seems to influence the outcome. Due to the small patient number, this report could be only a step forward to prospective randomized trials, bringing hopefully an answer to these questions.
Disclosure of Interest: None Declared.

PH-P429
THERAPY WITH ACITRETIN IS EFFECTIVE IN REFRACTORY SCLERODERMATOUS CHRONIC GRAFT VERSUS HOST DISEASE

C. De Miguel[1,], A. Bermudez[1], P. Ibarrondo[1], L. Yañez[1], A. Insunza[1], E. Conde[1]*
[1]Hematology, Hospital Universitario Marques de Valdecilla, Santander, Spain

Introduction: The sclerodermatous form of cutaneous chronic graft versus host disease (SCL-cGVHD) affects quality of life of patients and it is usually refractory to different systemic immunosuppressive treatments. Acitretin is an oral retinoid used in the treatment of different dermatological diseases as well as in systemic sclerosis and its use has been hardly explored in the context of allogeneic transplantation. The objective of this study is to analyze retrospectively the efficacy of acitretin treatment in patients with SCL-cGVHD refractory or intolerant to systemic immunosuppressive therapy considering rate and duration of response, development of side effects and reduction or cessation of concomitant systemic immunosuppression (IS). Response was measured after a minimum of 3 months of therapy.
Materials (or patients) and Methods: From 2001 to 2012, 8 patients with SCL-cGVHD, defined clinical and histological, were treated with acitretin in our center. Four patients were male and median age at time of transplant was 49 years (22-72). All patients underwent an allogenic stem cell transplantation (allo-SCT) from an HLA-identical donor (2 unrelated donor). The stemcell source was peripheral blood (5) and bone marrow (3). Three patients had a previously allo-SCT. All patients had extensive moderate/severe cGVHD with cutaneous involvement, mucosal involvement (7) and/or lung involvement (6). The median therapy lines for cGVHD before acitretin treatment were 2,5 (0-5). Seven patients were refractory to several IS lines that included systemic corticosteroids in all cases and extracorporeal photopheresis in 4 cases. In one patient acitretin was started for severe cutaneous and muscular morphea at the beginning of cGVHD. The initial dose of acitretin was 25-35 mg/day added to previous IS, and it was reduced based on clinical course and toxicity. In all patients, liver function tests and lipids were monitored. The median time from the onset of cGVHD to the beginning of acitretin was 23 months (1-129).
Results: The median time of acitretin therapy was 10 months (3-20). All patients have shown improvement while receiving acitretin: 4 had cutaneous complete response (CR) and 4 partial response (PR). Two patients with PR showed cutaneous progression (at 4 and 9 months respectively) after withdrawal of the drug due to side effects. The median time to response was 2 months (1-3) and the median of duration of response was 13 months (1-111). Six patients developed side effects being the most frequent xerosis (6) and hyperlipidemia/hypertriglyceridemia (2).Two patients discontinued treatment due to severe xerosis and alopecia. In three cases, a 10 mg dose was used as maintenance treatment (1 for a good clinical outcome and 2 for mild xerosis). IS therapy could be diminished in 5 patients and withdrawal in 3. After acitretin discontinuation, there was no relapse or progression of SCL-cGVHD in those patients who achieved CR.
Discussion: In our experience, acitretin is an effective and well tolerated treatment in refractory SCL-cGVHD. It produces a high rate of complete responses and allows the reduction of systemic immunosuppression in most cases.
Disclosure of Interest: None Declared.

PH-P430
HIGH LEVELS OF SOLUBLE HUMAN LEUKOCYTE ANTIGEN G FAVOR TOLERANCE AFTER

L. Kordelas[1,], B. Wagner[2], P. A. Horn[2], D. W. Beelen[1], V. Rebmann[2]*
[1]Department of Bone Marrow Transplantation, [2]Institute for Transfusion Medicine, University Hospital Essen, Essen, Germany

Introduction: Human leukocyte antigen G (HLA-G) is a non-classical class I molecule. Membrane-anchored HLA-G and its soluble forms (sHLA-G) exert multiple functions favoring immune tolerance being operative in allogeneic situations such as pregnancy

and transplantation. The release of soluble HLA-G molecules is known to be influenced by genetic factors within the HLA class I and II regions of chromosome 6.
Materials (or patients) and Methods: Here, we studied the course of sHLA-G levels in 22 patients undergoing HLA-identical (*N*=13) or HLA-mismatched (*N*=9) allogeneic stem cell transplantation (alloSCT) due to acute myeloid leukemia (AML, *N*=17), secondary AML (sAML, *N*=4) and myelodysplastic syndrome (MDS, *N*=1). Plasma samples were serially procured from the patients before and 1, 2, 3, 4, 5, 6, 9 and 12 month(s) after transplantation. The samples were analyzed by sHLA-G specific ELISA and related to acute and chronic Graft-versus-host disease (GvHD).
Results: In line with the expected tolerance-inducing function of HLA-G, the sHLA-G levels were overall significantly increased in patients with low or no GvHD (*N*=13) compared to patients with acute GvHD grade 2 – 4 (*P*=0.02, *N*=9; two-way ANOVA). The difference in the sHLA-G courses was more prominent when patients with no or limited chronic GvHD (*N*=11) were compared to patients suffering from extended chronic GvHD (*P*=0.0008; *N*=11). Similar results were obtained for patients undergoing HLA-identical allo-SCT for acute (*P*=0.01) and chronic GvHD (*P*=0.0001), s. Figure 1. However, in HLA-mismatched alloSCT the sHLA-G levels did not differ in patients with and without severe acute or chronic GvHD.
Discussion: This study clearly demonstrates that high levels of sHLA-G favor tolerance after HLA-identical alloSCT, whereas in HLA-mismatched alloSCT genetic factors must be taken into consideration responsible for the release of HLA-G molecules which in turn influence the transplantation outcome.
Disclosure of Interest: None Declared.

PH-P431
CD4+CD25++ LYMPHOCYTE LEVELS ARE CLOSELY ASSOCIATED WITH AN OVERT MANIFESTATION OF AGVHD BUT IF LOW AT HEMATOLOGICAL RECONSTITUTION MAY BE PREDICTIVE OF HERPES VIRUSES REACTIVATION AND POORER SURVIVAL POST ALLOHSCT

D. Dlubek[1,2], J. Lange[2], E. Jaskula[1], M. Sedzimirska[2], M. Mordak-Domagala[2], A. Tarnowska[2], A. Borowik[2], A. Lange[1,2,]*
[1]L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, [2]Lower Silesian Center for Cellular Transplantation with National Bone Marrow Donor Registry, Wroclaw, Poland

Introduction: CD4+CD25++ lymphocytes are recognized as cells representing T regulatory cells subpopulation. Indeed, more than 80% of CD4+CD25++ are equipped with the suppressor cells machinery being FoxP3+ Baecher-Allan *J. Immunol*., 2001, *Roncador Eur. J. Immunol*., 2005).
Materials (or patients) and Methods: 99 patients (age: 7 to 65 years, median 42; 59 unrelated (MUD) and 40 sibling (SIB) transplantations: all except one MUD transplanted patients received anti-lymphocytes antibodies - ATG: 51 patients or Campath: 7 patients, in variance in the SIB group ATG or Campath received only those on reduced intensity conditioning – 13 and 3 patients, respectively) transplanted due to hematological malignancies were followed for the proportions and numbers of CD4+CD25++ lymphocytes in blood. Observation started on the day of hematological reconstitution (granulocytes > 500/µl), and was continued in one week interval for four weeks. Blood cells phenotyping was performed with the use of monoclonal antibodies (CD45, CD3, CD4, CD8, CD16, CD56, CD57, CD25, CD14, DR) and the results were read in FACS Calibur four color cytometer (BD, Mountain View, CA). The patients were followed for the pace of hematological recovery, aGvHD and infectious complications until one year post HSCT. For surveillance of Herpes viruses (CMV, EBV and HHV6) infections/reactivations Real Time PCR was used.
Results: It was found: 18 and 26 patients had symptoms of aGvHD (grade≥1) at haematological recovery (median 12 days post HSCT) or later (median 35 days) post-HSCT, respectively.
· In 18, 26 and 14 patients EBV, CMV, or HHV6 DNA copies were found in blood at least on one occasion during 1 year post-HSCT observation time, respectively.
· Patients having aGvHD symptoms at the time of haematological recovery had lower proportions (mean±SEM: 0.331%±0.047 vs 0.523%±0.045, *P*=0.007) and numbers (mean: 1 vs 4 cells/µl, *P*<0.001) of CD4+CD25++ cells at that time than those not having aGvHD any time post-HSCT.
· Patients showing aGvHD later post-transplant had lower percentages of CD4+CD25++ lymphocytes at the manifestation of aGvHD (median 35 days) as compared to CD4+CD25++ values found at the last determination prior to this complication (median 24 days post HSCT, 0.302%±0.042 vs 0.414%±0.065, *P*=0.005) which were similar to those seen at haematological recovery (0.414%±0.065 vs 0.425%±0.043, *P*=ns).
· Patients having Herpes virus(es) infections/reactivations during one year post HSCT had lower proportions of CD4+ (21.52%±1.88 vs 27.79%±1.84, *P*=0.010) and CD4+CD25++ (0.360%±0.030 vs 0.542%±0.044, *P*<0.001) lymphocytes at haematological recovery.
· Patients with high proportions of CD4+CD25++ lymphocytes (0.4%) at haematological recovery enjoyed better survival as compared to those with lower values (60% vs 34%, 2-year survival, *P*=0.039).
Discussion: In conclusion: (i) low proportions and numbers of CD4+CD25++ lymphocytes are associated with an overt clinical manifestation of aGvHD (ii) low proportions of CD4+ lymphocytes and their CD25++ subpopulation constitute a risk factor of Herpes viruses reactivation which may appear even later post HSCT and (iii) high proportions of CD4+CD25++ lymphocytes at haematological recovery are beneficial for long term survival.
Disclosure of Interest: None Declared.

PH-P432
SUPERIOR OVERALL SURVIVAL AFTER SIMULTANEOUS SALVAGE THERAPY BY PENTOSTATIN AND MYCOPHENOLATE MOFETIL FOR SEVERE INTESTINAL STEROID REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE

S. Klein[1,], T. Schmitt[2], G. Bug[3], J. Schetelig[4], R. Schwerdtfeger[5], K. Schaefer-Eckart[6], C. Schmid[7], G. Kobbe[8], D. Heidenreich[1], E. Rühe[1], M. Bornhaeuser[4], H. Martin[3], P. Dreger[2] and german cooperative transplant study group*
[1]III. Medizinische Klinik, Universitätsmedizin Mannheim, Mannheim, [2]Medizinische Klinik V, Universität Heidelberg, Heidelberg, [3]Medizinische Klinik II, Johann Wolfgang Goethe-Universität, Frankfurt, [4]Medizinische Klinik I, Universitätsklinikum Carl Gustav Carus, Dresden, [5]Zentrum für Knochenmarktransplantation, DKD, Wiesbaden, [6]5. Medizinische Klinik, Klinikum Nürnberg, Nürnberg, [7]2. Medizinische Klinik, Klinikum Augsburg, Augsburg, [8]Klinik für Hämatologie, Onkologie und Klinische Immunologie, Heinrich Heine Universität, Düsseldorf, Germany

Introduction: Steroid refractory intestinal acute GvHD (aGvHD) is a severe complication with a poor prognosis. Pentostatin has shown efficacy as salvage therapy in this situation. Here we report on a retrospective analysis on patients with histologically proven severe intestinal steroid-refractory aGvHD treated with pentostatin at 8 centres. Since pentostatin had been combined frequently with additional immunosuppressive therapies in the participating centres it was a specific aim of this study to elucidate the effect of such simultaneous immunosuppressive approaches.
Materials (or patients) and Methods: 124 patients who had received pentostatin as salvage therapy due to III° (58) or IV° (66) intestinal steroid-refractory aGvHD between 2000 and 2011 were included. Steroid-refractory aGvHD was defined as progression or no improvement despite treatment with prednisolone (≥2mg/kg/d) for ≥ 3 days. Pentostatin was infused at a dose of 1mg/m^2 for 3 days. Patients received 1-4 cycles. Steroids and calcineurin inhibitors were continued. Response was classified as complete (CR, no ongoing symptoms), very good partial (VGPR, residual symptoms) or partial (PR). 50 females and 74 males with a median age of 50 (range: 19-70) years were included. The underlying diseases were

AML (71), ALL (15), MPN (6), lymphoma (11), MDS (11), multiple myeloma (9) and aplastic anaemia (1). Patients had been transplanted from matched related (39), matched unrelated (54) or mismatched donors (31). Patients received pentostatin as first line salvage (106) or beyond first line salvage therapy (18).
Results: 70 (56%) patients responded after salvage therapy with pentostatin: 42 x CR, 14 x VGPR and 14 x PR. Median survival was 104 days; 2-year survival was 25% (median follow up: 45 months). Among 106 patients who received pentostatin as first line salvage therapy 64 (60%) responded (40 x CR [38%], 12 x VGPR [11%] and 12 x PR[11%]). Median and 2-year survival was essentially the same as in the total cohort. Responding patients had a significantly (P<0.0001) higher probability of survival in comparison with non-responders (2-year survival 44 vs 14%). Patients who had been transplanted from a matched related donor had a significantly (P=0.04) higher probability of survival in comparison with patients with other donors (2-year survival: 38 vs 21%). Out of the 106 first line salvage therapy patients 15 started with MMF simultaneously in addition to pentostatin. Patients of this group showed a significantly increased survival rate in comparison to all other patients after first line salvage therapy with pentostatin: median survival 465 vs. 89 days, 2-year survival rate 46 vs 22% (P=0.04). Further 27 patients out of the first line salvage group received one or more additional simultaneous non-MMF immunosuppressive approaches (MSC, infliximab, basiliximab, ATG, tacrolimus, alemtuzumab or ECP). These patients demonstrated a lower 2-year survival rate (19%, n.s.) and a median survival of 89 days.
Discussion: In line with previous reports the outcome after salvage therapy of III/IV° steroid-refractory intestinal aGvHD with pentostatin is within the range as reported for other salvage approaches. However, this analysis suggests that the combined use of pentostatin and MMF can improve survival rates. In contrast, the addition of other immunosuppressive therapies had no beneficial effect.
Disclosure of Interest: S. Klein Conflict with: Hospira, T. Schmitt: None Declared, G. Bug: None Declared, J. Schetelig: None Declared, R. Schwerdtfeger: None Declared, K. Schaefer-Eckart: None Declared, C. Schmid: None Declared, G. Kobbe: None Declared, D. Heidenreich: None Declared, E. Rühe: None Declared, M. Bornhaeuser: None Declared, H. Martin: None Declared, P. Dreger: None Declared.

PH-P433

HYPERACUTE GRAFT-VERSUS-HOST DISEASE (GVHD): SINGLE CENTER EXPERIENCE

S. Alonso[1,], O. López-Godino[1], M. Cabrero[2], R. Cabral[3], J. Dávila[4], J. C. Caballero[1], D. Sánchez-Nieto[5], R. Pérez-López[5], J. M. Bastida[2], M. López-Parra[2], F. Sánchez-Guijo[2], L. Vázquez[1], A. Redondo[2], C. del Cañizo[2], L. López-Corral[2], D. Caballero[2]*
[1]Hematology department, [2]Hospital Universitario de Salamanca, Salamanca, Spain, [3]Hematology department, Hospital de San Antonio, Porto, Portugal, [4]Hematology, [5]Pharmacy department, Hospital Universitario de Salamanca, Salamanca, Spain

Introduction: Acute GVHD is one of the major limiting factors in successful allogeneic hematopoietic stem cell transplantation (HCT), and early onset (hyperacute GVHD –haGVHD-) may be associated with lower response rate to first-line therapy and a higher non-relapse mortality rate. Few studies have been reported in the last years focusing on this aGVHD type.
Materials (or patients) and Methods: Between 1996 and 2013, 564 patients have received HCT in our center, 49 of them (8,7%) developed haGVHD defined as acute GVHD occurring before day +14, independent of neutrophil engraftment. Aims of this study were to analyse the outcome of these patients and to identify risk factor predicting survival.
Results: Median age was 45 years (16-65). Diagnosis were: 34% acute leukemias, 14% MDS, 32% NHL, 4% MM, 14% other diagnosis. Status of the disease at HCT was in 57% complete response (CR), 10% partial response, 10% stable disase and 22% non-response. In 41% of patients, conditioning regimen was myeloablative (22% including total body irradiation). 59% received HCT from unrelated donor (75% HLA 10/10). The cell source was peripheral blood in 81%. GVHD prophylaxis was based on cyclosporine in 50% (42% plus methotrexate and 8% plus MMF) or tacrolimus (45%; 10 % plus methotrexate and 35% plus rapamicine), 2 patients had received rapamicine-bortezomib; in 2% ATG was added. Median day of neutrophil engraftment (count > $0.5x10^9$/L) was day +15 (8-29). Median day to development haGVHD was 9 (2-14) and the clinical characteristics were: 12% fever, 75% rash, 67% gastrointestinal symptoms, 26% hepatic dysfunction and 10% weigh gain.
haGVHD grades were: 14% grade I, 57% grade II, 22% grade III and 6% grade IV. Biopsy was performed in 75% of patients, and the clinical diagnosis was proven in 26%. Overall response to first line treatment was 85%, with 75% of CR. 63% of patients achieving CR, relapsed in a median time of 52 days from the first episode; 38% with grade III-IV; 66% of them responded to the next line therapy. 66% of the patients at risk alive at day + 100 developed chronic GVHD.
With a median follow-up of 43 months for patients alive (13-174), the estimated 6 months, 1 and 5 years OS was 67%, 53% and 20%. Overall transplant related mortality was 61% (23% at day +100). The main cause of death was GVHD (61%). In the univariate analysis, factors significantly associated with a better OS an PFS were: reduce-intensity regimen (not achieved (NA) vs 6 months, P=0.002), GVHD grade I-II Vs III-IV (NA vs 4 months, P=0.013) and CR with first line treatment (NA vs 3 months, P=0.02). Regarding to aGVHD reactivation, if it was >100 days after the first episode (NA vs 12 months, P=0.04) or grade I-II-II vs IV (NA Vs 8 months) were associated with favourable outcome.
Discussion: Although it is assumed that engraftment is a requisite for the appearance of acute GVHD, in almost 10% of our patients it appears before. Histology confirms the diagnosis in only about a quarter of the patients. Although in our experience CR is high, it seems to be associated with a higher relapse and mortality. Much more biological knowledge is needed in these patients in order to identify them and improve prognosis.
Disclosure of Interest: None Declared.

PH-P434

SWITCH OF CYCLOSPORINE TO TACROLIMUS IN CHILDREN AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

F. Alby-Laurent[1,], J.-H. Dalle[1]*
[1]Hemato-Immunology, Robert Debré, Paris, France

Introduction: Because of its efficacy and for historical reasons, Cyclosporine A (CSA) still remains the basis of immunosuppression used in allogeneic hematopoietic stem cell transplantation (HSCT) as graft versus host disease (GVHD) prophylaxis at least in Europe. However, some published studies showed that Tacrolimus (FK) could be equivalent to, even better than CSA in this indication. Finally, some patients experimented evolutive acute GVHD despite CSA or CSA intolerance, then we may switch CSA for FK. We report here our experience about this switch.
Materials (or patients) and Methods: We included all the children who received an HSCT and a switch from CSA to FK between 2007 and 2012 in our pediatric hemato-immunology department.
Results: 302 allogeneic HSCT were performed during this period in 287 patients. All of them received CSA based GVHD prophylaxis. Among these 287 patients, 23 switched from CSA to FK. The median age of the patients who switched to FK was 7,2 years old and the sex ratio was 47.8%. 86.9% of them had malignant disorders including 65% with acute disorders in either CR1 (46%) or CR2 (54%) before the HSCT. 65.2% of the donors were unrelated and bone marrow was the most common source of stem cells (78.2%). The initial GVHD prophylaxis was CSA alone (26.1%), CSA + Methotrexate (39.1%) or CSA + Mycophenolate Mofetil (34.8%). 43.4% of the patients received antithymocyte globulin as pre-HSCT. The median follow-up was 556 days after the HSCT (range 31-2101). The OS and the DFS were 78.2%. The cumulative incidence of TRM was 8.6%. Only 1 patient died because of GVHD. 69,6% of the patients were alive and free from disease and GVHD. The switch between CSA and FK was made averagely on day 59 (range: 15-134) after the HSCT. The main cause of the switch was a CSA-

refractory acute GVHD despite association with corticosteroids +/- other immunosuppressive drugs (95.7%). The average time for obtaining FK efficient serum level (T0 ≥5ng/ml) was 7.5 days. The average minimal posology to have an efficacy T0 of FK was 0.01 mg/kg/day IV and 0.12mg/kg/day p-o. Before the switch, 39.1% of patients had grade I-II acute GVHD, 47.8% had acute GVHD grade III-IV and 13% had chronic GVHD. The average grade of GVHD was 2.56 +/– 0.90. After the switch we observed a significant reduction of GVHD with an average grade of 1.57 +/– 1.41 (P<0.01) when the T0 was obtained. The difference continues being significative 3 months after the switch with an average grade of 0.038 +/– 0.89 (P<0.01). 17.3% of the patients stopped tacrolimus because of side effects but 47.3% of the rest of the patients no longer needed the administration of other immunosuppressive therapies. Thrombotic microangiopathy episode in two patients - one of them had previously experienced it under CSA -, one seizure episode and 2 diabetes mellitus cases which could be also be explained by the concomitant administration of corticosteroids, were reported as side effects of FK. 43.4% of pts had presented an episode of viral replication. 3% needed antiviral treatment. 13% had documented bacterial events and no patients had fungal events.
Discussion: This study suggests that FK may be helpful for some patients with CSA resistant GVHD and may be used safely. It would be interesting to do a prospective study comparing CSA and FK as GVHD's prophylaxis.
Disclosure of Interest: None Declared.

PH-P435

IMPACT OF SMALL BOWEL EXPLORATION USING VIDEO-CAPSULE ENDOSCOPY IN THE MANAGEMENT OF DIARRHEA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

R. Dulery[1,], F. Jourdain[2], V. Maunoury[3], L. Magro[1], V. Coiteux[1], J.-F. Colombel[1], I. Yakoub-Agha[1]*
[1]Hematology and Bone Marrow Transplantation, [2]CHRU Lille, Lille, France, [3]Gastro-Enterology, CHRU Lille, Lille, France

Introduction: Diarrhea is a frequent and common symptom after allogeneic stem cell transplantation. Etiological investigation can be challenging as treatment options are often opposed. The purpose of this study was to evaluate the impact of the global diagnostic approach on the outcome of patients suspected of having acute (a) gastrointestinal (GI) graft-versus-host disease (GVHD).
Materials (or patients) and Methods: Patients who had diarrhea underwent extensive gastrointestinal examination and screening for infection. Small bowel exploration using video-capsule endoscopy (VCE) (associated or not with colonoscopy and/or upper endoscopy with systematic biopsy) was performed within 48 hours for each patient with atypical symptoms (without evidence of stage II or higher acute graft-versus-host disease (GVHD)-related skin lesions).
Results: A total of 37 VCE were performed on 30 consecutive patients who underwent allogeneic stem cell transplantation. The indications were isolated diarrhea (n=15), persisting or relapsing diarrhea (n=5), febrile or hemorrhagic diarrhea (n=17). The mean time between transplantation and the exploration was 48 days. The final diagnoses were GVHD (n=19), functional diarrhea without any other cause (n=9), viral infection (n=9), of which 3 were associated with GVHD. The evolution was favorable (n=24), treatment change (n=8), or death (n=5). The correlation between VCE results and the final diagnosis was perfect. For all diagnosis, the positive predictive value was 100% and the negative predictive value was 80%. Of the 20 patients with GVHD lesions observed with VCE, the final diagnosis was identical (2 cases with co-infections). Of the 10 patients with normal VCE, diarrhea was either toxic (n=8) or infectious (n=2). In 2 cases, VCE showed infectious lesions matching with the final diagnosis. Of note, VCE could not be interpreted in 3 cases because the capsule stayed in the stomach sack. In these 3 cases, the diagnoses were GVHD, motor diarrhea and GVHD with co-infection. In the 8 cases with uncertain or non-specific histological diagnosis, VCE results were normal (ruling out GVHD diagnosis) or showed GVHD lesions (leading to immunosuppressive treatment prescription).
Discussion: The VCE, associated with infectious screening, has enhanced the authors' ability to establish the right diagnosis and adapt treatments in patients suffering from diarrhea. This approach appears highly useful in patients with atypical GVHD-like symptoms, particularly with a view to avoiding unnecessary immunosuppressive treatment.
Disclosure of Interest: None Declared.

PH-P436

LOW INCIDENCE OF LATE NRM AND SEVERE CGVHD IN A PHASE II TRIAL OF THYMOGLOBULIN®, TACROLIMUS AND SIROLIMUS FOR GVHD PREVENTION

Z. Al-Kadhimi[1,], Z. Gul[2], W. Chen[3], D. Smith[3], A. Mitchell[3], M. Abidi[4], L. Ayash[4], L. Lum[4], A. Deol[4], V. Ratanatharathorn[4], J. Uberti[4]*
[1]Hematology/Bone Marrow Transplant, Emory University/ Winship Cancer Center, Atlanta, [2]Hematology/Bone Marrow Transplant, University of Kentucky/ Markey Cancer Center, Lexington, [3]Biostatistics, [4]Hematology/Bone Marrow Transplant, Wayne State University/ Karmanos Cancer Center, Detroit, United States

Introduction: Chronic graft-versus-host disease (cGVHD) is a major factor determining long term outcome and quality of life following allogeneic hematopoietic cell transplantation (AHSCT). In fact, cGVHD is the leading cause of late non-relapse mortality (NRM) and morbidity after AHSCT. The rates of severe cGVHD using NIH consensus criteria reported in the literature are 15-38%. Effective regimens are needed to reduce the severity of cGVHD and improve NRM. We analyzed and updated the long term outcomes of our phase 2 trial evaluating the efficacy of intermediate dose rabbit anti-thymocyte globulin (Thymo) in combination with tacrolimus and sirolimus for the prevention of acute and chronic GVHD after unrelated donor transplantation.
Materials (or patients) and Methods: In a Phase II trial, 47 adult patients (pts) underwent AHSCT from unrelated donors using Thymo, tacrolimus, and sirolimus for GVHD prophylaxis. Thymo was given as follows: 0.5 mg/kg day -3, 1.5mg/kg day -2, and 2.5mg/kg day -1. Chronic GVHD was measured using NIH consensus criteria.
Results: Twenty-two pts received 8/8 and 25 received 7/8 HLA matched unrelated grafts. Thirteen pts received a reduced intensity preparative regimen, while 34 pts received a full intensity regimen. The median follow-up duration was 45.2 months (95% CI 37.7-48.8), with minimum follow up of 30 months. At 4 years of follow up, the cumulative incidence of NIH severe cGVHD a, was 6.4 % (95% CI 1.6-15.9), and overall cumulative incidence of cGVHD was 48.9% (95% CI 33.6-62.6). Of 20 pts who are alive and disease free, only 4 pts continue to need systemic immune suppression at the last follow up. At 4 years of follow-up, the cumulative incidence of NRM and disease relapse were 27.7% (95% CI 15.7- 41.0) and 30.0% (95% CI 17.5- 43.6), respectively. There has been one non relapse death beyond 12 months and none after 18 months of follow up. Only one pt died from cGVHD and bronchiolitis obliterans, while two other pts had minimal symptoms from bronchiolitis obliterans. Progression free survival (PFS) and overall survival (OS) at 2 years were 50% (95% CI 35-64) and 56% (95% CI 42-70). Median OS is 33.9 months [95% CI (9.8 -*) *the upper limit of the CI was not calculated due to the pattern of censoring] All patients were censored after 40 months, so PFS and OS could not be calculated at 48 months. The median karnofsky performance status at 2 years was 90%.
Discussion: At long term follow up, the combination of Thymoglobulin, Tacrolimus, and Sirolimus was associated with low incidence of severe cGVHD, low incidence of late NRM, and good performance status. Most of the acute complications associated with this regimen could be minimized with maintaining Tacrolimus and Sirolimus at therapeutic levels. Further validation of these results in randomized phase III trials is needed.
Disclosure of Interest: None Declared.

Graft-versus-host Disease–Preclinical and Animal Models

PH-P437
INCREASED TH17/TREG RATIO IN LIVER CHRONIC GRAFT-VERSUS-HOST-DISEASE

F. Malard[1,2,], C. Bossard[3], E. Brissot[1,2], P. Chevallier[1], T. Guillaume[1], J. Delaunay[1], J.-F. Mosnier[3], P. Moreau[1], M. Grégoire[2], B. Gaugler[4], M. Mohty[2,5]*
[1]Hematology department, CHU de Nantes, [2]CRCNA UMR 892 INSERM 6299 CNRS, [3]Pathology department, CHU de Nantes, Nantes, [4]INSERM UMR 1098, Besancon, [5]Hematology department, AP-HP Hopital Saint Antoine, Paris, France

Introduction: Chronic GVHD is the main cause of late non-relapse morbidity and mortality after allo-SCT. Pathophysiology of chronic GVHD remains poorly understood. Chronic GVHD presents clinical features that mimic autoimmune diseases. The identification of proinflammatory Th17 cells which contribute to autoimmune diseases pathophysiology, raised the issue of the role of Th17 cells in human chronic GVHD. Indeed, the contribution of Th17 cells in chronic GVHD was assessed in GVHD mouse models. Furthermore, theses studies indicates that the expansion of Th1 and Th17 cells is favored by the progressive loss CD4+CD25+Foxp3+ regulatory T cells (Treg) leading to chronic GVHD. This report investigated the role of Th17 cells and Treg in liver biopsies taken from patients with or without chronic GVHD.
Materials (or patients) and Methods: The cohort included 17 patients who underwent allo-SCT for different hematological malignancies (cGVHD group) with a median age 54 (range, 27-71) y. The stem cell source was PBSCs in 9 cases (53%) and BM in 8 cases (47%). 13 patients received transplant from a MRD, and 4 patients from a MUD. A RIC regimen was used in 8 patients (47%) and a MAC regimen in 9 patients (53%). The control group included 8 patients without hematological malignancies who underwent liver biopsy for sleeve gastrectomy for morbid obesity (n=2) or adjacent tumor surgery (n=6). Immunohistochemistry was performed on deparaffinized tissues sections. A quantitative evaluation of antigens expression was performed by counting the number of positive cells in the whole biopsy.
Results: In the cGVHD group, based on standard pathology criteria, all 17 patients had a histologically proven liver chronic GVHD. Biopsies were taken at time of first hepatic symptoms declaration or during their reappearance and prior to corticosteroid treatment initiation or resumption. In the control group, all patients present histologically normal liver biopsy. In order to identify the Th17 cell population, biopsies were tested for expression of CD161 CCR6 and RORgt, the key transcription factor for the differentiation of Th17 cells. Significantly higher numbers of RORgt+, CD161+ and CCR6+ cells were counted in the liver of patients with chronic GVHD compared with the control group, mainly found in the portal space (P=0.0001, P=0.03 and P=0.03 respectively). We also assessed the presence of T cells expressing Tbet (the transcription factor characterizing Th1 cells) and Foxp3 (the master regulator gene of Treg cells) in the liver biopsies. There was no difference in the number of Th1 and Treg cells between the 2 groups (P=0.88 and P=0.12 respectively). Finally we look at the Th17/Th1 and Th17/Treg ratios, considering Th17 cells as RORgt positive cells as RORgt is the more specific hallmark of Th17 cells. Both Th17/Th1 and Th17/Treg ratios were significantly increased in the liver of patients with liver cGVHD (P=0.005 and P=0.002 respectively).
Discussion: The current study shed some light on the role of Th17 cells in the context of liver chronic GVHD. We show that Th17 cells infiltrate liver biopsies from patients with chronic GVHD. In addition, Th17/Treg ratio was significantly increased in the liver of patients with liver chronic GVHD, suggesting a regulatory defect in liver chronic GVHD. These data raise the prospect of future innovative approaches to optimize immunosuppression regimens for the treatment or prophylaxis of chronic GVHD by targeting the Th17 response.
Disclosure of Interest: None Declared.

PH-P438
THE ROLES OF DONOR T CELLS FROM DISTINCT ORIGIN IN CLINICALLY RELEVANT MURINE CHRONIC GVHD MODEL

M. Kosugi[1,2,], S. Ueha[1], K. Matsushima[1], T. Teshima[2]*
[1]Molecular Preventive Medicine, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, [2]Department of Hematology, Hokkaido University Graduated School of Medicine, Sapporo, Japan

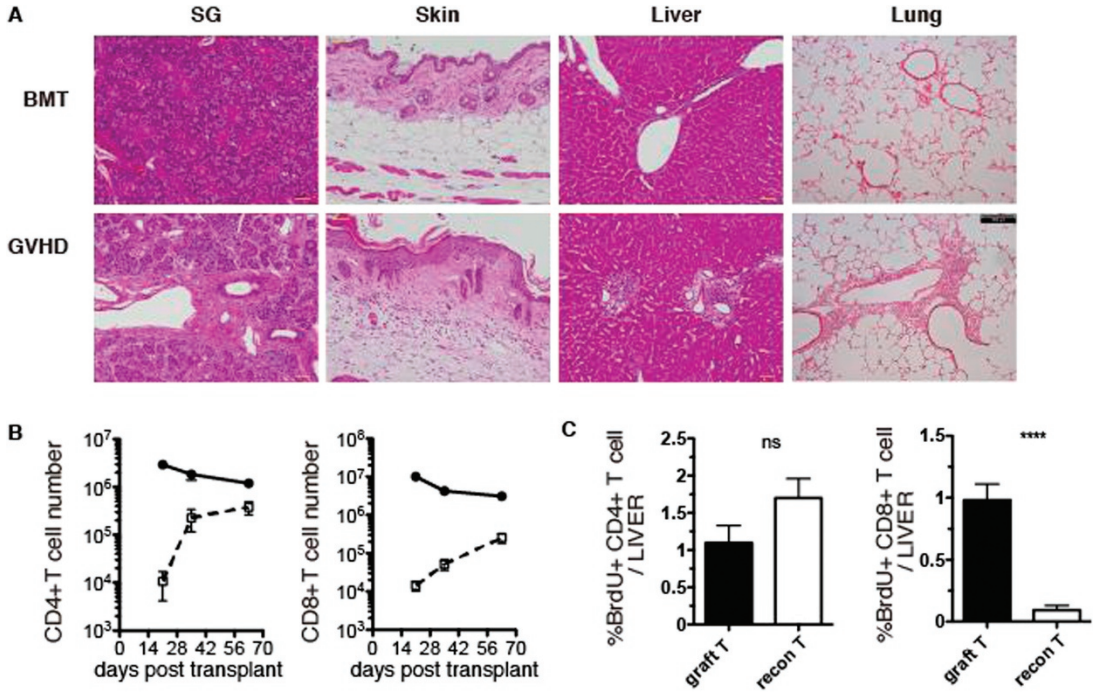
Introduction: The pathogenic of chronic graft-versus-host disease (cGVHD) is still elusive. Prior acute GVHD (aGVHD) increase the risk of cGVHD and *in vivo* T cell depletion decrease it. These preclinical findings indicate that cGVHD is strongly linked to donor T cell; not only reconstituted T cell (recon-T) but also graft derived T cell (graft-T). To understand the roles of distinct T cells, murine model that replicating clinical features including T-cell reconstitution in human is required. Here, we established clinically relevant murine cGVHD model and characterize each T cells from distinct origin during cGVHD.
Materials (or patients) and Methods: C3H.Sw (H2b) recipients were received 9Gy TBI and transferred T cell depleted BM (TCD-BM) with or without spleen CD4 and CD8 T cells from B6 (H2b) donor. After 9 weeks, histological analysis was performed in cGVHD affected organ (lung, liver, skin and salivary gland) following NIH criteria. The kinetics and function of graft- and recon-T in affected organ and secondary lymphoid organ (SLO) by using congenic system. Interventions included use of administration of Thy1.2 monoclonal antibody (mAb) from day 21 to deplete distinct T cell selectively.
Results: In this model, recipient mice develop multiorgan cGVHD (Figure 1A) with immunodeficiency. Both graft- and recon-T involved in affected organ more extent to SLO. Notably, graft-T was long-lasting and predominated over recon-T even at 63 days post-transplant (Figure 1B). Moreover, not all graft-T were exhausted and retained higher proliferation potential as compared to recon-T (Figure 1C). To demonstrate the contribution of long-lasting graft-T or recon-T in cGVHD pathogenesis, we performed selective depletion in chronic phase. Graft-T depletion failed to block cGVHD development because of recon-T proliferated and activated compensatory just in affected lesion. On the other hand, recon-T depletion resulted in acute exacerbation and immediately death due to graft-T reactivation. These data showed that recon-T was pathogenic enough to induce cGVHD independently, and graft-T possessed high-cytotoxicity and induce acute exacerbation in chronic phase. These compensatory activations indicated that each T cells was regulated competitively, and the extent of its activation depended on T cell pool size in affected organ.
Discussion: These findings elucidate the distinct roles of each T cell in chronic phase by using clinically relevant murine cGVHD model, and also suggest that the extrinsic regulation determine the extent of pathogenicity.
Disclosure of Interest: None Declared.

PH-P439
LENALIDOMIDE POTENTIATES HUMAN T CELL ALLORESPONSES BY SELECTIVELY INCREASING PROLIFERATION OF ALLOREACTIVE CD8+ CELLS WHICH EXHIBIT A NOVEL GENE EXPRESSION PROFILE - IMPLICATIONS FOR IMID THERAPY AFTER ALLOGENEIC HAEMATOPOIETIC CELL TRANSPLANT

C. Besley[1,], E. Kotsiou[1], R. Petty[1], A. Sangaralingam[1], R. LeDieu[1], J. Gribben[1], J. Davies[1]*
[1]Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University, London, United Kingdom

Introduction: IMiDs such as lenalidomide (Lena) have immunostimulatory effects and therefore potential to reduce relapse after allogeneic (A) HCT by increasing GvT effects. However, early clinical experience using IMiDs after AHCT has been limited by increased GvHD. Although Lena has been shown to augment mitogen-stimulated T cell responses in various ways, the effects of Lena on T cell alloresponses that mediate both GvT and GvHD have not been well defined. We determined the effects of Lena on

[PH-P438]



functional human T cell alloresponses using a HLA-mismatched *in vitro* model.
Materials (or patients) and Methods: We cocultured CFSE-labelled PBMC from healthy donors with irradiated allogeneic PBMC in the presence of 1μM Lena or vehicle control. Functional alloresponses were quantified after 9 days of allo-coculture by flow cytometry. In addition, allo-coculture responders were flow-sorted into allo-proliferative or non-proliferative fractions and extracted RNA used for gene expression profiling. Data was interrogated by O-miner, R and Ingenuity Pathway Analysis.
Results: Addition of Lena to allo-cocultures (n=12) led to a median 17% increase in the total number of responder T cells (P<0.001) due to an increase in proliferation of allospecific responder CD8 (alloCD8) T cells (P<0.001). In contrast, Lena had no effect on proliferation of CD4 cells. Proliferation kinetic analysis showed that Lena did not increase the number of times alloCD8 cells divided, but increased the precursor frequency of these cells within the responder cell pool (median 4% vs 11%, P=<0.001) consistent with a lowering of the activation threshold of alloCD8 cells. However addition of Lena to allo-cocultures did not increase the proportion of alloCD8 cells secreting TNFα or IFNγ or expressing CD107a. As expected, alloCD8 cells from untreated allo-cocultures demonstrated >2-fold altered expression of >500 genes mostly associated with DNA synthesis/cellular proliferation pathways when compared to non-proliferative CD8 cells. Lena treated alloCD8 cells when compared to treated non-proliferative CD8 cells showed further increases in expression of many of these genes, consistent with potentiation of pathways intrinsic to proliferative CD8 alloresponses. Importantly, Lena treated alloCD8 cells also demonstrated significant changes in expression of additional genes when compared to untreated alloCD8 cells. These included >8 fold increases in expression of multiple genes known to potentiate T cell immune responses in other settings including *PFKFB4*, *PIR* and *SOCS2* (part of the E3 ubiquitin ligase complex known to associate with cereblon and Lena) and >5 fold decreases in expression of genes which suppress T cell activation and memory differentiation including *FAIM3* and *PMCH*.
Discussion: We have shown for the first time that Lena potentiates human alloresponses by selectively increasing proliferation of alloreactive CD8 T cells. Lena likely mediates this effect by increasing expression of genes common to the intrinsic CD8 alloproliferative response but also by modulating expression of additional genes important in control of T cell activation and differentiation. These findings could be exploited to use Lena more effectively to potentiate GvT without increasing GvHD after AHSCT.
Disclosure of Interest: None Declared.

PH-P440
MICRORNA-181A ALTERS GRAFT-VERSUS-HOST-DISEASE BY MODULATING T CELL ALLOREACTIVITY

C.-W. Lee[1,], I. Dallmann[1], A. Krueger[2], A. Ganser[1], M. Scherr[1], M. Eder[1], C. Koenecke[1]*
[1]Hematology, Hemostasis, Oncology and Stem Cell Transplantation, [2]Institute of Immunology, Hannover Medical School, Hannover, Germany

Introduction: MicroRNA (miR)-181a enhances T cell receptor signaling by repression of several downstream phosphatases. Thus, we hypothesized that manipulation of miR-181a expression in donor T cells may alter Graft-versus-Host Disease (GvHD) after allogeneic bone marrow transplantation (BMT).
Materials (or patients) and Methods: Lentiviral gene transfer was used to over-express miR-181a and eGFP in murine T cells. To study miR-181a loss-of function, T cells from miR-181a/b-1 deficient (*miR-181a/b-1*$^{-/-}$) mice were used. *miR-181a*-over-expressing (*miR-181a-high*) and *miR-181a/b-1*$^{-/-}$ T cells were tested *in vitro* and *in vivo* using a murine haploidentical BMT-model (C57BL/6 into BDF1). T cell proliferation, apoptosis, alloreactivity and trafficking of donor T cells were assessed at different time points after gene transfer and BMT, respectively. GvHD symptoms were monitored using a clinical GvHD scoring system and survival was analysed using Kaplan-Meier statistics.
Results: miR-181a was over-expressed up to 200-fold upon lentiviral gene transfer into primary T cells with transduction rates

between 50% and 70%. In *in vitro* cultures stimulated with anti-CD3 and anti-CD28 beads, proliferation of *miR-181a-high* T cells was reduced 5-fold as measured by ^{3}H-thymidine incorporation. Furthermore, competitive *in vivo* homing assays showed significantly reduced numbers of *miR-181a-high* T cells in GvHD target organs seven days after BMT as compared to controls as assessed by FACS-analysis of eGFP positive $CD4^{+}$ and $CD8^{+}$ cells. Further analysis of donor T cells revealed significantly increased Fas ligand expression on *miR-181a-high* T cells as compared to control-T cells four days after BMT. Interestingly, recipient mice receiving *miR-181a-high* T cells showed no or fewer signs of GvHD and survived significantly longer as compared to control-T cell recipients. In contrast, *miR-181a/b-1*$^{-/-}$ T cells showed a trend towards more severe GvHD than wild-type T cells in our GvHD model.
Discussion: Our gain- and loss-of function data indicate a role of miR-181a in T cell allo-responsiveness. Although the precise molecular mechanism is not yet known over-expression of Fas ligand and activation induced cell death of *miR-181a-high* T cells may contribute to their reduced presence in GvHD target organs and concomitant prolonged survival in our haploidentical GvHD model. Hence, manipulation of miR-181a expression in allogeneic T cells modulates T cell allo-responses after BMT and miR-181a may thereby serve as a potential immunotherapeutic target to prevent or ameliorate GvHD.
(*MS, ME and CK contributed equally to this work).
Disclosure of Interest: None Declared.

PH-P441
PENTRAXIN 3 AS GRAFT-VERSUS-HOST DISEASE (GVHD) BIOMARKER IN A COHORT OF PEDIATRIC PATIENTS WITH HEMATO-ONCOLOGIC DISEASES

E. Dander[1,], P. De Lorenzo[1,2], F. Masciocchi[3], P. Vinci[1], F. Pasqualin[4], S. Bonanomi[3], A. Di Gregorio[1], M. Migliavacca[3], F. Pavan[3], L. Di Maio[3], C. Garlanda[4], A. Mantovani[4,5], A. Biondi[1], M. G. Valsecchi[2], A. Balduzzi[3], B. Bottazzi[4], A. Rovelli[3], G. D'Amico[1]*
[1]Centro Ricerca Tettamanti, Clinica Pediatrica, University Milano Bicocca, Ospedale San Gerardo/Fondazione MBBM, [2]Centro di Biostatistica per l'Epidemiologia Clinica, Università di Milano-Bicocca, [3]Centro Trapianti Midollo Osseo, Clinica Pediatrica, University Milano Bicocca, Ospedale San Gerardo/Fondazione MBBM, Monza, [4]Dipartimento di Immunologia e Infiammazione, Istituto Clinico Humanitas, [5]Dipartimento di Biotecnologie e Medicina Traslazionale, Università di Milano, Rozzano, Italy

Introduction: GvHD is a major obstacle to safe HSCT. Reliable markers facilitating the monitoring of this invalidating disease are warranted to improve its management. The long pentraxin (PTX) 3 increases in different human inflammatory pathologies and the correlation between PTX3 levels and disease severity suggests the potential usage of this molecule as GvHD marker.
Materials (or patients) and Methods: We analyzed plasma samples from 70 pediatric patients with hemato-oncologic diseases, who received HSCT at S. Gerardo Hospital (Monza), collected before, at HSCT and weekly thereafter, until day +100. Blood samples were further collected at the day of GvHD onset, before the beginning of GvHD-specific drug therapy. PTX3 plasma levels were monitored by ELISA. 34 patients developed acute GvHD very early (i.e. within day +28), 20 between day +28 and day +100 and 16 did not show GvHD in the monitored time-frame (jointly called 'no early GvhD' group).
Results: PTX3 plasma levels were significantly higher at the onset of early GvHD compared to levels of 36 patients in the 'no early GvHD' group: the median was 33.1 ng/ml (range: 9.4-847.4) versus 14.2 ng/ml (range 4.1-200.6), respectively (*P*-value<0.0001). Among patients who developed GvHD anytime within day+100, 17 with grade I GVHD showed significantly lower levels of PTX3 at disease onset (median 15.7 ng/ml, range: 7.3-38.3) as compared to levels of 36 patients with grade >I GVHD (median 37.1 ng/ml, range: 7.1-847.4, *P*-value=0.0012). In addition, we investigated the potential of PTX3 measured at day +0, +7 and +14 since HSCT in the prediction of early GvHD occurrence. After normalization with respect to baseline levels, i.e. before conditioning regimen, individual change in PTX3 at each time-point was calculated for patients in the 'early GvHD' and 'no early GvHD' groups. Comparison of median change at day +0 in the 2 groups showed that the conditioning regimen induced an increase in PTX3 levels in both groups, but with no statistically significant difference between them (*P*-value=0.62). However, at day +7 the median increase in PTX3 level was significantly higher in patients experiencing early GVHD versus those who did not (2.5-fold versus 1.2-fold increase, *P*-value=0.014). Data at day+14 showed a similar although less marked trend (0.98-fold versus a 0.3-fold increase, *P*-value=0.045). Finally, we also investigated the role of PTX3 plasma levels in a murine model of acute GvHD. PTX3 increased in allogeneic transplanted mice upon GvHD occurrence, compared to syngeneic controls. Interestingly, PTX3 levels were higher in the allogeneic group also before GvHD onset. This finding is consistent with the evidence of our pediatric cohort and supports the predictive value of PTX3 for GvHD onset.
Discussion: Our data showed that PTX3 plasma levels not only increased in patients experiencing GvHD, but they could also help in predicting patients at high risk for developing GvHD early after HSCT.
Disclosure of Interest: None Declared.

PH-P442
EFFECT OF PERI-TRANSPLANT PRO-INFLAMMATORY MILIEU ON HEPARANASE EXPRESSION IN DONOR CELLS BY STIMULATION OF PB AND CB MNC WITH LPS: STRONG ASSOCIATION WITH RS4693608 SNP

O. Ostrovsky[1], A. Shimoni[1], P. Baryakh[1], Y. Morgulis[1], M. Mayorov[1], K. Beider[1], A. Shteingauz[2], N. Ilan[2], I. Vlodavsky[2], A. Nagler[1,]*
[1]Bone Marrow Transplantation, Chaim Sheba Medical Center, Ramat Gan, [2]Cancer and Vascular Biology Research Center, Rappaport Faculty of Medicine, Technion, Haifa, Israel

Introduction: The success of allogeneic stem cell transplantation, a standard therapy for hepatopoietic melignancies and inherited hematopoietic disorders, is limited by graft-versus-host disease (GVHD) morbidity and mortality. The impact of the microbiota on GVHD development is known to be significant. Our previous studies revealed a highly significant correlation of HPSE gene rs4693608 SNP with the risk of developing graft vs. host disease (GVHD). The discrepancy in this SNP between recipients and donors was found to be a more significant factor for the risk of acute GVHD, than patient genotype. Moreover, heparanase is up-regulated in response to pre-transplantation conditioning, followed by a gradual decrease thereafter. Expression of the HPSE gene correlated with the rs4693608 both before and after conditioning.
Materials (or patients) and Methods: Analysis of HPSE gene expression before and after LPS treatment was performed in 128 umbilical cord blood (CB) samples and 104 peripheral blood (PB) samples from healthy individuals. Evaluation of heparanase expression in cell subsets was analyzed by RT-PCR and immunocytochemistry. Statistic analysis was performed using the NCSS software.
Results: Heparanase (both mRNA and protein) is expressed primarily in PB and CB derived neutrophils, monocytes and macrophages. In addition, we found, for the first time, that heparanase is expressed in NK cells. LPS treatment was found to up-regulate HPSE gene expression ($P<10^{-7}$) through TLR4. Our results indicated that monocytes are the key cells involved in the observed LPS, TLR4, HPSE cascade. Analysis of heparanase expression in resting MNCs did not reveal differences among individuals with various HPSE gene genotypes in both PB and CB samples Post-treatment heparanase expression correlated with rs4693608 SNP in both PB and CB MNCs. RQ (relative quantification) of AA carriers after LPS treatment of PB MNCs was 16.3 (7.5-34.5), while RQ of GG possessors was 6.4 (3.3-19.9), *P*=0.014. Analysis of HPSE gene expression in CB MNCs showed similar results (RQ of AA possessors was 24.9 (15.2-40.5), while RQ of GG carriers was 5.4 (3.1-13.0), *P*=0.0006). Ratio test revealed that MNCs with the AA genotype increased the level of heparanase to a higher extent than their counterparts with the GG genotype (*P*= 0.001 for PB MNCs and *P*=0.0006 for CB

MNCs). Moreover, exposure of PB and CB MNCs with genotype AA and GG to increasing amounts of LPS revealed up-regulation of the HPSE gene in MNCs with the AA genotype, while MNCs with the GG genotype disclosed non-responsiveness to increasing amounts of LPS.
Discussion: The present study indicated that the level of heparanase strongly correlates with the rs4693608 SNP and depend on cell type. The observed correlation between rs4693608 and heparanase expression in neutrophils and in LPS-treated MNCs suggests a yet undefined specific switch occurring only in activated cells. The present study helps to clarify how discrepancy in rs4693608 SNP between recipient and donor may elevate risk of acute GVHD.
Disclosure of Interest: None Declared.

PH-P443
PROTEOMIC PEPTIDE PROFILING IN THE LIVER DURING ACUTE GRAFT-VERSUS-HOST DISEASE
Y. Shi[1,], D. Pérez-Hernandez[2], S. Cordes[1], J. Mengwasser[1], G. Dittmar[2], O. Penack[1]*
[1]Department of Hematology and Oncology, Charite University Hospital, [2]CF Mass Spectrometry, Max-Delbrück-Centrum for Molecular Medicine, Berlin, Germany

Introduction: To add knowledge on the mechanisms contributing to acute GVHD, we first established a chemotherapy-based minor mismatch mouse model. We then studied the proteins expression in the liver during GVHD using a mass spectrometry-based proteomic approach.
Materials (or patients) and Methods: We used Bu/Cy conditioning in the LP/J→C57BL/6 model. At the time of maximum clinical manifestation of GVHD (day+23) the liver proteins were isolated. Quantification data of labeled peptides were measured considering N-termini and lysine dimethylation on light (+28Da), medium (+32Da) or on heavy (+36Da) modification per free primary amine. Protein and peptide quantitation information were extracted from MaxQuant 1.2.2.5. Protein interactions were studied with "String 9.05" (http://string-db.org/).
Results: We compared the results of WT mice to syn-BMT recipients and to allo-BMT recipients. In total, 2238 proteins and 14489 peptides were quantified by dimethylation labelling. 120 and 48 proteins were up (fc > 4) and down (fc >2.8) regulated in the liver of allo-BMT recipients as compared with syn-BMT recipients. Tap1 (28), ICAM1 (6), Cnx (4.6), H2-K1 (5.8) and H2-L (17) were up regulated; Iigp1 (13), Tgtp1 (38), Gbp2 (24) and Igtp (14) were remarkably overexpressed (Fig1, red circle). Another cluster of proteins, like Steap4 (10), Fmo5 (4) and Cytochrome P450 family proteins (around 4.5), were all significantly overexpressed (Fig1, blue circle).
Lrg1 and ICAM1 were significantly up regulated in GVHD liver (Fig2).
Discussion: During GVHD two clusters of proteins were over expressed, which contribute to activation of recipient antigen-presenting cells and donor T cells (Fig1, red circle), and are important to oxidation-reduction process and cellular metabolic process (Fig1, blue circle). Another cluster of proteins involved in cellular iron ion homeostasis was down regulated, possibly reflecting a disturbed iron metabolism during GVHD.
Interestingly ICAM1 and Lrg1, proteins that are involved in endothelial biology and angiogenesis, were significantly up regulated. Further proteomics studies, with more time points/organs, may help us to elucidate the mechanisms, and ultimately may help to identify novel potential therapeutic targets for GVHD therapy.
Disclosure of Interest: None Declared.

PH-P444
TARGET ORGAN TROPISM OF EFFECTOR T CELL POPULATIONS IN CHRONIC GRAFT-VERSUS-HOST DISEASE
R. Whittle[1,2,], H. Denney[1], T. Maher[1], M. Foster[1], A. Alfred[1], A. Chantry[2], P. Taylor[1]*
[1]Haematology Dept, Rotherham General Hospital, Rotherham, [2]Oncology Department, University of Sheffield, Medical School, Sheffield, United Kingdom

Introduction: Chronic graft-versus-host-disease may affect a broad range of organs, including the skin, gut, liver, eyes and lung. Patients may exhibit single or multiple organ pathology. Factors which influence particular organ involvement are poorly understood. Migration molecules including integrins & chemokines have been illustrated to licence allogeneic T cell entry to target organs. We have previously reported associations between differential chemokine expression and cGVHD organ profile (EBMT 2013, O325). We used a panel of chemokine receptor & tissue specific adhesion molecule expression profiles to determine whether organ involvement is reflected in circulating effector T cell populations
Materials (or patients) and Methods: Peripheral blood lymphocytes from 20 cGVHD adult patients were examined using flow cytometry. Effector, memory & regulatory T cell populations were

[PH-P443]

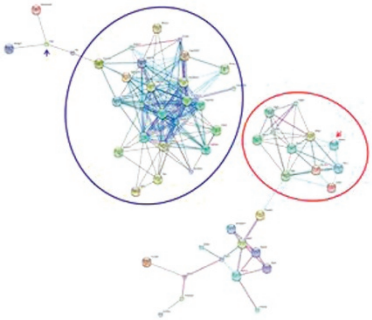


Fig1: Protein interaction networks of the over expressed proteins in the liver of GVHD mice: Some over expressed proteins were divided into 2 clusters: cluster I (red circle, ICAM1, red arrow) and cluster II (blue circle) proteins. Lrg1 is indicated with blue arrow. n=4 in wt and syngeneic group, n=3 in allogeneic group.

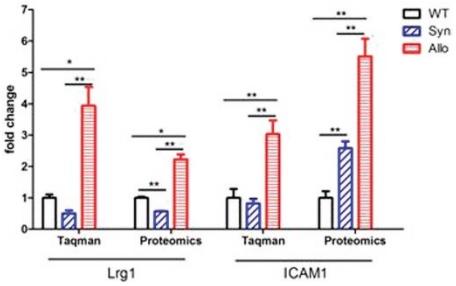


Fig2: The expression of Lrg1 and ICAM1 in the liver: Both mRNA and protein expression were measured. All the fold changes were compared to the WT untreated mice. Data are shown as mean±s.e.m. n=4-6 of Taqman and n=3-4 of proteomics per group. *P<0.05; **P<0.01.

identified. Expression of skin homing receptor, cutaneous lymphocyte antigen (CLA), gut tropic β7 integrin vascular addressin & a panel of chemokine receptors were examined & compared with normal controls (NC). 15/20 patients had active skin disease, with 6/15 exhibiting isolated cutaneous symptoms, 6/20 liver cGVHD, 5/20 current or recent gut GVHD.
Results: The proportion of circulating CLA+ CD4+ effector T cells were significantly elevated in patients with active skin disease (*n*=15), compared to patients without skin disease (*n*=5) (Median 13% vs 6%, *P*=<0.05). Proportion of CLA+ CD4+ effectors was highest in patients with isolated skin disease (*n*=6, median 18%). CD4+ effector T cells bearing chemokine receptor CCR5 were elevated in skin only disease patients compared with non-skin (Median 60% vs 20% (*P*=<0.05). In 5/6 skin patients with isolated skin disease a CLA+ CCR5+ CD25+ CCR7- CD4+ T cell population could be identified which was absent in patients without skin disease. Proportions of CXCR3+, CCR5+ & dual CXCR3+ CCR5+, CD4+ effector T cells of a Th1 phenotype were all elevated compared to NC (*P*=<0.01), however no association with different organ involvement could be determined. There was a trend toward higher proportions of CXCR6+ T cells in patients with liver or gut disease compared to those without (Median 3% vs 0.5%. *P*=0.10).
Discussion: The skin homing receptor CLA and its interaction its ligand, E-selectin, represent an important axis in the ingress of T cells into the skin. The majority of CLA+ T cells are resident in the skin under resting conditions so whilst recruitment of additional T cells from circulation may be a feature of cutaneous inflammatory conditions, it may not be prerequisite. However the presence of elevated proportions of CLA+ effector T cells in skin cGVHD patients, and in skin disease only patients, the existence of an activated phenotype CLA+ effector T cell population, which in lacking CCR7 is unlikely to recirculate to the lymph node, is supportive of a skin specific effector cell population which may be contributing to differential target organ pathology. The elevated effector CCR5 & CXCR3 T cell chemokine receptor expression is consistent with allogeneic expansion and cellular migration / tissue entry studies in GVHD (Palmer *et al,* 2009, Croudace *et al,* 2012). However, the lack of association of CXCR3+ CD4+ effector T cells with specific organ involvement may reflect the diverse tissue production of CXCR3 ligand chemokines. Our findings may contribute toward understanding differential organ phenotypes in cGVHD
Disclosure of Interest: None Declared.

PH-P445
BONE MARROW DERIVED MESENCHYMAL STROMAL CELLS (BMSC) ARE EFFICIENT MODULATORS OF XENOGENEIC GRAFT VERSUS HOST DISEASE

A. Barrett[1,], S. Amarnath[2], B. Salem[3], A. Laurence[4], M. Eckhaus[5], J. J. Melenhorst[6], J. J. Rose[7], M. Sabatino[8], D. Stroncek[8], D. H. Fowler[3]*
[1]Hematology, National Heart Lung and Blood Institute, NIH, [2]Experimental Transplantation and Immunology Branch, [3]Experimental Transplantation and Immunology Branch, National Cancer Institute, NIH, [4]Molecular Immunology and Inflammation Branch, National Institute of Arthritis, Musculoskeletal and Skin Diseases, [5]Division of Veterinary Services, Office of Research Services, NIH, Bethesda, [6]Department of Pathology and Laboratory Medicine, Clinical Cell and Vaccine Production Facility, University of Pennsylvania, Philadelphia, [7]Experimental Trnasplantation and Immunology Branch, NCI. NIH, [8]Cell Processing Section, Department of Transfusion Medicine, Clinical Center, NIH, Bethesda, United States

Introduction: Graft versus Host Disease (GVHD) is a major complication of bone marrow transplantation therapy. Here we sought to investigate if BMSCs are ideal candidates for the treatment of human GVHD by utilizing our previously well-established mouse model of humanized x-GVHD.
Materials (or patients) and Methods: Mice were allowed to develop lethal GVHD and monitored for >50% human Th1 cell engraftment. On sufficient Th1 cell engraftment and disease development, murine recipients were treated with BMSC (2M per mouse) three times every four days. Control cohorts received irradiated allogeneic human monocytes (2M per mouse). At the end of treatment, mice were evaluated for weight loss, long-term survival and pathological changes in the GVHD target tissues.
Results: We found that BMSCs were (1) efficient at treating x-GVHD long term (2) diminished T cell numbers in the GVHD target organs and (3) maintained T cell immune competence *ex vivo*. Cohorts post BMSC treatment showed increased weight gain compared to control cohorts (*P*=0.04), diminished pathological damage of GVHD target organs (*P*=0.03), with significant changes in the liver (*P*=0.0005), lung (*P*=0.03) and skin (*P*=0.05). Immunological parameters were measured in secondary lymphoid organs such as the spleen and BM. Interestingly, murine recipients that were treated with BMSCs showed significant decrease in human cytokine production in the spleen (control vs. treated; IFNgγ; *P*=0.04, TNF-α; *P*=0.04). A similar phenotype was noted in the BM compartment reaching a trend towards significance (control vs. treated; IFNg; *P*=0.07, TNF-α; *P*=0.08). We next explored the immune-phenotype of human T cells in the GVHD target organs. In contrast to the secondary lymphoid organs, a significant decrease in human Th1 cell number was noted in the liver of the treatment cohorts (*P*=0.01) with no difference in the cytokine profile of the human Th1 cells. A similar trend towards decreased cell numbers were also noted in the lungs of the treated cohorts. Finally we sought to determine the mechanism by which BMSCs diminished GVHD in the treatment cohorts as compared to the controls. We found that the human T cells from the treatment cohorts exhibited diminished proliferation capacity as measured by Ki67 (*P*=0.06) and had a significant decrease in Bcl_2 expression (*P*=0.01) as compared to the control cohorts. On *ex-vivo* stimulation, human Th1 cells from the treatment cohorts exhibited strong allo-responses as compared to the control cohorts (*P*=0.03).
Discussion: These data taken together may suggest a novel mechanism by which BMSCs can inhibit human Th1 cell mediated x-GVHD. In summary, our study is the first to assess the function of clinical grade BMSCs in inhibiting x-GVHD. The data presented here suggests that BMSC can efficiently decrease inflammation by decreasing T cell number while maintaining T cell immune competence. These results may suggest that BMSCs are ideal cellular therapeutic candidates for inhibiting Th1 mediated allo and autoimmunity.
Disclosure of Interest: None Declared.

PH-P446
DEFINING THE BIODISTRIBUTION AND IMMUNOMODULATORY MECHANISMS OF HUMAN MESENCHYMAL STROMAL CELLS FOLLOWING ALLOGENEIC BMT

J. J. Auletta[1], S. K. Eid[2], P. Wuttisarnwattana[3], M. D. Keller[2], I. Silva[4], D. L. Wilson[3], K. Robert Cooke[4,]*
[1]Pediatrics, Nationwide Children's Hospital, Columbus, [2]Pediatrics, Case Western Reserve University School of Medicine, [3]Biomedical Engineering, Case Western Reserve University, Cleveland, [4]Oncology, Johns Hopkins University School of Medicine, Baltimore, United States

Introduction: Defining the biodistribution and immunomodulatory mechanisms of human mesenchymal stromal cell (hMSCs) following allogeneic BMT (alloBMT) is critical to optimizing the clinical utility of these cells for the treatment and prevention of GvHD. We initially reported that hMSC can modulate murine T-cell (TC) alloreactivity *in vitro* and *in vivo* (*ASH 2011*). Importantly, hMSCs had no effect on CTL activity and potent GvL effects were preserved following hMSC administration. We now use novel, cryo-imaging technology to determine where hMSCs co-localize with donor T-cells to attenuate activation and proliferation.
Materials (or patients) and Methods: Cryo-imaging provides high-resolution anatomic imaging as well as single-cell detection and can facilitate unique quantitative analysis of fluorescently-labeled cells within an organ of interest. In initial experiments, irradiated, B6D2F1 mice were IV injected with 1.0×10^6 fluorescently labeled (Qtracker 625 red quantum dots) hMSCs on D1. Mice were sacrificed at several time points, embedded en bloc in optimal cutting

temperature compound and snap frozen. In subsequent experiments, CFSE labeled donor (B6 or B6D2F1) splenic T cells were infused on D0 followed by the infusion of Q-dot labeled hMSCs on D1.
Results: Six hours after infusion, fluorescent cryo-imaging with single cell resolution demonstrated that the majority of labeled hMSCs can be found in the lung (83%) and liver (17%), with relatively few dispersed in other locations. At 24 hours (D2 after BMT) early re-distribution of cells to the liver and spleen was detected. Specifically, we found that hMSCs migrated to splenic marginal zones after BMT. Greater numbers of hMSCs were found in the spleens of alloBMT recipients (compared to syn) at all time points through 96hr (D4) after BMT (P <0.05). Interactive visualization enabled imaging hMSC co-localization with T-cells within the spleen at 48, 72 and 96 hours after BMT. For each mouse, white pulp volume was first quantified and then CFSE intensity was measured. Since CFSE dye intensity decreases with T-cell expansion, intensity histogram analysis of T-cell brightness served as an *in vivo* T-cell proliferation assay. Analysis revealed that T-cells in allo-BMT mice given hMSCs retained their CFSE fluorescence intensity to a greater degree than allogeneic mice not given hMSCs. Correspondingly, whole spleen and white pulp volumes were also significantly reduced compared to alloBMT controls. Finally, MLRs containing hMSCs demonstrated that decreased T-cell proliferation was associated with increased PGE_2 levels. Indomethicine and E-prostanoid 2 (EP2) receptor antagonism reversed, and EP2 agonism restored, hMSC-mediated T-cell suppression, confirming a role for PGE_2 *in vitro*, and cyclo-oxygenase inhibition after alloBMT abrogated the protective effects of hMSCs *in vivo*.
Discussion: In sum, novel, whole-mouse, cryo-imaging shows for the first time that hMSCs migrate to the marginal zone of the spleen within 24 hours of administration. hMSCs subsequently accumulate, co-localize with donor T-cells in the splenic white pulp and attenuate alloreactive T-cell proliferation and expansion in a manner that is PGE-2 dependent and preserves allo-specific GvL responses. These results provide crucial insights connecting biodistribution with local regulatory effects of hMSCs that may serve to optimize the delivery of hMSCs to regulate clinical GVHD.
Disclosure of Interest: None Declared.

Haemoglobinopathy and Inborn Errors of Metabolism

PH-P447
HAPLOIDENTICAL STEM CELL TRANSPLANTATION WITH CD3+/19+ DEPLETED PBSC FOR ADVANCED STAGE SICKLE CELL DISEASE

B. Carolin Heinzelmann[1,], M. Kleis[1], J. Föll[1], S. Corbacioglu[1]*
[1]Pediatric Hematology/Oncology, University Hospital Regensburg, Regensburg, Germany

Introduction: Sickle cell disease (SCD) with approximately 300.000 newborns diagnosed each year is one of the most prevalent hematological diseases worldwide. Despite significant advancement in the prevention and treatment of SCD-related complications, associated morbidity and mortality remains substantial.
Allogeneic hematopoietic stem cell transplantation (SCT) is currently the only curative option. Until recently SCT was offered to patients with advanced SCD only if an HLA identical sibling was available. As MSD are available only for a minority of patients haploidentical SCT is becoming an intriguing alternative.
Materials (or patients) and Methods: Three adolescent and young adult patients with homozygous SCD, 1 male, two female (age 18, 17, 13 years) with a Turkish background and a high degree of disease burden were transplanted from a haploidentical donor.
Results: Conditioning consisted of ATG, Fludarabin, Thiotepa and Treosulfan in two patients, who achieved a complete donor chimerism without developing any relevant SCT related complications. The other patient received Campath, Fludarabin, Thiotepa and Melphalan. The graft of his mother contained only 2.93 x 10^6/kg CD3/CD19 depleted cells as a result of poor mobilization. Due to graft failure the patient received an ineffective boost of 4 x 10^6/kg CD 34+ cells on day +30. The patient was reconditioned with ATG (Fresenius), Fludarabine, Cyclophosphamide and 2 Gy TBI and was retransplanted from his father with a total of 8.4 x 10^6/kg CD3/CD19 depleted cells. The patient achieved a complete chimerism on day +20 and maintains currently a stable mixed chimerism without any further complications.
In all three patients immunosuppression consisted of CsA and MMF. Transplant related morbidity was mild. Two patients developed aGvHD of the skin that resolved immediately with prednisolone alone. Currently the patients are 410, 260 and 92 days post SCT in excellent condition with stable chimerism of 89%, 100% and 100% respectively.
Discussion: Socioeconomically the costs of conventional medical care outweigh by far the costs of SCT. Haploidentical SCT is a safe and curative option for patients with SCD, even in advanced stage and can be a feasible option for SCD patients with poor donor availability in developing countries.
Disclosure of Interest: None Declared.

PH-P448
HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MUCOPOLYSACCHARIDOSIS VII

L. Sisinni[1,], M. Torrent[1], N. Pardo[1], M. Pineda[2], M. Coll[3], L. Gort[3], E. Fernandez[1], I. Badell[1]*
[1]Pediatric BMT Unit, H. Santa Creu i Sant Pau, [2]H.Sant Joan de Deu, [3]H. Clínic, Barcelona, Spain

Introduction: The Mucopolysaccharidosis VII is an inherited disease characterized by the cellular accumulation of undegraded glycosaminoglycans due to the deficiency of the lisosomal enzyme B-glucuronidase. The usefulness of HSCT is due to the ability of hematopoietic cells to distribute and differentiate as microglial cells in the brain, alveolar macrophages in the lungs and Kuppfer cells in the liver and to a cross-correction phenomenon by which the engrafted cells provide normal enzyme to the neighboring deficient cells. At our knlowledge only one case of MPS VII submitted to HSCT is described in literature.
Materials (or patients) and Methods: We describe a case report of a two years old female. Clinically she presented skeletal deformations, hepatomegaly, umbilical hernia and a history of frequent upper respiratory infections. She did not show neurocognitive neither cardiac alteration. The height and weight were at a lower centile for the age (3$^{rd\ centile}$). The diagnosis of MPS VII was carried out by the demonstration of high levels of glycosaminoglycans in urine, a low level of B-glucuronidase in leucocytes and mutations in the B-glucuronidase gene. In order to ameliorate skeletal problems and to avoid a possible neurocognitive involvement the patient was submitted to an allogeneic bone marrow transplantation from an unrelated donor with HLA identity. A reduced intensity conditioning regimen based on fludarabine, melphalane and alemtuzumab was used. The prophylaxis of GVHD was based on cyclosporine and mofetil mycophenolate. After 1 year, because of the loss of the engraftment, she received a second stem cell transplantation. The source of progenitors was blood cord from HLA-matched unrelated donor. A myeloablative conditioning regimen based on busulfan (adjusted doses), cyclophosphamide and rabbit ATG was used. Cyclosporine was given for the GVHD prophylaxis.
Results: After the first transplantation the patient achieved total chimerism on day +26. The laboratory tests confirmed progressive normalization of B-glucuronidase level from day +55 until day +272 when started decreasing. The chimerism was progressively lost with final rejection of engraft. On day +300 post HSCT the B-glucuronidase level was <3% of normal. In order to improve the engraftment six infusions of donor lymphocytes were carried out,

nevertheless no improvement was observed. After the second transplant full chimerism and normal B-glucuronidase level were achieved on day +26 and +116 respectively and persisted during the 48 months after transplant. Glycosaminoglycans in urine presented a progressive decrease.
Discussion: HSCT may be a valid therapeutic option in patients with mucopolysaccharidosis VII. In our report total engraftment and normalization of B-glucuronidase level were achieved after both transplantations nevertheless only with a myeloablative conditioning regimen stable engraftment was maintained allowing persistent metabolic correction. Clinically we observed the stabilization of skeletal abnormalities, improvement of phenotype and resolution of hepatomegaly. During the 48 months after the second transplantation the patient has been showing a normal neurocognitive development and physical growing, being able to perform normal daily activities.
Disclosure of Interest: None Declared.

PH-P449
ALLOGENEIC STEM TRANSPLANTATION IN PATIENTS WITH CLASS 2 AND CLASS 3 THALASSEMIA MAJOR: A SINGLE-CENTER EXPERIENCE FROM SOUTH INDIA

S. Bhat[1,], S. Badiger[1], N. K.S[2], S. Damodar[2]*
[1]Pediatric Hematology and Oncology, [2]Hematology, Mazumdar Shaw Medical Center, Narayana Health City Bangalore India, Bangalore, India

Introduction: Hematopoietic stem cell transplantation (HSCT) is the definite treatment for patients with thalassemia major.
Materials (or patients) and Methods: We retrospectively analyzed the outcomes in thalassemia major patients classified as Class 2 and Class 3 by the Pesaro criteria, who underwent allogeneic HSCT between November 2004 and December 2012.
Results: Forty one patients (Males *n*=27; females *n*=14) with a median age of 6 years (range 3 to 22 years) underwent HSCT during this time. Thirty five patients were classified as Class 2 and 6 as Class 3. The conditioning regimens used consisted of Bu-Cy-ATG (*n*=24), Bu-Cy (*n*=2), Flu-Bu-Cy-ATG (*n*=8) and Treo-Flu-Thio (*n*=7) with cyclosporine and methotrexate as GVHD prophylaxis. Stem cell source was marrow, peripheral stem cells and cord in 33, 7 and 1 patients respectively. The median TC cell dose was $6x10^8$/kg (range 3 to 16.3 $x10^8$/kg) and median CD34 cell dose of 3.8 $x10^6$/kg (range 1.0 to 11.0 $x10^6$/kg). Hematological recovery was seen in all patients except three patients, who died before engraftment. Neutrophil and platelet engraftment occurred at a median of 13 days (range, 12-20 days) and 18 days (range, 16-35 days), respectively. Sixteen patients developed veno-occlusive disease, 4 patients developed Gr II-IV acute graft-versus-host disease (GVHD), and 4 patients had chronic GVHD. At day +28, twenty two patients showed more than 90% donor chimersim and rest had mixed chimerism. Two patients experienced secondary graft rejection. Treatment-related mortality (TRM) for the whole cohort was 14.6% at 100 days and 19.5% at 6 months post transplant. TRM was 14.2% and 50% for Class 2 and class 3 patients respectively. At a median follow up of 1144 days, overall survival and thalassemia free survival are 80.4%, and 75.6% respectively.
Discussion: Conclusion:
Similar outcomes have been reported from developed countries. Outcome of Class 3 patients still continues to be poor and VOD rates are high in this patient group.
Disclosure of Interest: None Declared.

PH-P450
A COMPARISON BETWEEN PERIPHERAL BLOOD STEM CELL TRANSPLANTATION AND BONE MARROW TRANSPLANTATION IN THALASSEMIA MAJOR

A. Ghavamzadeh[1,], K. Alimoghaddam[1], A. A. Hamidieh[1], A. Jalali[1], L. Sharifi Aliabadi[1], S. A. Mousavi[1], B. Bahar[1], M. Vaezi[1], M. Jahani[1]*
[1]Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of medical Sciences, Tehran, Iran, Republic Of Islamic

Introduction: HSCT is considered the only curable treatment option for patients with thalassemia. Here, we report our hematopoietic stem cell transplantation experience in the treatment of thalassemia major patients, using PBSCT versus BMT.
Materials (or patients) and Methods: From 1991 to 2013, 574 patients underwent HSCT in our centre. 221 patients underwent HSCT from BMT and 353 patients received PBSCT. The median age in the BMT group was 7years (2-26years) and in the PBSCT group was 8 years (2-29 years) (*P*-Value=0.001). In the BMT group, 89(40.3%) patients were class III, whereas in the PBSCT group 121(34.3%) were class III (*P*-Value=0.196).
Results: Acute graft versus host disease (GvHD) occurred in 141(63.80%) and 253(71.70%) of patients in the BMT and PBSCT groups, respectively (*P*-Value=0.048). Chronic GvHD was 19.30% in the BMT and 32.70% in the PBSCT (*P*-Value=0.001) survivors after 100 days. With a median follow-up of 50 months, the 5-year thalassemia-free survival rate (TFS) of BMT and PBSCT was 76.3% and 67.5%, respectively (*P*-Value=0.294). The 5-year overall survival rate (OS) in the BMT and PBSCT groups was 80.1% and 73.8%, respectively (*P*-Value=0.119). The rate of rejection was 16.3% and 6.5% in the BMT and PBSCT groups, respectively. The most common causes of death were GvHD and infections in both groups. TFS and OS results stratified by disease classes showed better survival rates in lower classes (Table1).
Discussion: According to results of the study, HSCT is considered for younger patients with lower class of thalassemia. Appropriate GvHD prophylaxis regimen should be used to decrease the mortality rate in these patients. Compared to BMT, PBSCT with short time hospitalization has a lower cost and is an easy procedure to perform for donor Engraftment was faster in the PBST group, compared to BMT group. Acute and chronic GvHD were more frequent in the PBSCT group, but most of them were limited and manageable and chronic GvHD was more mild but was alleviated over time.
Disclosure of Interest: None Declared.

[PH-P450]

Table 1- Overall survival rate (OS) and thalassemia-free survival rate (TFS) of BMT and PBSCT stratified by disease classes:

BMT					**PBSCT**				
OS	1 y	2 y	5 y	p-value	OS	1 y	2 y	5 y	p-value
I	92.3%	92.3%	90.1%	0.148	I	81.7%	80.7%	78.0%	0.011
II	79.7%	79.7%	77.6%		II	87.9%	85.2%		79.5%
III	79.7%	78.3%	76.3%		III	75.1%	69.5%		64.0%
TFS	1 y	2 y	5 y	p-value	TFS	1 y	2 y	5 y	p-value
I	80.8%	80.8%	78.8%	0.167	I	78.9%	77.9%	75.2%	<0.001
II	68.4%	67.0%	65.4%		II	85.5%	81.9%		76.1%
III	67.7%	64.7%	60.6%		III	66.0%	59.7%		55.2%

PH-P451

THIRTY YEARS' EXPERIENCE OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR GAUCHER DISEASE IN SWEDEN

M. Machaczka[1,2,], C. Kämpe Björkvall[3], K. Myhr-Eriksson[3], A. Markuszewska-Kuczyńska[1,4], H. Hägglund[1,2], B. Lauri[3], M. Remberger[5], P. Ljungman[1,2], C.-G. Groth[6], O. Ringdén[5,7]*
[1]Hematology Center Karolinska, Karolinska University Hospital Huddinge, [2]Division of Hematology, Department of Medicine at Huddinge, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, [3]Department of Medicine, Sunderby Regional Hospital of Norrbotten County, Luleå, [4]Department of Radiation Sciences, Section of Hematology, University of Umeå, Umeå, [5]Division of Clinical Immunology and Transfusion Medicine, [6]Division of Transplantation Surgery, Department of Clinical Science, Intervention and Technology, [7]Center for Allogeneic Stem Cell Transplantation, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden

Introduction: After the early successes in therapy of Gaucher disease (GD), allogeneic hematopoietic stem cell transplantation (allo-HSCT) was completely abandoned in developed countries in the 1990s in favor of life-long medical treatment, the most often in form of enzyme replacement therapy (ERT).
The aim of this study was to describe a long-term outcome of Swedish patients who successfully underwent allogeneic bone marrow transplantation (allo-BMT) for GD at Karolinska University Hospital between 1982–1991.
Materials (or patients) and Methods: Six patients underwent allo-BMT because of severe GD; 4 patients for Norrbottnian, neuronopathic type 3 Gaucher disease (N-GD3) and 2 patients for non-neuronopathic, type 1 GD (GD1). The median age of the patients at the time of transplantation was 2.5 years (range 2–9 years). All patients were splenectomized before transplantation (two of them underwent partial splenectomy). Five patients underwent BMT from related donors (4 HLA-identical siblings and 1 from a one HLA-B antigen locus-mismatched father) and one patient was grafted from an unrelated donor (HLA-A, -B and -DR matched, with one -DP antigen mismatched graft). The myeloablative conditioning in all cases was based on BuCy in 4 patients and CyTBI in 2 patients.
Results: All patients engrafted; however, the parental graft was rejected (the patient is still alive on ERT). Four patients developed mild acute GVHD, although requiring prednisolone therapy. One patient developed chronic extensive GVHD requiring combined immunosuppressive therapy with prednisolone, cyclosporine A, and PUVA in long periods over many years, until his death from sepsis 7 years after allo-HSCT. Four patients (2 F, 2 M) are still alive with fully functioning graft (2 pts with GD1 and 2 pts with N-GD3) after a mean and median follow-up time of 27 years (range 22–31 years). Unfortunately, both N-GD3 patients (2 F), with a follow-up time of 31 and 27 years after allo-BMT, developed epilepsy which is a debilitating sign of brain involvement in GD3 (after 16 and 22 years after allo-BMT, respectively). All four successfully transplanted patients have normal complete blood counts, no signs of visceral disease as well as skeletal and neurological status fully comparable with other Swedish patients with GD1 and N-GD3 receiving ERT. Analysis of plasma chitotriosidase activity (control range: <40 nkat/L), a biomarker of GD, disclosed completely normal levels (10–14 nkat/L) in patients who underwent allo-HSCT as compared with patients treated with ERT (96–1398 nkat/L). One woman with N-GD3 gave birth to a healthy child at the age of 20 years (18 years after allo-BMT).
Discussion: The literature on the topic of allo-HSCT in GD is limited, restricted to only case reports and small series of patients. Our long-time results indicate that Gaucher disease patients can benefit from allo-HSCT. It seems reasonable that, under some circumstances, allo-HSCT could offer a valuable treatment option for Gaucher patients failing medical therapy, as well as for patients suffering from GD3, before development of neurological damage. However, as long as transplant-related mortality of allo-HSCT will not be close to 0% and the risk of GVHD remains significant, it would be questionable to implement allo-HSCT for GD in developed countries, where safe but extremely expensive medical therapy is available for GD patients.
Disclosure of Interest: None Declared.

PH-P452

IMPROVED ENGRAFTMENT AND TRANSFUSION INDEPENDENCY AFTER CYCLOSPORINE WITHDRAWAL IN PATIENT WITH MIXED CHIMERISM AFTER MATCHED SIBLING TRANSPLANT FOR THALASSEMIA MAJOR

S. Khalid[1,], N. Yaqub[2], P. Marwah[3], R. Soni[3], F. Itrat[2], A. Khan[2], S. Khan Gilani[2], M. El Missiry[1], M. Hamed Hussain[1], C. Uderzo[1], L. Faulkner[1]*
[1]Cure 2Children Foundation, Firenze, Italy, [2]Hematology-Oncology, Children's Hospital Pakistan Institute of Medical Sciences, Islamabad, Pakistan, [3]South East Asia Institute for Thalassemia, Jaipur, India

Introduction: Rejection after matched related bone marrow transplantation (BMT) for thalassemia major (TM) is observed in 5% to 30% of BMTs, may occur up to 2 years after transplant and is heralded by falling donor chimerism and hemoglobin.
We describe three patients transplanted from a matched sibling for thalassemia major who had mixed chimerism post-BMT with hemoglobin persistently ≤7 g/dL and seemed to responded favourably to cyclosporine discontinuation. To our knowledge this is the first report of such observation in the context of BMT for TM.
Materials (or patients) and Methods: Two children were transplanted at the Children's Hospital, Pakistan institute of Medical Sciences, Islamabad, Pakistan, and one at the South–East Asia Institute for Thalassemia, Jaipur, India, within a cooperation program supported by the Cure2Children Foundation, Florence, Italy, between 2009 and 2013. Conditioning consisted of oral busulfan (14 mg/kg), thiotepa (10 mg/kg) and cyclophosphamide (200 mg/kg) followed by GVHD/rejection prophylaxis with cyclosporine A, methotrexate and prenisolone (Lucarelli's Protocol 6.1). The table below summarizes critical time points relatively to chimerism, hemoglobin levels and cyclosporine discontinuation in our three patients.
Results: All patients are alive and well, transfusion-independent, with 100% performance score and no GVHD.
Discussion: In some patients with falling chimerism and/or hemoglobin after matched related BMT for TM, engraftment and transfusion independency may be promoted by cyclosporine withdrawal.
Disclosure of Interest: None Declared.

[PH-P452]

Patient	Age at BMT	Minimum chimersim*	Minimum Hb (day post-BMT	Day of CsA stop	Day of spontaneous Hb increase to > 8	Last Chimerism*	Current Hb off RBC Tx
1[§]	1.2 y	50% (+382)	6.5 (+395)	+449	+463	50% (+528)	8 to 9 (+625)
2	5.7 y	80% (+120)	6.5 (+164)	+160	+178	99% (+366)	10 to 11 (+648)
3	3.6 y	20% (137)	7 (+148)	+175	+186	96% (+281)	11 to 12 (+1685)

CsA (Cyclosporine A); Hb (hemoglobin in g/dL); RBC Tx (red blood cell transfusion); *Donor chimerism assessed on whole peripheral blood; [§]This patient received 5 donor lymphocyte infusions, the last of which on day +372 at a dose of 10^7 T-cells/kg but did not seem to improve until CsA was discontinued.

PH-P453

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR BETA-THALASSEMIA MAJOR: A SINGLE CENTER EXPERIENCE

M. Ertem[1], T. Ileri[1,], E. Unal Ince[1], Z. Uysal[1]*
[1]Pediatric Bone Marrow Transplantation Unit, Ankara University School of Medicine, Ankara, Turkey

Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only therapy to cure patients with thalassemia major (TM) who have a human leukocyte antigen identical donor.
Materials (or patients) and Methods: All the pediatric patients who received HSCT from full-matched related donor for thalassemia major between March 1997 and September 2013 at the Pediatric Hematopoietic Stem Cell Transplantation Unit of Ankara University School of Medicine were evaluated retrospectively.
Results: Median age of the patients was 8.8 y (1.9 -18.5 y). According to Pesaro risk criteria 28 of them were low risk (Class I: 6and Class II: 22) and 46 of them were high risk (Class III) patients. All donors were HLA identical, 75 were siblings and 3 were family member. Stem cell sources were bone marrow (BM) in 55, peripheral blood (PB) in 18, cord blood (CB) in 2, CB plus PB in 2 and BM plus CB in one patient. Preparative regimen consisting of bus ulfan+cyclophosphamide (BU+CY) was used in low risk patients, BU+CY+thiotepa was used in CB transplanted patients. All class III patients except one and second transplanted patients received Pesaro protocol 26 plus ATG and one class III patient received Pesaro protocol 26. The patients whose stem cell source was CB received only cyclosporine A (CsA), all other patients received CsA+methotrexate for GVHD prophylaxis. Engraftment was achieved in all transplants except two (97%). Autologous recovery was seen in 5 patients and three of them underwent second HSCT. Aplastic bone marrow was observed in one patient and she underwent second HSCT. All patients achieved engraftment after second HSCT. In our series, overall, 73 patients (93%) were alive at a median follow-up time of 54 months (range: 3-201 months) and thalassemia-free survival rate is found as 91%. During follow-up period five patients died (pneumonia: one, acute GVHD: one and very late sepsis: three patients). The total mortality rate was found as 6.5% and mortality was found significantly increased patients with high risk criteria (0% vs 11%). On the other hand, two of three patients who died because of late sepsis were also splenectomized patients.
Discussion: In conclusion, this evaluation demonstrated that developing countries are be able to perform HSCT successfully. Unfortunately, during the long term follow-up period risk of infection increases significantly because of additive effects of immunosuppression to the unfavorable effect of splenectomy and it is not possible to achieve early intervention after hospital discharge in a developing country.
Disclosure of Interest: None Declared.

PH-P454

ALLOGENEIC UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING (RIC) IN CHILDREN WITH HURLER SYNDROME (SINGLE CENTRE EXPERIENCE)

A. Svyatoslavovna Borovkova[1,], N. Vasilievna Stancheva[1], A. Alekseevna Ratc[1], P. Valerievna Kozhokar[1], S. Victorovna Razumova[1], T. Alexandrovna Bykova[1], E. Vladimirovna Semenova[1], O. Vladimirovna Paina[1], I. Victorovna Marcova[1], M. Mihaylovna Smirennikova[1], K. Alexandrovich Ekushov[1], Y. Gennadievna Fedukova[1], L. Stepanovna Zubarovskaya[1], B. Vladimirovich Afanasyev[1]*
[1]R.M.Gorbacheva Memorial Institute of Children Oncology, Hematology and Transplantation, First Pavlov State Medical University of St.Petersburg, St.Petersburg, Russian Federation

Introduction: Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder with progressive, multiorgan involvement caused by deficiency of the lysosomal enzyme α-L-iduronidase (AIDU), leading to accumulation of glycosaminoglycans. Patients with most severe form of MPS I (Hurler syndrome) (HS) have an early-onset, rapidly progressive disease with CNS involvement, resulting in death by 8-10 years of age.
Allogeneic HSCT (alloHSCT) is still the treatment of choice for children with HS. Traditionally myeloablative regimens (MAC) were used for conditioning of MPS I patients (pts). But this group of patients has a multisystem involvement and high risk of complications. MAC is associated with a lot of treatment related toxicity that limits its usage in pts with severe disease's complications. So reduced intensity conditioning regimens (RIC) may have some advantages because they are associated with lower rates of severe toxicity.
The aim of study: to evaluate efficacy of allo-HSCT with RIC in children with HS.
Materials (or patients) and Methods: From 2009 to 2013 alloHSCT were performed in 7 pts with HS. Median age at the time of diagnosis was 14 months (3-24 months), at transplantation - 29 months (19 months-40 months). All patients received enzyme replacement therapy before HSCT for 10,7 months on average (5–19 months). Matched unrelated (*n*=5) or partially matched (9/10) (*n*=2) unrelated HSCT were performed. Peripheral blood stem cells (PBSC) (*n*=6) and bone marrow (*n*=1) were used as source of hematopoietic stem cells. The conditioning regimen contained Fludarabine 150mg/m^2+Melphalan 140 mg/m^2+ATG 60 mg/kg. CsA+short course of methotrexate or MMF were used for GvHD prophylaxis. In 3 cases we used CD34-selection or CD3/CD19-depletion of PBSC (CliniMACS) for prevention of GVHD.
Results: 4-years overall survival is 72%. 5 pts are alive with median follow-up of 17,8 months (1.5-44 months). During RIC alloHSCT none patients developed severe toxicity (III-IV stage according to WHO classification).
The median time to neutrophil recovery >0.5x10^9/L was 19 days (range12-23 days). All patients were engrafted with achievement of full donor chimerism and normal activity of AIDU on D+30. Median range of AIDU activity in leucocytes was 61.3 nM/mg/18h on D+30 and 77.6 nM/mg/18h on D+100 (normal AIDU activity range-61.0-175.5 nM/mg/18h).
Complications: grade II aGvHD of skin and gut – in 2 pts, 1 pt had grade III gut GvHD, which was successfully treated by steroids and monoclonal antibody therapy. 1pt died on D+65 from grade IV aGvHD of gut. 1 pt died from TRALI-syndrome on day+45.
Discussion: AlloHSCT is an effective treatment option for HS, but efficacy of usage of different conditioning regimens is controversial. In general, the outcome of children undergoing HSCT is varied and depends on degree of clinical involvement and the child's age at the time of transplantation (Lorne A Clarke, Jonathan Heppner *et al*., 2011), HS pts usually have multiorgan involvement, that lead to an increased risk of complications post-HSCT, especially with MAC. RIC significantly decreases development of different complications. But some previous studies (JJ Boelens *et al*., 2009, 2012) showed, that use of RIC was the risk factor for graft failure in HS patients.
In our study we demonstrate that RIC is effective in children with HS, has low toxicity and is the promising alternative to MAC.
Disclosure of Interest: None Declared.

PH-P455

CONDITIONING WITH TREOSULFAN, FLUDARABINE, THIOTEPA AND ATG FOR MATCHED FAMILY DONOR TRANSPLANTATION IN CHILDREN WITH SICKLE CELL ANEMIA

B. Hartz[1,], J. Schrum[1], R. Grosse[1], U. Kordes[1], M. Bleeke[1], I. Müller[1]*
[1]Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: Allogeneic bone marrow transplantation (BMT) currently is the only curative therapy for patients with sickle cell anemia (SCD)[1]. These patients are at risk for developing vaso-occlusive disease (VOD)[2]. Treosulfan has been used in several conditioning regimens showing a low risk for VOD and may thus represent an interesting alternative to classic regimens[3]. Patients with thalassaemia major undergoing allogeneic BMT after treosulfan-based conditioning regimens have a high rate of successful engraftment, a low rate of complications and no case of VOD[4].

However, there is no published evidence on efficacy and toxicity of treosulfan as part of conditioning regimens in patients with sickle cell anemia.
We present seven children with sickle cell disease receiving a treosulfan-based conditioning regimen before 9/10 or 10/10 HLA-matched sibling donor BMT.
Materials (or patients) and Methods: Children with homozygous sickle cell anemia, at least one severe sickle cell anemia related event and a well HLA-matched sibling donor were eligible for allogeneic BMT. The conditioning regimen consisted of treosulfan (3 x 14 g/m^2), thiotepa (2 x 5 mg/kg), fludarabine (5 x 30 mg/m^2) and anti-thymocyte globulin (ATG Genzyme®, 10 mg/kg). GvHD prophylaxis was maintained with ciclosporine A, methotrexate, metronidazole and IVIGs. All patients received G-CSF from day +5 until engraftment.
Results: Seven patients were enrolled in our centre. The chemotherapy was well tolerated and all patients showed successful engraftment. The median leukocyte engraftment was observed on day +13 (range 11 - 17). Some patients experienced mild complications as mucositis or fever of unknown origin. Three patients developed transient skin GvHD grade I/II. There was no evidence of VOD in any of our patients. After a median follow-up of 16 months all children were alive, disease-free and free of GvHD. On day +100 four children had a complete donor chimerism and three children had a mixed donor-chimerism. Two of these children had a donor-chimerism of >80% and >90%, respectively. Only one child who also suffered from a glucose-6-phosphate dehydrogenase deficiency, had a declining donor-chimerism and required analgetics for diffuse pain during an febrile infection of the upper respiratory tract early after transplantation while in rehabilitation. It is unlear if this was due to the remaining sickle cells at this point, as he has not encountered complications during following infections. None of the patients required erythrocyte transfusions or had direct evidence for any SCD-related event.
Discussion: To our knowledge we present the first data of treosulfan, fludarabine, thiotepa and ATG-G as conditioning regimen for children with sickle cell disease. Chemotherapy as well as the post-transplantation course proceeded without severe adverse events. All children showed a successful engraftment. There was no case of VOD. From these pilot data, we conclude that treosulfan-based conditioning prior to MFD BMT seems to be safe and effective in patients with sickle cell anemia. Larger cohorts are necessary to substantiate these observations.
References
1. Locatelli, F. & Pagliara, D. *Pediatr Blood Cancer* 59, 372–376 (2012).
2. McPherson, M. E. *et al*. *Bone Marrow Transplant*. 46, 27–33 (2011).
3. Danylesko, I. *et al. Bone Marrow Transplant*. 47, 5–14 (2012).
4. Bernardo, M. E. *et al. Blood* 120, 473–476 (2012).
Disclosure of Interest: None Declared.

PH-P456
DEPLETION OF TCRA/B +/CD19+ CELLS AS A NOVEL APPROACH IN SCT FOR OSTEOPETROSIS
C. S[1], G. S[1], B. F[2], B. R[2], B. A[2], L. A[2], P. F[2,]*
[1]UO Oncoematologia Pediatrica e Trapianto Midollo Osseo, [2]Servizio di Medicina di Laboratorio,Sezione di Ematologia e Emocoagulazione,Laboratorio Cellule Staminali, Ospedale dei Bambini, Brescia, Italy

Introduction: Osteopetrosis is an heterogeneous group of bone remodelling disorders characterized by an increase in bone density due to a defect in osteoclastic bone resorption. We previously presented a retrospective study of 20 children, 11 males and 9 females; the disease is diagnosed at a mean age of 4 months (range 10 d to 9 m). We found 9 children with mutations of the ATP6i (TCIRG1) gene, 2 patient of the CLCN7 gene, 3 of OSTM1 gene and one of the RANKL gene. The remaining 6 children have unknown genetic defects. 14 children underwent BMT, 8 of whom are alive: 5 a genotype HLA identical sibling donor, 4 an haplotype mismatched related donor and 9 a phenotype HLA matched unrelated donor (MUD). 4 children underwent 2 BMT. 6 patient didn't receive a BMT(3 severe neurological impairment; 1patient parents refused MUD-BMT, 2 children died before BMT.
8 children developed forms of acute GVHD minimal or moderate, 2 children developed chronic GVHD, 1 extensive, 1 limited.
Disease free survival was 80% for HLA matched, 33% for haploidentical HLA matched donor and 60% for (MUD). To improve engraftment rate and decrease intensity and severity of GVHD we applied depletion of TCRα/β +/CD19+ cells as a novel approach in SCT in osteopetrosis in 3 cases (1 RANKL, 2 CLCN7).
Materials (or patients) and Methods: We applied the CliniMacs system depletion of TCRα/β+/ CD19+ cells on PBSC of MUD donors with the aim to avoid GVHD at all and to enhance engraftment that in our previous experience didn't exceeded 60%, with 4 patients who required a second SCT. We planned to infuse a high number of both CD34+ and TCR γ/δ+ cells together with a controlled number of TCRα/β+ cells (not exceeding 25x10^6 cells/Kg).
Results: The data on the infused cells are reported in Table 1.
The mean number of infused CD34+ cells was 18x10^6/Kg, the number of TCR g/d+cells 66x10^6/Kg and the number of TCR a/b 21.85 x10^6 /Kg obtained from the "non target" fraction. Overall the procedure allowed us, in the first 2 cases, to achieve engraftment 100% donor by day 12 with no evidence of acute GVHD with a mean follow up of 5 months. One case too early to be evaluated.
Discussion: This seems a promising procedure to enhance engraftment due to an high CD34+ inolculum combined with a good number of γ/δ TCR +cells in a genetic disease difficult to be grafted such as osteopetrosis. We have added a controlled number of α/b+ TCR +cells that in our previous experience didn't provoke GVHD in the MUD setting.
Disclosure of Interest: None Declared.

PH-P457
30 YEARS OF EXPERIENCE OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR INBORN ERRORS OF METABOLISM
M. Solders[1,2,], M. Remberger[1,2], B.-M. Svahn[1,2], B. Gustafsson[3], G. Solders[4,5], K. Naess[6,7], M. Machaczka[8,9], J. Mattsson[1,2], O. Ringdén[1,2]*
[1]Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital, [2]Division of Therapeutic Immunology, Karolinska Institutet, [3]Department of Clinical Science, Intervention and Technology, [4]Department of Clinical Neurophysiology, [5]Department of Neurology, Karolinska Institutet, Karolinska University Hospital, [6]Centre for Inherited Metabolic Diseases, Karolinska University Hospital, [7]Department of Laboratory Medicine, Karolinska Institutet, [8]Hematology Center Karolinska, Karolinska University Hospital, [9]Department of Medicine at Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Introduction: For the last 30 years, allogeneic hematopoietic stem cell transplantation (HSCT) has been used to cure many inherited non-malignant disorders. The transplanted stem cells differenti-

Table 1

PZ	AGE	HPC	WBCx10^6/kg	CD34+x10^6/Kg	CD3+ TCRα/ βx10^6/kg	CD3+ TCRγ/ δ X10^6/Kg	Engraftment ₊30 days
F.L.	1YEAR	HPCA	1302,500	14,700	25,800	127,460	PBL=100% PMN=100%
R.M.	4YEARS	HPCA	2155,840	19,380	19,760	62,700	PBL=100% PMN=100%
L.M.	10YEARS	HPCA	605,340	19,950	20,000	7,150	

ates, migrates and replace the deficient enzyme, abnormally shaped, non-functional or missing cells.
Materials (or patients) and Methods: We report 30 years of experience in 137 patients. The disorders include 40 immunodeficiencies, 24 benign hematological disorders, 31 histiocytic disorders,15 mucopolysaccharoidoses, 14 leukodystrophies, 6 Gaucher's disease, 1 Sandhoff's disease, 1 erythropoietic protoporphyria, 1 Shwachman's syndrome, 1 amyotrophic lateral sclerosis, 1 osteopetrosis and 2 aspartylglucosaminuria. Their median age was 3 (0–63) years. The donors were 50 HLA-matched related (MRD), 54 matched unrelated (MUD) and 33 HLA-A, -B or -DR mismatches (MM). The stem cell sources were 108 bone marrow (BM), 14 peripheral blood stem cells (PBSC) and 15 cord blood (CB). 85 patients received a myeloablative conditioning regimen (MAC) and 52 patients received a reduced intensity conditioning regimen (RIC).
Results: Among recipients of HLA-identical sibling grafts, 10% developed acute GVHD grades II-IV as opposed to 22% in the group receiving MUD-grafts and 29% in the MM group. The overall cumulative incidence of acute GVHD grades III-IV was 4%. The overall cumulative incidence of chronic GVHD was 15%. The rejection rates were low in the MRD group (6%), in the MAC group (9%) and the group receiving PBSC (7%) and high in the MM group (24%), the RIC group (29%) and the group of patients receiving a cord blood graft (27%).
The 5-year survival rates were 96%, 84%, and 55% in recipients of grafts from HLA-identical siblings, MUD and MM, respectively. The overall 10-year survival rate was 76%. The patients transplanted in between 1984–2004 had an overall 5-year survival rate of 77% (*n*=66) compared to 86% for the patients transplanted in between 2005–2013 (*n*=71) (*P*=0.18).
The surviving patients with immunodeficiencies and hematological disorders are all well. The patients with mucopolysaccharoidoses are all well except for their skeletal problems. All patients with Gaucher's disease are alive. One rejected the graft and is well with enzyme replacement therapy, the others are well with donor enzyme levels. Among the leukodystrophies, the treatment failed to halt the disease in the infantile cases, but arrested the progression in the juvenile and adult cases.
Discussion: In many patients with inborn errors of metabolism, HSCT remains the only curable treatment. HSCT however often remains controversial. The efficiency and safety is difficult to prove in studies because of the small number of patients representing each disease. In this study, we looked at the whole group of patients transplanted for non-malignant disorders, mainly to evaluate the safety of the treatment. The overall 5-year survival for the group has improved in more recent years, conformably with the overall development of HSCT. In a vast majority of cases, the HSCT managed to either permanently halt or even to reverse the disease. In conclusion, HSCT provides a high overall survival in patients with inborn errors of metabolism, with an increased survival rate in the last years. The best survival rates are seen using PBSC from HLA-matched donors and the most beneficial effects on the disease are seen in those who are transplanted early after time of diagnosis.
Disclosure of Interest: None Declared.

PH-P458
T-CELL DEPLETED HAEMATOPOIETIC STEM CELLS TRANSPLANTATION WITH ADD BACK OF CD45RA NEGATIVE DONOR LYMPHOCYTES INFUSION: ABOUT 5 CASES
B. Neven[1,], F. Touzot[1], L. Dal Coritvo[1], G. Cros[1], M. Chomton[1], R. Creidy[1], A. Brignier[1], D. Moshous[1], S. Blanche[1], A. Fischer[1], M. Cavazzana[1]*
[1]Necker Hospital, Paris, France

Introduction: Combined immunodeficiencies (CIDs) are genetically heterogeneous inherited diseases that can only be cured by allogeneic hematopoietic stem cell transplantation (HSCT). However morbidity and mortality of this procedure are high in these patients in particular with donor other than geno-identical sibling. These poor results are related to high rate of severe graft-versus-host disease (GVHD) and serious viral infectious complications often present before the HSCT procedure. Mice studies suggest that GVHD is mostly mediated by naïve T cells while memory T cells have anti-infectious properties with absent or strongly reduced capacity to induce GVHD (Anderson BE *et al*., 2003). Thus, removing CD45RA cells from donor lymphocytes could clear opportunistic and viral infections without induction of GVHD. This procedure was evaluated in 5 patients.
Materials (or patients) and Methods: 5 CID patients of age between 6 and 24 months (1 ORAI1 deficiency, 2 MHC class II deficiency, 2 CD25 deficiency) with >1 viral infections (*n*=5) and autoimmunity (*n*=3) were transplanted with an immunoselected CD34+ graft followed by infusion of CD45RA negative T cell fraction. They were all transplanted with a 9/10 HLA donor (intrafamilial *n*=1, unrelated *n*=4). Conditioning regimen was myeloablative, based on busulfan, fludarabine (and thiothepa in 4 cases). Serotherapy was administrated in 4 patients (upfront in 3 cases) CD34+ cell selection and CD45RA cell depletion procedures were performed using the Clini Macs system (Miltenyi Biotec). Grafts contain 2.9 to 12.3 $x10^6$ CD34+ cells/kg (median 8.8 $x10^6$ CD34+ cells/kg) with less than 5000 T lymphocytes/kg and 0.9 and 9.2$x10^6$/kg CD45RO+ T cells (median 2.52$x10^6$/kg). GVH prophylaxis was based on ciclospirine (D-1 to 6 months post HSCT) and mycophenolate mofetyl (D0 to D+45).
Results: Four patients engrafted, one presented a mixt chimerim requiring unmanipulated donor lymphocytes infusion (DLI) on D+35. One patient rejected and died of infection in the absence of autologous reconstitution. Grade II GVHD was noticed only in P1 on D48 post transplant (D12 post DLI) suggesting a role of DLI in onset of GVHD. No chronic GVH occurred. Clinical outcome was remarkably good in all 4 patients who engrafted and rapidly cleared viral infection and autoimmunity within 45 days post HSCT. Kinetics of immune reconstitution was fast with complete immune reconstitution 6 to 9 months post HSCT in all 4 patients who engrafted. Early specific anti-viral response could be documented in 2 patients.
Discussion: This procedure may be promising for high risk HSCT in patients with CID allowing fast anti-viral immune reconstitution and minimizing risk of GVHD. It should be evaluated in a clinical trial.
Disclosure of Interest: None Declared.

PH-P459
HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR HEMOGLOBINOPATHY(SICKLE CELL DISEASE) IN NIGERIA– 2013 UPDATE BY NIGERIAN GROUP FOR BLOOD AND MARROW TRANSPLANT (NGBMT)
N. Godwin Bazuaye[1,], B. Enomamien[1]*
[1]Hematology, University of Benin Teaching Hospital, Benin, Nigeria

Introduction: Hematopoietic Stem Cell Transplantation (HSCT) remains the only curative therapy for some malignant and Non – malignant diseases. The world celebrated 1 millionth HSCT in late December 2012 in honor of the death of Donnall Thomas (Nobel prize winner) who died in October 2012.However East Mediterranean/Africa represents only 2% the of global HSCT.
Materials (or patients) and Methods: The first successful HSCT was performed in Nigeria in September 2011 for a seven years old sickle cell disease (SCD) patient with cerebrovascular accident (CVA). The second HSCT was a 12-years old SCD patient with recurrent vaso-oclusive crises (VOC) in August 2012 and a re-transplant in May 2013. The third HSCT was for a 15years old SCD patient with recurrent VOC in July 2013. All had Allogeneic HSCT from identical siblings and conditioning regimen was with reduced intensity conditioning (RIC) (FLU/BU) Fludarabin 160mg/m^2 days -6 to -2, oral Busulphan 16mg/kg days -5 to -2. GVHD prophylaxis and immunosuppression was with ATG(ATGAM) 73mg/kg total dose days -6 to -4, cyclosporine A and MMF. There was primary rejection for the second HSCT and had a second HSCT with CY/BU (cyclophosphamide 100mg/kg before and 100mg/kg day +3 and +4).
Results: There was no recorded acute or chronic GVHD in the patients. First patient had complications of infection with pseudomonas organism at the catheter site sensitive to ofloxacine.

Chimerism for first HSCT patient was 95% at 2 years post HSCT, 57% at day +27 post HSCT for the third patient and 0% for the second patient after the second re-transplant. The second transplant patient at 6 months post HSCT had a total of 15 units of irradiated red cell concentrates, developed iron over load (serum ferritin of 1800ng/ml) and currently on oral iron chelator. He also had a total of 32 irradiated platelet concentrate and current parameters show a platelet count of 50,000/ul, hemoglobin of 7g/dl, WBC of 4500/ul and ANC of 1800/µl.
Discussion: Postulated reasons for rejection include a lower dose of transplanted total bone marrow stem cells (nucleated cells of $5.7X10^8$/kg) compared to $9.8X10^8$/kg for the first and $8.2X10^8$/kg for the third. Also there was minor ABO incompatibility between patient/donor (A+/O+) and the inability of the centre to monitor serum cyclosporine levels which was done for the third patient. Reported overall survival and disease free report from our centre is comparable with other centres. New developments for improved HSCT in Nigeria include a Bone marrow registry established at the University Nigeria Teaching Hospital Enugu to improve the prospect of unrelated stem cell transplantation. There is also HLA laboratory at the Obafemi Awolowo University Teaching Hospital Ile-Ife Nigeria. The Nigerian Group for Blood and Marrow transplant (NGBMT) and African Blood and Marrow Transplant (AFBMT) society was inaugurated in 2013 to coordinate and regulate HSCT in Nigeria and Africa respectively.
Disclosure of Interest: None Declared.

PH-P460

INDICATION FOR STEM CELL TRANSPLANTATION IN SICKLE CELL DISEASE: IS OLDER AGE A CONTRAINDICATION? SINGLE CENTER EXPERIENCE IN BRAZIL

B. Pinto Simões[1,], R. Cunha[1], D. Moraes[1], J. EB Dias[1], L. Guilherme Darrigo Jr.[1], C. ES Grecco[1], A. B. PL Stracieri[1], G. MN Barros[1], F. Pieroni[1], M. Carolina Oliveira[1]*
[1]Internal Medicine, Medical School Ribeirão Preto University of São Paulo, Ribeirão Preto, Brazil

Introduction: Sickle cell disease is the most prevalent monogenic hereditary disease in Brazil. In 2001 a national newborn screening program was started in several states of the country. In 2005 the ministry of health launched the first official program for the treatment of SCD in Brazil. This program regulated the use of antibiotic prophylaxis, vaccination and family education. In 2009 the use of hydroxyurea was also introduced in the public health system for defined population of patients. We estimate that yearly about 3500 children with sickle cell diseases (SCD) are born in Brazil and more than 30 000 patients with the diseases are alive nowadays. Most of them came from the northern states of the country and from low incoming families. Stem cell transplant is the only curative approach but has been performed mostly in children. Since the newborn screening program finally covered the whole country only in 2012, many patients with SCD in Brazil are adult candidates for stem cell transplantation (SCT). We started a SCT program at our center and accepted patients without upper age limit.
Materials (or patients) and Methods: We have transplanted 16 patients (9 females and 7 males) transplanted between the ages of 8 and 38 (median age 18 years) and compared the outcome of patients older and younger than 18 years. Indications for transplant were: recurrent priapism (2 patients), neurological alterations (4 patients, one with MoyaMoya), repeated vaso-occlusive episodes unresponsive to hydroxyurea (9 patients) and recurrent and severe acute chest syndrome with pulmonary hypertension (one patient). Each patient received bone marrow from a healthy HLA identical sibling; in 4 cases the donor had a sickle cell trait. Fifteen patients (the 38 years old patient with liver hemosiderosis grade 3) received myeloablative conditioning regimen consisting of Fludarabine 120 mg/kg, Busulfan per os 12 mg/kg and ATG 4.5 mg/kg, one patient with liver hemosiderosis grade 3 was conditioned with Fludarabine and Cyclophosphamide. GVHD prophylaxis consisted of CsA and a short course of MTX.
Results: All patients engrafted (median day for neutrophils 18 and platelets 22). The median follow up is 2 years and 15 of 16 patients are alive. One patient died on day 416 of hemorrhagic stroke as a result of Moya Moya. Severe acute GVHD was not observed, the incidence of grade I and II was 25%. None of the patients has yet developed chronic GVHD. Actuarial overall survival is 94%. We have not seen any difference in terms of complications and mortality in patients younger or older than 18 years using the same protocol. The death occurred in a 16 years old girl with severe neurological complications already before transplant.
Discussion: In Brazil the incidence of SCD is high and transplantation is the only curative approach; it should be offered for every patient with a complicated course independently of his age. Careful evaluation and preparation for transplant are essential for the best outcome; iron chelation, reduction of hemoglobin S, treatment of ulcers and prolonged use of anticonvulsant are indispensible. Patients should be offered before irreversible damage has occurred.
Disclosure of Interest: None Declared.

PH-P461

PRE-CONDITIONING IMMUNOMODULATION AND REDUCED TOXICITY CONDITIONING (RTC) IN ALLO-SIBLING HSCT OF ADOLESCENTS AND YOUNG ADULTS WITH SICKLE CELL ANEMIA (SCA) AND THALASSEMIA MAJOR (TM)

S. Y. Mohamed[1,], F. Al-Mohareb[1], H. Alzahrani[1], S. A. Osman[1], G. AL Dawsari[1], M. Aljurf[1], K. Ibrahim[1], M. H. Almahayni[1], B. Al-Haj Ali[1], S. AbuJaafar[1], R. Youniss[1], T. ElHassan[1]*
[1]Hematology-Transplantation, KFSHRC, Riyadh, Saudi Arabia

Introduction: SCA and TM are associated with short survival. Allogeneic sibling HSCT offers high overall and disease- free survivals (OS & DFS) when done in childhood and young adolescents. Studies in adults report significantly lower eligibility rate due to significant co-morbidities accumulated with age leading to higher transplant related mortality (TRM) and rejection. Reducing the intensity of conditioning (RIC) increased rejection while myeloablative conditioning (MAC) may be associated with increased toxicity and mortality.
We aimed to study the feasibility and outcome of delivering steroids and cytoreduction to reduce rejection in a pre-conditioning phase followed by reduced toxicity conditioning (RTC) with lower doses of ATG in adolescents and young adults with SCA and TM.
Materials (or patients) and Methods: 9 patients with SCA (*n*=8) and thalassemia major (*n*=1) (5 F/4M) with a median age 18 y (14–30y) transplanted between 1/11 and 10/213 from identical siblings (6 bone marrow, 2 peripheral blood and 1 both BM+PB) with a median dose of CD34+ cells of 5.79 $x10^6$/kg (range 2.88–8.58). Preconditioning anti-rejection phase included intense cyto-reduction with hydroxyurea (to achieve an acceptable level of leukopenia (TLC >3,000/µl, ANC >1500–<3000/µl, Platelet >150–<250 $x10^3$/µl), prednisone 0.5 mg/kg every other day for at least 3 weeks, hyper-transfusion and intense chelation. In case of avascular necrosis prednisone is replaced by Cellcept. Reduced Toxicity Conditioning: Fludarabine 40mg/m^2/d on d-9 to d-6, Busulfan 0.8mg/kg/dose Q 6h x 14 doses (total 11.2mg/kg), ATG (Thymoglobulin; Genzyme) 1.5mg/kg/d d-4 to d-2 (total dose 4.5mg/kg). (1 patient received 7mg/kg and 2 received 5.5 mg/kg),GVHD prophylaxis included: Cyclosporine (CSA) starting d-1 and ending 9 months after SCT and short course of Methotrexate.
Results: 9 patients were followed for a median of 11 months (2-32 mo).
All patients tolerated Immunmodulation and cytoreduction before conditioning with only one episode of short (3 days) neurtopenic fever. TRM was 0% and all patients engrafted and living (OS 100%) and disease free (DFS 100%) and Transfusion-free (TFS 100%). Median time to ANC engraftment for the eligible patients (n:8) was 21 days (16–24) and for platelets 17 d (9-36 d). Only 2 of 7 evaluable patients developed grade II-III a-GVHD that completely resolved on steroid therapy and one of the 5 evaluable patients (20%) developed cGVHD that resolved on systemic steroids. 7 patients were evaluable for chimerism studies and all

(100%) were chimeric with 100 % myeloid in 7/7 (100%) and lymphoid chimerism was 100% in 1 (14%), high (≥50-95% in 4 (57%) and low (<50%) but stable in 2 (29%).
Discussion: In hemoglobinopathies (SCA & TM), pre-conditioning immunomodulation with steroids and cytoreduction hydroxyurea and hypertransfusion followed by reduced toxicity conditioning regimens with relatively small doses of ATG is associated with no transplant-related mortality, stable chimerism and very high disease free survival in adolescents and young adults transplanted from identical sibling donors.
Further expansion of the study to include older adults and larger number and to use other immunomodulating agents should be tried before conditioning to allow better engraftment and with minimal toxicity with an acceptable rate of GVHD.
Disclosure of Interest: None Declared.

Infectious Complications

PH-P462
DOSE CYTOMEGALOVIRUS INFECTION PREDICT A LOWER RISK OF DISEASE RELAPSE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION? A META-ANALYSIS OF OBSERVATIONAL STUDIES
X.-H. Luo[1,], Y.-L. Zhang[1], L. Liu[1]*
[1]Department of Hematology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

Introduction: Cytomegalovirus (CMV) infection has long been a challenging obstacle in the hematopoietic stem cell transplantation (HSCT) procedure. However, uncertainties remain concerning the influence of CMV infection on transplant outcome. This meta-analysis was conducted to assess the association between CMV serostatus or reactivation and primary malignancy relapse after transplantation.
Materials (or patients) and Methods: PubMed, EMBASE, the Cochrane Library, SCI, and Chinese Biomedicine Databases were searched for all studies that investigated CMV infection and relapse in transplant patients with hematologic malignancies. The quality of the included studies was evaluated using the Newcastle-Ottawa Scale (NOS). A literature search identified 14 cohort studies evaluating such an association.
Results: Meta-analysis showed that patients with a negative pre-transplantation CMV donor and recipient (D-/R-) serostatus had an increased risk of developing relapse [pooled hazard ratio (HR) = 1.26, 95% confidence interval (CI), 1.13-1.41; P<0.001] compared with other donor/recipient combination pairs, and the risk was increased especially in acute leukemia (AL) subgroup (pooled HR = 1.35, 95% CI, 1.16-1.57; P < 0.001). D-/R- serostatus was significantly associated with reductions in CMV reactivation after transplantation when compared with other donor/recipient combination pairs [pooled relative risk (RR) = 0.12, 95% CI, 0.03-0.47; P < 0.01]. Unexpectedly, there was no association between CMV reactivation and relapse in combined studies (pooled HR = 0.83, 95% CI, 0.53-1.30; P=0.42); however, the association was observed in chronic myeloid leukemia (CML) subgroup (pooled HR = 0.59, 95% CI, 0.40-0.89; P= 0.01). After excluding studies using anti-CMV prophylaxis and T-cell deplete grafts, CMV reactivation was significantly associated with a decreased risk of relapse in AL patients (pooled HR = 0.41, 95% CI, 0.19-0.90; P= 0.03).
Discussion: Our results suggest that D-/R- serostatus increases the risk of developing post-HSCT disease relapse. In AL and CML patients, CMV reactivation is a protection factor for relapse.
Disclosure of Interest: None Declared.

PH-P463
CLINICAL DESCRIPTION OF CRYPTOCOCCAL INVASIVE FUNGAL INFECTION (IFI) IN HEMATOPOIETIC CELL TRANSPLANTATION (HCT); A SINGLE CENTER EXPERIENCE
S. Singh Dadwal[1,], B. R Tegtmeier[1], J. Kriengkauykiat[1], J. I. Ito[1]*
[1]Infectious Disease, City of Hope National Medical Center, Duarte, United States

Introduction: Cryptococcal infection is a rare occurrence in the HCT patients and mostly described as case reports. The outcomes of Cryptococcal infection in HCT are unknown.
Materials (or patients) and Methods: Chart review of patients who underwent HCT for hematologic malignancy (>18 years of age) diagnosed with proven or probable IFI caused by *Cryptococcus spp.*, from January 1987 to December 2011, as defined by the European Organization for Research and Treatment of Cancer/ Mycosis Study group.
Results: 8138 HCT were carried out between 1987 and 2011 for hematologic malignancies. 12 cases of Cryptococcal IFI were identified (9 proven and 3 probable) with an incidence of 0.15%. Median age was 47 years and 8 were males. 6/12 had allogeneic HCT (4 unrelated, 2 related donor) and 6 autologous HCT. Underlying hematologic disorder: 6 had acute leukemia, 4 had lymphoma and 2 had multiple myeloma. 6/12 patients had relapsed disease at the time of IFI diagnosis. 4/6 allogeneic HCT's had graft versus host disease (GvHD). 8/12 patients were on steroids. 5/6 allogeneic HCT patients were receiving antifungal prophylaxis at diagnosis (3 were on echinocandin, 1 posaconazole, 1 voriconazole) and none of the autologous HCT patients. Of the12 isolates of *Cryptococcus spp*., 6 were *C. neoformans*, 3 *C. albidus* and 2 *C. humicolus*. Only 5/11 had fever at presentation. 6/11 had positive blood cultures (2 with meningitis, 1 had skin/ tongue lesion and 1 patient had cardiac/renal involvement noted on autopsy), and 8/12 had pulmonary infiltrates. 7/12 had co-infection (1 CMV, 4 bacteremia (*coagulase negative Staphylococci* and *Staphylococcus aureus*), 1 with *Mycobacterium xenopi/Mycobacterium avium intracellulare* and 1 patient with mucormycosis of sinus/fusariosis). Treatment: 7 received amphotericin B product (2 also got flucytosine), 4 fluconazole, 1 voriconazole. 5 (42% - 3 allogenic and 2 autologous HCT patients) died within 90 days of onset of IFI (3 attributable to Cryptococcus). 4 of 5 deaths occurred in patients who had relapsed disease (80%).
Discussion: *Cryptococcal spp.* IFI in the HCT population is rare and associated with high mortality despite antifungal therapy, especially in patients with relapse of underlying hematologic disease.
Disclosure of Interest: None Declared.

PH-P464
EFFICACY AND TOXICITY IN CLINICAL PRACTICE OF ANTIFUNGAL COMBINATION THERAPY FOR PROVEN OR PROBABLE INVASIVE MOLD DISEASES IN HEMATOPOIETIC STEM CELL TRANSPLANTATION
A. Candoni[1,], S. Cesaro[2], A. Busca[3], D. Lazzarotto[1], M. Caira[4], D. Russo[5], R. Fanin[6], L. Pagano[7]*
[1]Division of Hematology and HSCT, University of Udine and AOUD, Udine, [2]Pediatric Hematology Oncology, Verona, [3]Department of Hematology, San Giovanni Battista Hospital, Torino, [4]Institute of Hematology, Università Cattolica del Sacro Cuore, Rome, [5]Unit of Blood Diseases and Stem Cell Transplantation, University of Brescia, Brescia, [6]University of Udine and AOUD, Udine, [7]Università Cattolica del Sacro Cuore, Rome, Italy

Introduction: The Combination (Combo) antifungal therapy is a very hot topic in haematology. This multicentre observational study reported the efficacy and toxicity, in clinical practice, of antifungal Combo therapy as treatment of proven or probable Invasive Mold Diseases (IMDs) in Hematopoietic Stem Cell Transplantation (HSCT) recipients.
Materials (or patients) and Methods: Between June 2006 and June 2012, 30 cases of IMDs (16 proven and 14 probable) treated with Combo were reported from 7 HSCT Centers in Italy. Median age of

HSCT patients was 29 yrs (range 2-61) and 26% had less than 18 yrs. Acute Leukemia was their most common underlying hematologic disease (17/30; 57%); 10/30 (33%) of cases were in complete remission and 20/30 (67%) had refractory or relapsed hematologic disease. The main site of infection was lung with or without other sites. The causative moulds were: *Aspergillus sp* in 22 cases (74%), *Zygomycetes* in 4 cases (13%), *Fusarium sp* in 3 cases (10%) and *Paecilomyces sp* in 1/30 (3%).
Results: The most used Combo therapy were caspofungin+voriconazole (13/30 pts-43%), caspofungin+liposomal amphotericin B (L-AmB) (7/30 pts-22%), and L-AmB+voriconazole (8/30 pts-26%). The median duration of Combo therapy was 30 days (range 7-154). The overall response rate (ORR) was 67% (20/30 responders) without significant differences between the Combo regimens. The granulocyte (PMN) recovery did not significantly influenced the response to the Combo therapy in HSCT recipients. Only 29% of patients experienced mild and reversible adverse events (hypokalemia, ALT/AST increase, creatinine increase). The mortality IFD related was low (24%).
Discussion: This observational study indicates that Combo therapy was well tolerated and effective in HSCT with proven or probable IMDs. The most used Combo regimens in clinical practice were caspofungin+voriconazole (with a response rate of 80%) and caspofungin+L-AmB (response rate 70%). The ORR was 67%.
Disclosure of Interest: None Declared.

PH-P465
COMPARISON OF LEVOFLOXACIN PROPHYLAXIS VERSUS NO PROPHYLAXIS IN PATIENTS RECEIVING AUTOLOGOUS OR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION. RETROSPECTIVE COHORT STUDY FROM A SINGLE CENTER

M. Batlle[1,], J. Fernandez[1], M. Morgades[1], S. Vives[1], J.-M. Sancho[1], B. Xicoy[1], M. Moreno[1], C. Ferrrà[1], J. Vila[1], J.-M. Ribera[1]*
[1]Clinical Hematology Department, Institut Català d'Oncologia-Hospital Germans Trias i Pujol. Josep Carreras Research Institute. Badalona. Universitat Autònoma de Barcelona, Badalona, Spain

Introduction: Bacterial infections are an important cause of morbidity and mortality in patients receiving hematopoietic stem cell transplantation (HSCT). This justifies the wide use of antibacterial prophylaxis with fluoroquinolones in the period of neutropenia. This retrospective cohort study was performed to compare prophylaxis with levofloxacin (LF) 500 mg oral per day *versus* no bacterial prophylaxis (no-LF). LF was administered from hospitalization for HSCT to the recovery of neutropenia (>1x10^9/L) or the emergence of fever (intravenous antibiotic treatment). The variables analysed were the rate of microbiologically documented infection (MDI), the frequency of LF-resistant bacteria and the outcome of infection in patients receiving LF or not.
Materials (or patients) and Methods: From October 2006 to October 2010, 239 consecutive patients were enrolled. Two consecutive groups were included: no-prophylaxis (no-LF) (October 2006- October 2008, 107 procedures) and prophylaxis with LF (October 2008-October 2010, 132 procedures). Data collected: age, gender, ECOG, hematologic disease, type of HSCT (autologous or allogeneic), MDI rate (with or without bacteremia), infection site, antibiotics used, frequency of LF-resistant bacteria and outcome of infection.
Results: Table 1 summarizes the main data in the LF and no-LF groups. No differences were observed with regards to the baseline characteristics in the two cohorts, as well as according to the type of HSCT. The rate of MDI with or without bacteremia was similar in the two groups; however, gram-negative bacteria were isolated more frequently in the no-LF cohort. Significant differences were observed with respect to the empirical use of antibiotics (imipenem+amikacin and teicoplanin was more used in the no-LF group). The incidence of resistant-bacteria was significantly higher in the LF group. No significant differences were observed on comparing of the outcome of infection in the two groups.
Discussion: In this study no significant differences were observed in the incidence of microbiologically documented infections and in the outcome of infections in HSCT patients according to the use of antibiotic prophylaxis with levofloxacin or not. Prophylaxis with levofloxacin reduced the frequency of infections by gram-negative bacteria in patients receiving HSCT.
Supported by grant RD 12/0036/0029 from RTICC, Instituto Carlos III, Spain.
Disclosure of Interest: None Declared.

[PH-P465]

	Levofloxacin n: 132	No-Levofloxacin n: 107	p
Age (years) Mean (SD)	50.48 (12,72)	48.82 (13,31)	0.278
Age (years) Median	54 [16-69]	52 [14-70]	0.078
Gender			
Male	82	60	0.344
Female	50	47	
ECOG			
0	89	64	0.073
1	20	30	
2-3	5	3	
Hematological disease			
ALL	9	1	0.349
AML	33	32	
MM	39	29	
NHL	23	24	
HL	11	7	
MDS	6	4	
Other*	11	10	
Type of HSCT			
Autologous	79	59	0.279
Allogeneic related donor	28	32	
Allogeneic unrelated donor	25	16	
Graft source			
PB[1]	118	96	0.887
BM[2]	4	2	
UCB[3]	10	9	
MDI**			
With bacteremia	54	43	0.266
Without bacteremia	3	5	

	Levofloxacin n: 132	No-Levofloxacin n: 107	p
Pathogens			
Gram-positive	48	39	0.001
Gram-negative	15	41	
Site of infection			
Abdominal	16	13	0.424
Catheter	3	4	
Mucositis	11	16	
Pneumonia	0	2	
Urinary	0	3	
Other	3	3	
Antibiotics used			
Cefepime+amikacin	50	17	<0.001
Imipenem+amikacin	11	30	
Meropenem	25	16	
Glycopeptide			
Vancomycin	36	9	<0.001
Teicoplanin	15	36	
Antifungal			
Caspofungin	14	10	0.859
Liposomal amphotericin B	1	0	
Levofloxacin resistance			
Yes	52	15	<0.001
No	7	33	
Outcome of infection			
Resolution	52	41	0.917
Need of Intensive Medical Care	2	2	
Death	11	8	

*CML (6), amyloidosis (2), aplasia (1), CLL (5), medulloblastoma (2), Mingie syndrome (1) and Sézary syndrome. ** Microbiologically documented infection. [1]Peripheral blood, [2]Bone marrow, [3]Umbilical cord blood.

PH-P466

FRENCH REAL LIFE COHORT ON THE USE OF MICAFUNGIN FOR INVASIVE FUNGAL INFECTIONS: RESULTS IN HAEMATOLOGY PATIENTS FROM THE MYRIADE STUDY

N. Milpied[1], J. Timsit[2,], G. Leverger[3], B. Gachot[4]*
[1]Haematology Department, University Hospital of Haut Levêque, PESSAC, [2]Intensive Care Unit, University Hospital of Bichat, [3]Department of Pediatric Hematology and Oncology, University Hospital of Trousseau, Paris, [4]Infectious department, Gustave Roussy Institute, Villejuif, France

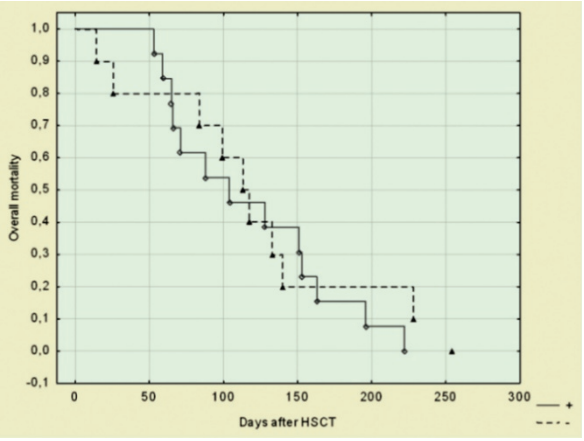
Introduction: Mycamine® (micafungin sodium) is an echinocandin indicated in adults (>=16 years old) and children (<16 year old, including neonates) for the treatment of invasive candidiasis, and for prophylaxis of *Candida* infection in patients undergoing allogeneic haematopoietic stem cell transplantation or patients who are expected to have neutropenia for 10 or more days. Mycamine® is also indicated in adult for the treatment of esophageal candidiasis.
MYRIADE was an observational, multicenter, national, prospective, longitudinal study conducted between January 2010 and December 2012, in patients treated with micafungin, among 117 physicians pre-selected for their potential involvement in treating invasive fungal infections. The main objective was to describe the use of micafungin in daily practice, in terms of reasons for prescriptions, therapeutic scheme, patient and physicians profiles and to assess prophylactic or curative patient response to treatment and potential adverse events as reported by physicians. Here we report the results from haematology patients.
Materials (or patients) and Methods: Treatment and all management procedures/exams were chosen by physicians according to their normal practice. Inclusion criteria: informed patients who were receiving a treatment with micafungin. Exclusion criteria: patient who were participating in another micafungin clinical trial or who couldn't be followed up. Data collection was performed at the initiation of micafungin (baseline) and at the end of treatment (withdrawal or not).
Results: Overall, 523 patients were recruited in the study, including 203 haematology patients treated by 28 haemato-oncologists: 138 adults and 65 paediatric patients including 19 less than 2 years old.
Micafungin was given in prophylactic intent for almost all patients (97.6%). Among adults, 39.1% had undergone allogeneic hematopoietic stem cell transplantation (27.7% of paediatrics). Malignancies predominated the underlying pathologies (86.2% of adults and 80.0% of paediatrics). The most common concurrent treatments were antineoplastic therapy (79.7%), prolonged broad-spectrum antibiotic therapy (60.2%), immunosuppressants (33.1%) and long-term high-dose corticosteroid therapy (18.6%). Compliance with the prophylactic daily dose for adults (50 mg) was observed for three quarters of patients (74.6%). The median daily dose per kg was 1.1 mg in haematology paediatric patients, and ranged from 1.0 to 1.4 across increasing patients age. Efficacy results were achieved for 81.0% of adults and 75.3% of paediatric patients.
Regarding Clinical laboratory results in this subpopulation, the proportion of patients within the normal range had increased at the end of treatment for Gamma-GT (from 27.4% to 30.4%) and Total Bilirubin (from 71.8% to 73.3%). This proportion decreased slightly for AST (from 76.4% to 65.5%), ALT (from 53.8% to 50.6%), Alkaline Phosphatase (from 71.3% to 64.8%) and Creatinine (from 55.0% to 50.8%).
Discussion: Compliance to Marketing Authorisation indications of micafungin was almost complete and recommended usage was generally respected. Real life data, collected through the first large observational study on micafungin in France confirmed its efficacy in prophylaxis and good safety profile.
Disclosure of Interest: None Declared.

PH-P467

INCIDENCE OF ADENOVIRAL DNA IN POLISH ADULTS UNDERGOING ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

S. Rynans[1], T. Dzieciatkowski[1,2,], M. Przybylski[1,2], G. W. Basak[3], A. Tomaszewska[4], K. Halaburda[3,4], W. W. Jedrzejczak[3], G. Mlynarczyk[1]*
[1]Chair and Department of Medical Microbiology, Medical University of Warsaw, [2]Department of Microbiology, Central Clinical Hospital, [3]Department of Haematology, Oncology and Internal Medicine, Medical University of Warsaw, [4]Department of Haematopoetic Stem Cell Transplantation, Institute of Haematology and Transfusion Medicine, Warsaw, Poland

Introduction: Adenoviruses are recognized as important viral pathogens in HSCT recipients. Early detection of HAdV in peripheral blood using real-time PCR has been suggested as a useful monitoring tool. Only few haematology centers use molecular methods for regular surveillance of adenoviral infection, and treatment strategies have never been evaluated in multicenter clinical trials.
Materials (or patients) and Methods: A group of 115 alloHSCT recipients from two hospitals in Warsaw, Poland was examined in early post-transplant period using quantitative real-time PCR. 1280 serum samples, collected from 60 (52%) men and 55 (48%) women, were evaluated for presence of HAdVs DNA. The 68 (59%) HSCT patients underwent unrelated grafts and the other 47 (41%) from sibling donors.
Results: HAdV DNA was detected in 67 (58%) of 115 patients. In twenty four of them (21%) DNA was detected only in a single positive sample, while 43 (37%) had positive results in two or more subsequent tests. In total, DNAemia was present in 221 sera samples (17.3%) with median time to isolation of 45 days. GvHD was observed in 19 (28%) HAdV-infected transplant recipients and a significant correlation between HAdV infections and GvHD clinical presentation was found (P= 0.03124).
Discussion: There is a high prevalence of adenovirus infection in HSCT recipients in Poland during early post-transplant period. HAdV DNAemia is also significantly related to GvHD symptoms, enforcing the important pathogenic role of these viral infections in clinical complications post-alloHSCT.
Disclosure of Interest: None Declared.



PH-P468

PREVENTION PNEUMOCYSTIS JIROVECII PNEUMONIA BY PROPHYLACTIC INTRAVENOUS PENTAMIDINE IN HEMATOLOGIC PATIENTS: EFFICACY AND SAFETY

L. Alvarez Pequeño[1,], L. Iglesias Domínguez[1], C. Albo López[1], J. Vázquez Álvarez[1], E. Fernández Mellid[1]*
[1]Hematología, Complejo Hospitalario DE Vigo, Vigo, Spain

Introduction: Pneumocystis jirovecii (PJ) pneumonia is a serious complication in immunocompromised patients such as those with

HIV infection, hematological malignancies and transplant recipients. The most effective prophylactic therapy is TMP-SMZ but its administration is often associated with intolerance and toxicity. Other prevention strategies as dapsone or inhaled pentamidine have been associated with higher incidence of pneumonia. Several studies have suggested that intravenous (IV) pentamidine could be an effective alternative.
Materials (or patients) and Methods: Data from clinical histories of all hematologic patients receiving at least one dose of IV pentamidine in the Hospital Xeral-Cies of Vigo between January 2002 and February 2013 were retrospectively collected.
Risk factors to promote development of PJ infection were analyzed: CD4 lymphocytes count <200/mm^3, transplant type, GVHD and previous high dose steroid, purine analogues, bendamustine or rituximab therapy.
Results: 93 patients (39.78% male, 60.22% female) received IV pentamidine. Median age was 51 years old. 87 as transplant prophylaxis: 53 (56.98%) autologous and 34 allogeneic (16 unrelated donor, 13 family related donor and 5 cord blood transplant). 6 pts received IV pentamidine prophylaxis associated to immunosuppressive chemotherapy.
The underline diagnosis was: acute leukemia 29 pts (31.18%), NHL 27 (29.0%), multiple myeloma 19 (20.43%), HD 7 (7.52%), lymphoproliferative disorders 4, myeloproliferative disorders 4, myelodysplastic syndromes 2 and Ewing´s sarcoma 1.
Previous chemotherapy included purine analogs, bendamustine and/or rituximab in 39 pts. 67 received high dose steroid. At start of prophylaxis the median CD4 count was 236.5, 30 pts showed <200. Chosen treatment was TMP-SMZ plus folinic acid and reasons to be replaced by IV pentamidine were: pancitopenia in 42 pts (45.16%), neutropenia in 22 (23.65%), intolerance in 27 (29.03%) (gastrointestinal in 19 and skin reactions in 8) and others in 2 (poor compliance and odynophagia).
Treatment was IV pentamidine, a dose of 4 mg / kg given in one hour infusion monthly. Patients received a median of 9.5 doses (range 1–25). One case of Pneumocystis jirovecii pneumonia was documented by BAS positive immunofluorescence in a patient seventh month after allogenic cord blood transplantation. She had received 9 doses of prophylactic IV pentamidine. 7 cases of adverse events were reported. 6 Grade 1 -gastrointestinal symptoms in 3 pts (3.22%), paresthesia in 2 (2.15%), and 1 (1.07%) chills- and 1 Grade 2 skin reaction. All cases responded to premedication and no patients required therapy discontinuation.
Discussion: Our data shows that prophylactic IV pentamidine can be a safe and effective alternative when standard prophylaxis with TMP-SMZ is toxic or poorly tolerated.
Disclosure of Interest: None Declared.

PH-P469
RISK FACTORS FOR THE DEVELOPMENT OF INVASIVE INVASIVE FUNGAL INFECTION IN PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
P. Topcuoglu[1,], E. Berna Koksoy[2], M. Ozen[1], O. Arslan[1], M. Ozcan[1], N. Konuk[1], H. Akan[1], M. Beksac[1], G. Gurman[1]*
[1]Hematology, [2]Internal Medicine, Ankara University, Faculty of Medicine, Ankara, Turkey

Introduction: Invasive fungal infections (IFIs) are seen in gradually increasing frequencies in allo-HSCT recipients. In this study, the risk factors before and after transplantation for IFIs development were investigated in the patients underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Materials (or patients) and Methods: We retrospectively analyzed 370 patients underwent allo-HSCT between May 2005 and 2012 in Ankara University Faculty of Medicine Hematology Department Stem Cell Transplantation Unit. IFI development in allo-HSCT recipients and also IFI history before transplantation were determined with patient characteristics, clinical, radiologic and microbiologic evidences and categorized as possible, probable and proven IFI as EORTC/MSG criteria. Probable and proven IFIs were accepted as IFI development. Time of IFI development was examined in three periods; until day +21, between day +21 and +90, after day +90.
Results: In 46 patients, there was the history of IFIs [probable (*n*=39) or proven (*n*=7)] prior to the transplantation. At transplant and post-transplant period, probable and/or proven IFI development were found in 63 patients (16.6 %). Most of the IFIs (63%) developed in the first three months following transplantation. IFI development was observed in 18.8% (*n*=54) among 287 allo-HSCT without the history of any IFIs prior to the transplantation. In contrast, we determined IFIs development in only 4 (8.7 %) among 46 patients allo-HSCT with the history of possible IFI. In the univariate analysis, statistically significant relationships were detected between IFI development and the intensity of conditioning regimens, type of GvHD prophylaxis, and the development of CMV infection, thrombocyte engraftment and transplant-related mortality. Multivariate logistic regression analysis showed that significant predictors of IFI development were the presence of CMV infection and the absence of thrombocyte engraftment (OR: 3.6 and 2.5, respectively).
Discussion: In conclusion, CMV infection and absence of thrombocyte engraftment should be predictable of the development of IFIs. In the aspect of these findings, prospective studies including more patients and more homogenous groups for the development of IFIs and/or CMV infection in allo-HSCT recipients should be designed.
Disclosure of Interest: None Declared.

PH-P470
A PROPHYLACTIC STRATEGY INCLUDING IGM-ENRICHED IMMUNOGLOBULIN AFTER ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION RESULTS IN LOW TRM, LOW RATE OF EARLY INFECTIONS AND NO SEVERE LIVER TOXICITY
G. Milone[1,], S. Leotta[2], A. Cupri[2], D. Berritta[2], P. Spina[2], M. Parisi[2], A. La Fauci[2], A. Di Marco[2], A. Spadaro[2]*
[1]Bone Marrow Transplantation Program, Azienda Policlinico Vittorio Emanuele, [2]Hematology, BMT UNIT, Catania, Italy

Introduction: Prophylaxis of infections using High dose IgG after allogeneic transplantation do not decrease infectious risk, furthermore it may be associated to increased liver toxicity. IgM-enriched immunoglobulin are able to neutralize endotoxin and, thus, it may reduce toxicity and TRM after allogeneic HSC transplantation.
Materials (or patients) and Methods: 114 patients affected by acute leukemia and treated with allogeneic transplantation were studied. All patients received as anti-infectious prophylaxis:-IgM-enriched Immunoglobulin (Pentaglobin) at dosage of 200 mg/weekly for 6 doses, -prophylactic systemic antibiotics, -Total GI tract decontamination. 54 patients received also prophylaxis with Defibrotide as a further anti-toxicity. 81 patients were affected by AML and 33 by ALL, 66% received BM and 33% PBSC, in 65% donor was a HLA identical sibling (SIB) while in 35% was an Unrelated volunteers, 88% received a Myeloablative conditioning. 56% percent of patients were in 1st CR while 44 % in more advanced phase. Early Infections were categorized as: "FUO intervening early post-HSC infusion", "FUO after day +2", "Gram positive bacteremia or CVC infections", "Gram negative pneumonia or focal pneumonia".
Results: No patients died because of early infections and overall TRM at 100 days was 5%. "FUO after day +2" was registered in 39% of patients and it was not associated to Conditioning type, Diagnosis, Advanced disease at transplant or to HSC source. In univariate analysis, "FUO (after day +2)" was significantly associated with low immunoglobulin level of at admission (*P*=0.01), "FUO (after day +2)" was more frequent also after MUD transplants (51% versus 32%, *P*=0.05) and in patients who previously had a prior transplant (*P*=0.01). In multivariable analysis, only low level of serum immunoglobulin at admission remained significant for an increased risk for this type of infection (*P*=0.02). Gram negative bacteremia or focal pneumonia interested 4.5% of patients and these types of infections were significantly more frequent in MUD transplant in respect to SIB (9.5% vs 1.5%, *P*=0.04). A low grade

(< 38.5 °C) and transient fever presenting early after infusion of HSC ("FUO intervening early post-HSC infusion"), interested 22% of patients (4% in family sibling and 55% in MUD, *P*=0.0001) no association of this type of FUO with immunoglobulin level, phase of disease or other factors was found. "Gram positive bacteremia or CVC infections" interested 26% of patients and was not associated to any factors. Overall incidence of Liver Sinusoidal Obstructive Syndrome (SOS) was 4.9%, no SOS graded as "severe" was registered. Patients presenting an increase in bilirubin above 2 mg/dl were 20%. The only factor associated to liver toxicity was advanced phase disease (*P*=0.004). Patients treated by Defibrotide had a reduction of liver toxicity, in terms of SOS and Bilirubin >2mg/dl, that, however, did not reach significance.
Discussion: Low serum Immunoglobulin level at admission is associated to an increased risk of FUO. A strategy that includes IgM-enriched Immunoglobulin leads to excellent results in terms of early TRM and infections. No clinically important liver toxicity associated to IgM-enriched immunoglobulin is evident. Role of IgM-enriched immunoglobulin remain to be precised further in a prospective controlled study.
Disclosure of Interest: None Declared.

PH-P471
TOXOPLASMA GONDII DNA POSITIVITY IN HSCT PATIENTS IS AGE- AND TIME-DEPENDENT FOR DIFFERENT BIOLOGICAL SAMPLES

L. Zubarovskaya[1], S. Bondarenko[1], Y. Eismont[2], M. Vladovskaya[1], B. V. Afanasyev[1], A. B. Chukhlovin[1,]*
[1]R. Gorbacheva Memorial Institute of Children Oncology, Hematology and Transplantology, [2]Department of Microbiology, St. Petersburg State I. Pavlov Medical University, St. Petersburg, Russian Federation

Introduction: Some clinical cases of *T. Gondii* infection were previously reported in patients following hematopoic stem cell transplantation (HSCT). However, age- and time dynamics of *T. Gondii* incidence was not studied for large groups of HSCT patients, while testing different biological samples.
Materials (or patients) and Methods: Patients and methods. From April 2010 to July 2013, we have observed 297 consecutive HSCT patients (1 to 60 years old, a median of 19 years), 57% males and 43% females. They we treated for: ALL (*n*= 101), AML (*n*= 105), MDS (*n*=31), CML (*n*=17), Hodgkin lymphoma (17), non-Hodgkin lymphoma (*n*=6), severe aplastic anemia (*n*=12), neuroblastoma (*n*=5), inborn metabolic disorders (*n*=4). Their conditioning regimens were myeloablative (35%), or non-myeloablative (65%) of cases. HSCT was performed from related (16%), matched unrelated (63%), or haploidentical donors (21%). The patients received either bone marrow, or peripheral blood stem cells (resp., 47% and 53% of the cases). Second transplants were excluded from the analysis. Anti-infective treatment included pre-emptive acyclovir therapy and antimicrobial treatment at a standard schedule. Conventional GvHD prophylaxis was performed with Cyclosporin A and methotrexate, while ATG was applied in most cases. DNA-based diagnostics of *T. Gondii* (a total of 2975 tests) was performed in blood leukocytes on a weekly basis, and, if available, in cerebrospinal fluid (CSF) cells, broncho-alveolar lavage (BAL), and urine sediments, using commercial gene-specific PCR kits, at a cutoff value of 1000 copies/mL, as verified by quantitative PCR kits.
Results: PCR tests for *T. Gondii* DNA were positive in 13% of blood samples, 9% of CSF specimens, 11% of BAL samples, and 5% of urine sediments. We could not find any significant correlations between Toxoplasma activation and type of donors or source of transplanted cells for individual cases. Initial incidence of the pathogen was similar for different age groups (1…4, 5…9, 10…14, 15…19, 20> years). *T. Gondii* activation (>2 consecutive positives within 100 days post-HSCT) was found in 12% of the patients, pointing to a special subgroup of patients prone to *T. Gondii* activation. Comparing subgroups of patients with different conditioning regimens, we have shown a sharply increased *T. Gondii* incidence in the patients of 10 to 14 years old who received myeloablative (MA) treatment, when compared with non-MA protocols (*P*=0.01), as revealed both for blood and BAL samples (*P*<0.05). When evaluating early post-transplant dynamics, we have revealed a significant increase of *T. Gondii* DNA excretion in urine samples during 1st month post-transplant (*P*=0.03).
Discussion: *T. Gondii* infection seems to be early activated in a sufficient subgroup of HSCT patients, as shown by urinary post-transplant excretion. Myeloablative treatment is associated with higher detectability of *T. Gondii* DNA in blood and BAL, at least in older children, thus presuming higher risks of respiratory involvement in this cohort. Further clinical studies will be focused on *T. Gondii*, as an individual risk factor for clinical lung disorders post-HSCT.
Disclosure of Interest: None Declared.

PH-P472
HEPATITIS B REACTIVATION FOLLOWING ALLOGENEIC HSCT

P. Bavaro[1,], P. Olioso[1], S. Santarone[1], G. Papalinetti[1], E. Pennese[1], P. Di Bartolomeo[1]*
[1]Hematology, BMT UNIT Pescara, Pescara, Italy

Introduction: Reactivation of hepatitis B virus (HBV) infection is a known complication after HSCT. It is most commonly observed in patients seropositive for hepatitis B surface antigen (HBsAg), however it is also reported in HBsAg-negative but anti HBc positive patients as the only sign of previous infection.
Materials (or patients) and Methods: We evaluated retrospectively the incidence, clinical impact and risk factors of HBV reactivation in 296 allogeneic transplant recipients who were followed for a median time of 46 (range: 5-108) months after HSCT. The median age at transplant was 42 (range: 1-71) yrs. The conditioning therapy was myeloablative (MAC) in 223 and reduced (RIC) in 73 transplants. In 96 transplants from unrelated donors and 62 transplants from haploidentical donors, anti-thymocyte globulin (ATG) was also used. Stem cell source was bone marrow in 166, peripheral blood cells in 125 and cord blood in 5 transplants.
Results: Regarding the prior exposure to HBV, we differentiated patients in naïve (47) or not naïve (249): no significant difference was observed in overall survival (54% vs 50%) and cumulative incidence of acute (19% vs 25%) and chronic GVHD (18% vs 22%). Within familiar donor/recipient pairs the incidence of antiHBc+ was significantly higher in recipients (14% vs 5%, *P*<0.001). Forty-six of 296 (15%) patients were at risk of HBV reactivation at transplant: 2 HBsAg+ recipients, 35 antiHBc+ recipients, 1 HBsAg- recipient who received transplant from HBsAg+ donor, 9 HBsAg-/antiHBc- recipients transplanted from anti HBc+ and antiHBs- donors. Five (11%) of 46 patients at risk had HBV reactivation. The cumulative incidence of HBV reactivation was 21% at 8 years. The incidence of HBV reactivation was not influenced by donor type (identical related, identical unrelated, familiar haploidentical), MAC or RIC conditioning, ATG use, bone marrow or peripheral stem cells source, recipient age, recipient sex. HBV reactivation was associated with serum HBsAg+, HBV DNA more than 8 log10 copies per ml and an ALT increase 1.5- to 36-fold (median 4). HBV reactivation was observed in 3 patients after immunosuppressive therapy withdrawal and in 2 patients under immunosuppressive therapy for chronic GVHD. All five patients had genotype D of HBV. Three patients showed acute icteric hepatitis B that evolved in acute liver failure in one case. Shortly after the diagnosis of HBV reactivation, one patient underwent a liver biopsy which evidenced both mild HBV-related inflammation and minimal colangiopathy suggesting chronic GVHD. At a median follow-up of 30 months after HBV reactivation, three patients are undergoing continuous antiviral therapy with tenofovir or entecavir. Regarding the virological status, at last follow-up one of them is HBV chronic hepatitis active carrier and two are inactive carriers. One patient died of AML relapse at 27 months from reactivation, as active hepatitis B carrier. Another patient while undergoing lamivudine prophylaxis, developed fatal antiviral resistance unresponsive also to therapy with entecavir and died of acute liver failure after 7 months from reactivation.

Discussion: HBV reactivation in highly immunosuppressed population may be a not infrequent and severe late complication. Active prophylaxis with nucleoside analogues are essential, but not sufficient to control HBV reactivation.
Disclosure of Interest: None Declared.

PH-P473
SUPPORTIVE CARE AND INFECTIOUS COMPLICATIONS IN CORD BLOOD AND HAPLOIDENTICAL STEM CELL TRANSPLANTATION IN ADULTS WITH HIGH RISK HEMATOLOGIC DISEASES.

J. Elcheikh[1,], R. Crocchiolo[2], A. Vai[2], S. Furst[1], S. Bramanti[2], B. Sarina[2], A. Granata[1], C. Faucher[1], B. Mohty[1], S. Harbi[1], R. Bouabdallah[1], N. Vey[1], A. Santoro[2], C. Chabannon[1], L. Castagna[2], D. Blaise[1]*
[1]Transplantation, Institut Paoli Calmettes, Marseille, France, [2]Transplantation, Humanitas Cancer Center, Milan, Italy

Introduction: Infections due to post transplant immune deficiency are a major problem following allogeneic stem cell transplantation, particularly in patients receiving cord blood (CBT) or haploidentical transplants (haplo-SCT). To our knowledge, there are only some heterogeneous studies comparing this important issue in this setting.
Materials (or patients) and Methods: We evaluated the incidence and type of infectious complications that occurred in these two types of transplant for 150 patients, 81 cord blood and 69 haploidentical, who received the same conditioning regimen: fludarabine (Flu), cyclophosphamide (Cy) and low dose TBI (2 Gy) combination in the two groups. The GVHD prophylaxis consisted of Cyclosporine A (CsA) and MMF in all patients in the two groups. In the Haplo group all patients received also 50 mg/kg Cy at day 3 and 4 post transplant. Of note, supportive care was the same during the whole study period. CMV infection management was also homogeneous.
Results: While incidence of infections appeared similar in both types of transplant, viral infections were more frequent than bacterial or fungal infections and were the most common cause of death in both groups. During the first year after stem cell transplant, the CBT group had 154 episodes of infection (31 infection episodes per 1,000 patient-days) versus 125 of the haplo patients (39 per 1,000 patient days). Viral infections were most common in both groups (83 vs 60 episodes) in CBT vs haplo group respectively. Patients in the haplo group were 1.2 times (95% CI: 1.1 to 2.5) more likely to have a viral infection (P=0.07). Pneumonia was the most common clinical syndrome and was higher in the haplo group 28% vs 11% in CBT. Both pneumonia and bacteremia prevalence occurred within the first 100 days in the majority of CBT patients while haplo patients had a bimodal distribution with more than one third of bacteremia episodes after 6 months post-transplant. Survival analysis showed that transplant source (CBT versus Haplo) did not have a significant effect on the probability to have an infection episode. (P=0.10) Bacterial, fungal and CMV infections still quite frequent and contributed to higher mortality in CBT vs haplo (P=0.06). The first cause of mortality was the viral infection (12 (15%) 3 (4%) P=0.060) followed by the bacterial infection cause (4 (5%) vs 3 (4%)) and the fungal infection (1 (1%) vs 0) in CBT and haplo group respectively.
Discussion: Our study is unique in comparing adult patients with advanced hematologic malignancies who received the same reduced-intensity conditioning regimen, which offers a common base for comparing the infections in these two types of transplant. In our hands, outcome is better for haplo donor decreasing the need of supportive measures and facilitating to perform the transplant in time.
Haploidentical transplants are a good and promising alternative option for high risk hematologic patients who lack an HLA-matched donor. Collectively, the impact of infectious complications after haplo-SCT was different from that in CBT patients, suggesting that a more intensive strategy for infection control in haplo patients is required to reduce infectious mortality.
Disclosure of Interest: None Declared.

PH-P474
PRE-ENGRAFTMENT INITIATION OF BRINCIDOFOVIR (CMX001) IN HEMATOPOIETIC CELL TRANSPLANT RECIPIENTS IS SUPPORTED BY LACK OF MYELOID TOXICITY

G. Papanicolaou[1,], M. Grimley[2], M. Morrison[3], T. Brundage[3], H. Mommeja-Marin[3]*
[1]Department of Medicine, Service of Infectious Disease, Memorial Sloan Kettering Cancer Center, New York, NY, [2]Division of Bone Marrow Transplant and Immune Deficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, [3]Clinical Research, Chimerix, Inc., Durham, NC, United States

Introduction: Currently available antivirals with anti-CMV activity have been associated with myeloid toxicity in hematopoietic cell transplant (HCT) recipients, limiting treatment initiation until after engraftment, and reducing the potential benefits of CMV prevention by increasing risks of other infections. Brincidofovir (BCV), currently in Phase 3 development for prevention of CMV infection post-HCT, is a nucleotide analogue with broad-spectrum double-stranded DNA antiviral activity and a favorable safety profile.
Materials (or patients) and Methods: Absolute neutrophil counts (ANC) from HCT recipients enrolled in two placebo (PBO)-controlled (Studies 201 and 202) and one open-label clinical trial (Study 350) of BCV were reviewed. In 201 and 202, study drug was initiated a median of 24 and 55 days post-HCT, respectively. Subjects from 201 and 202 receiving a total weekly dose of 200mg BCV or PBO, who had baseline (BL) ANC <1500 cells/μL, and who did not receive concomitant valganciclovir (vGCV) or ganciclovir (GCV) were included in the analysis. Last and maximum on-treatment ANC values were compared to baseline. Clinically identified graft failure rates were also compared.
Results: In 201 and 202, 40 subjects were analyzed: 24 on BCV and 16 on PBO. Subjects received a mean 6.0 (PBO) to 6.5 weeks (BCV) treatment. On-treatment ANC recovery to >1500 cells/μL occurred in 83% (20/24) on BCV compared to 81% (13/16) on PBO. Numerically improved ANC (>BL) was noted in 92% (22/24) BCV subjects versus 94% (15/16) on PBO. Due to the clinical importance of early ANC recovery, similar analyses were performed for the first 4 weeks of treatment: 68% of subjects on BCV versus 75% on PBO achieved ANC >1500, while 83% BCV versus 81% PBO subjects had an ANC >BL at week 4. Observed ANC through the first 4 weeks revealed a maximum ANC >1500 in 79% of BCV subjects compared to 81% of PBO subjects; evidence of early neutrophil recovery with a maximum ANC >BL was observed in 88% of BCV subjects compared to 94% of PBO subjects.
In Study 350, 41 post-HCT subjects had BL ANC <1500 cells/μL. Of these, prior vGCV or GCV use was reported in 41%, and 27% were enrolled due to concerns of cytopenia. Fourteen subjects were excluded due to concomitant vGCV or GCV use. The remaining 27 subjects received BCV for a median of 4 weeks; 19/27 (71%) had last on-treatment ANC >1500, and 21/27 (78%) had last on-treatment ANC >BL. Maximum on-treatment ANC was >1500 in 23/27 (85%), and >BL in 25/27 (93%).
In 201 and 202, graft failure was reported in 2/123 (1.6%) of the 200 mg/week BCV subjects, versus 4/77 (5.2%) of the PBO subjects. In 350, graft failure was reported in 1 (4%) subject on BCV monotherapy, versus 3/14 (21%) on BCV plus GCV/vGCV.
Discussion: These data suggest BCV has no negative impact on neutrophil recovery, supporting initiation of BCV in the immediate post-transplant period prior to engraftment, as per the design of the current Phase 3 SUPPRESS trial. The hematologic safety profile of brincidofovir and earlier initiation of antiviral prophylaxis in the post-HCT period may not only provide improved efficacy in the prevention of CMV infection and disease, but may also reduce rates of invasive bacterial and fungal infections post-HCT.
Disclosure of Interest: None Declared.

PH-P475

PROLONGED FLUCONAZOLE PROPHYLAXIS IS NOT REQUIRED IN PATIENTS WITH ACUTE LEUKEMIAS OR MYELODYSPLASTIC/MYELOPROLIFERATIVE DISORDERS AFTER REDUCED-INTENSITY CONDITIONING (RIC) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT): A PAIR-MATCHED ANALYSIS

E. Brissot[1,], X. Cahu[2], T. Guillaume[1], J. Delaunay[1], S. Ayari[1], C. Precupanu[1], P. Peterlin[1], J. L. Harousseau[3], N. Milpied[4], P. Moreau[1], M. Mohty[5], P. Chevallier[1]*

[1]Hematologie, Hotel Dieu, CHU Nantes, NANTES, [2]Hematologie, hopital Pontchaillou,CHU Rennes, Rennes, [3]Hematologie, Centre Rene Gauducheau, Nantes, [4]Hematologie, Hopital de Bordeaux, Pessac, [5]Hematologie, Hopital Saint Antoine, APHP, PARIS, France

Introduction: Invasive fungal infections (IFI) due to *Candida* and *Aspergillus species* remain a major complication of allo-SCT. In the myeloablative setting, previous studies have demonstrated that fluconazole, administered for 75 days after transplant (400 mg/d), in a prophylaxis setting, can allow for a decreased risk of gut GVHD and disseminated candida infections or candidiasis-related death, resulting in an overall survival benefit (Marr *et al*., Blood 2000). However, to our knowledge, there is currently no study, addressing the potential benefit of fluconazole prophylaxis in the setting of reduced-intensity conditioning (RIC) allo-SCT.

Materials (or patients) and Methods: This retrospective study included 105 patients with acute leukemia (AML, *n*=55, ALL, *n*=11) or myelodysplastic/myeloproliferative disorders (MDS, *n*=24; myeloproliferative disorder *n*=15) who received an allo-SCT between January 2000 and December 2011. In this series, patients received one (*n*=105) or two (*n*=4) RIC allo-SCT and PBSC as stem cell source for all. Among these 109 procedures, we compared those cases receiving (*n*=53) or not (*n*=56) fluconazole prophylaxis after transplant. The post-transplant fluconazole prescription or not was at the discretion of the attending physician and was homogeneous for each physician. There were not significant differences between both groups in terms of gender, median age (fluco: 55 vs 57 years), median follow-up (fluco: 42 months (range: 19-76) vs 37 months (range: 11-124)), median year of transplant (fluco: 2008 (range: 2003-2011) vs 2009 (range: 2000-2011)), type of disease and disease status at time of transplant or type of donor.

Results: The median time of fluconazole administration (400mg/day) after transplant was 88 days (range: 7-324). Fluconazole was stopped only in 2 patients because of (reversible) liver cytolysis. Before day +100, only 2 *Aspergillus* infections were documented in the fluconazole group vs 4 in the non-fluconazole group (*P*=NS). No *Candida* infections (septicemia or cutaneous candidiasis) developed in the fluconazole group compared to 2 in the non-fluconazole group (*P*=NS). After day +100, *Aspergillus* infections were documented in 5 patients in the fluconazole group versus 3 in the non-fluconazole group (*P*=NS). The number of patients receiving pre-emptive or curative antifungal treatment (voriconazole, caspofungin or ambisome) after transplant was higher in the non-fluconazole group (52% of cases vs 34%, *P*=0.09).

3-year OS, DFS and NRM were similar between both groups (fluco: 42% vs 51%, *P*=0.42, fluco: 38% vs 46%, *P*=0.62, and fluco: 28% vs 23%, *P*=0.67). Also, there were no significant differences in term of cumulative incidences of acute grade II-IV GVHD (fluco: 27% vs 36%, *P*=0.29), acute grade III-IV GVHD (fluco: 21% vs 18%, *P*=0.42) or chronic GVHD (fluco: 47% vs 33%, *P*=0.67).

Discussion: This is the first series reporting the comparison of the use or not of fluconazole as prophylaxis after RIC allo-SCT. Our results showed that the use of fluconazole has no impact in term of fungal infections or overall outcomes after RIC allo-SCT, suggesting that fluconazole is not required after RIC allo-SCT. Large prospective studies are warranted to further confirm this important therapeutic issue.

Disclosure of Interest: None Declared.

PH-P476

ANTIBODY RESPONSE TO IMMUNIZATION WITH LIVE-ATTENUATED VACCINES IN ADULT SURVIVORS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

M. Tsuji[1,], Y. Asano-Mori[1], M. Narita[1], R. Tsuchihashi[1], H. Ota[1], A. Nishida[2], K. Ishiwata[1], S. Takagi[2], H. Yamamoto[1], G. Yamamoto[1], N. Uchida[1], K. Izutsu[1], A. Wake[2], A. Yoneyama[3], S. Taniguchi[1]*

[1]Department of Hematology, Toranomon Hospital, Tokyo, [2]Department of Hematology, Toranomon Hospital Kajigaya, Kawasaki, [3]Department of Clinical Laboratory, Toranomon Hospital, Tokyo, Japan

Introduction: Immunization with live-attenuated vaccines (measles, rubella, mumps, and varicella) has been recommended after 2 years following allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients not receiving immunosuppressive drugs. There were several reports on effectiveness of vaccination for pediatric recipients, but few for adult recipients.

Materials (or patients) and Methods: We prospectively analyzed 45 adult allo-HSCT recipients who come to outpatient clinic for post-transplant immunization in Toranomon Hospital from Jun 2012 to October 2013. All patients fulfilled below criteria for immunization with live-attenuated vaccines; (1) two years or more after allo-HSCT, (2) one year or more after cessation of immunosuppressants, (3) no or under good control of chronic graft-versus-host disease (GvHD). Measles-rubella (MR), mumps, and varicella vaccines were subcutaneously injected once, respectively. Antibody titers for measles, rubella, mumps, and varicella were examined before and later than 2 months after immunization by enzyme-immunoassay methods.

Results: The median age was 52 years old (range, 23 - 72), 25 patients (55.6%) were male, and 21 (46.7%) underwent cord blood transplantation (CBT). The median counts of CD4-positive and CD19-positive cells were 540/uL (range, 202.8 - 1202.8) and 520/uL (122.2 - 2014.8), respectively. The median duration from allo-HCT to vaccination was 5 years (range, 2 - 12). The rates of sero-positivities before and after immunization were 11.1% and 37.7% for measles, 31.1% and 88.9% for rubella, 5.6% and 11.1% for mumps, and 42.2% and 57.8% for varicella, respectively. The rates of no seroconversion after vaccination were 57.9% for measles, 6.6% for rubella, 88.9% for mumps, and 42.2% for varicella, respectively. In the group of CBT, there were tendency of higher CD19-positive cells counts and higher incidence of seroconversion after immunization than the others, although the differences were not significantly significant.

Discussion: There was not a few patient without seroconversion after immunization, and only once immunization of live-attenuated vaccines was considered not enough for effective immunization. Different approach of immunization should be taken according to the type of donor sources. We are currently planning to vaccinate live-attenuated vaccines twice, and examine the relationship of immune recovery and effective immunization in each donor source.

Disclosure of Interest: None Declared.

PH-P477

ANALYSIS OF CD19+/CD38+/CD27HIGH PLASMA BLASTS IN ALLOGENEIC STEM CELL TRANSPLANT PATIENTS AFTER ROUTINELY PERFORMED RE-VACCINATION

J. Winkler[1,], M. Mach[2], A. Mackensen[1], T. Winkler[3]*

[1]Department of Hematology/Oncology, [2]Institute for Clinical and Molecular Virology, [3]Institute for Biology, Chair of Genetics, Nikolaus-Fiebiger-Center for Molecular Medicine, University Erlangen-Nuremberg, Erlangen, Germany

Introduction: After allogeneic stem cell transplantation (alloSCT) patients show a long lasting immune deficiency. To improve the humoral immune response we are planning for the first time in patients an adoptive transfer of purified donor-B-lymphocytes as a clinical phase I/II study. In addition to the analysis of the primary safety endpoints of the study (GvHD and EBV-viremia) we would like to test the activity of infused donor memory B cells *in vivo*. A

characteristic memory B cell response is defined as a mobilization of plasma blasts (PBs) 7 days after booster vaccinations into the peripheral blood (Odendahl *et al*., *Blood* 105: 1614-1621, 2005).
Materials (or patients) and Methods: In preparation of this study we analysed control patients (*n*=5) between day 180 to 240 after alloSCT in the course of routinely performed re-vaccinations with PENTAVAC. At the time of vaccination the patients received no immunosuppressive agents and showed no signs of active chronic GvHD. Before and 7 days after vaccination the number of $CD19^{+}$/$CD38^{high}$/$CD27^{high}$ plasma blasts was analysed by flow cytometry and by ELISPOT assays after enrichment for total B cells. Data from alloSCT patients were compared with results from a control group of healthy donors (*n*=10), which also received a booster vaccination with the same vaccine.
Results: After vaccination alloSCT patients showed no increase of circulating plasma blasts in the peripheral blood (mean 4.5% PB of B cells before vaccination and mean 4.6% PB of B cells d+7 after vaccination). In contrast, in the peripheral blood of healthy donors a 9.5-fold increase of plasma blasts was observed on 7 days after vaccination (mean 1.6% PB before and 15.5% PB d+7 after vaccination). In the ELISPOT assays, no increase of antigen-specific-IgG-secreting PBs was detectable in the alloSCT patients. In contrast, in the peripheral blood of healthy donors a profound increase of antigen-specific-IgG-secreting PBs against all vaccine-antigens tested could be observed 7 days after booster vaccination, as expected.
Discussion: In summary, our data show that a PB response after routine vaccinations is undetectable in alloSCT patients, suggesting that vaccine-specific memory B cells are absent at this timepoint after transplantation. The antigen-specificity of the IgG-secreting plasma-blasts, present in elevated frequency in the patients after alloSCT, is unknown. (Supported by BayImmuNet and DFG, SFB643).
Disclosure of Interest: None Declared.

PH-P478

PROSPECTIVE STUDY OF THE PERFORMANCE OF THE SERUM GALACTOMANNAN ASSAY AS PART OF A DIAGNOSTIC-DRIVEN STRATEGY FOR HIGH-RISK HEMATOLOGY PATIENTS ON POSACONAZOLE PRIMARY PROPHYLAXIS

I. Sánchez-Ortega[1,], I. Cuesta[2], B. Patiño[1], M. Arnan[1], A. Fernández de Sevilla[1], C. Gudiol[1], J. Ayats[3], M. Cuenca-Estrella[2], R. F. Duarte[1]*
[1]Institut Català d'Oncologia - Hospital Duran i Reynals, Barcelona, [2]Centro Nacional de Microbiología - Instituto de Salud Carlos III, Majadahonda (Madrid), [3]Hospital Universitari de Bellvitge, Barcelona, Spain

Introduction: We have recently reported that the preemptive use of the serum galactomannan assay (GM; Platelia, Bio-Rad) provides no diagnostic benefit to manage high-risk hematology patients on posaconazole prophylaxis, in whom the pre-test incidence of invasive aspergillosis (IA) is very low. In 262 such high-risk episodes, with a 1.9% incidence of IA, all evaluable serum GM tests performed as a surveillance tool in asymptomatic patients were always either negative or false positive. However, GM tests remained useful as part of the diagnostic strategy for symptomatic patients, both for their contribution to the diagnosis of breakthrough IA, as well as for their high negative predictive value [Sánchez-Ortega, *et al*; P950 EBMT 2013 and AAA 2014]. Here, we now present a prospective validation of our new strategy, which aims to use more efficiently serum GM tests in high-risk hematology patients on antifungal prophylaxis with posaconazole.
Materials (or patients) and Methods: From June 2013, our antifungal management strategy discontinued the preemptive use of serum GM tests in asymptomatic patients, and focused it, in combination with CT scans, microbiological samples and cultures from urine, blood, BAL or other possible infected sites, on a diagnostic strategy for persistently febrile symptomatic patients only. We present here prospective data until November 2013, on 49 consecutive high-risk episodes on posaconazole primary prophylaxis (31 AML-chemotherapy, 12 alloHCT and 6 GVHD) from 29 hematology patients from our centre.
Results: Since the implementation of our new diagnostic strategy we have performed only 8.3% the total number of GM tests of an equivalent 6-month period under our previous biweekly preemptive strategy (31 vs 372; $P<0.001$): only 35% of risk episodes (17/49) required serum GM tests for diagnosis of symptomatic patients, and the number of tests performed per episode was also significantly lower (median 1, range 1-5 *vs* 11, 3-30; $P<0.001$) (Table). GM test results were all negative in 15 of these 17 episodes, and positive (Optical Index [OI] >0.7 x1 or OI >0.5 x 2 consecutive samples) in only two (12%). These positive test results (2-3 per episode; OI: 0.59-2.25) were all false positive in both episodes, as patients remained on posaconazole prophylaxis, survived the episodes and never developed any other criteria of IA. In this prospective series, there have been so far no cases of breakthrough IA (0/49), and posaconazole prophylaxis was completed as initially planned in 88% of the risk episodes (43/49).
Discussion: Our analysis suggests that focusing the use of serum GM only for diagnostic purposes in symptomatic patients is a safe strategy to manage high-risk hematology patients on posaconazole prophylaxis. This approach translates into a very significant reduction of the use of GM tests, preventing asymptomatic patients from unnecessary assays and rationalizing resources utilization and diagnostic costs without an increase in breakthrough IA.
Disclosure of Interest: None Declared.

Table. Antifungal management according to a preemptive vs diagnostic-driven strategy of galactomannan use in high-risk hematology patients on antifungal prophylaxis with posaconazole.

	Preemptive GM strategy *(biweekly serum GM from the start of the risk episode)**	**Diagnostic-driven GM strategy** *(GM to diagnose persistently febrile symptomatic patients only)*
Study period	July 2007 – June2011	June 2013 – November2013
Risk episodes, n (%)	262 (100%)	49 (100%)
Risk episodes with >=1 serum GM test, n (%)	262/262 (100%)	17/49 (35%)
GM tests performed		
- All tests in series	2972	31
- Per 6 month period	372	31
- Per risk episode	11 (3-30)	1 (1-5)
Episodes stopping prophylaxis for IV antifungal treatment, n, %	34/262, 13%	6/49, 12%
Incidence of breakthrough IA	5/262 (1.9%)	0/49

* Sánchez-Ortega, et al. P950 EBMT 2013 and AAA 2014

PH-P479

EBV- ASSOCIATED DISEASES POST ALLOGENEIC SCT AFTER REDUCTION OF ATG DOSAGE, SINGLE CENTER EXPERIENCE

H. Hallböök[1,], A. Kinch[2], J. Arvidson[3], K. Sällström[4], K. Pauksens[5]*
[1]Medical Sciences, Hematology, [2]Medical Sciences, Infection, [3]Department of Women's and Children's Health, Pediatrics, Uppsala University Hospital, [4]Medical Sciences, [5]Medical Sciences, Infection, University Hospital, Uppsala, Sweden

Introduction: Ebstein-Barr Virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) is a life-threatening complication after allogenic stem-cell transplantation (SCT). EBV monitoring was initiated in 2000 in our department as a standard procedure. In a previous study, we found a high frequency of EBV-associated diseases including PTLD, which resulted in reduction in the ATG dosage in conditioning. This study aimed to evaluate the current practice of screening for EBV in plasma with PCR among high risk patients the first 100 days post-SCT and early pre-emptiv treatment with rituximab to prevent PTLD after the reduction of ATG dosage.
Materials (or patients) and Methods: Patients who underwent allogeneic SCT with an unrelated donor, a mismatched related donor and patients who received a T-cell depleted graft were regarded as high risk for EBV-associated disease and were followed weekly with EBV-PCR in plasma for at least 100 days post SCT. All other patients were sampled at clinical suspicion of EBV-associated disease. All 222 patients who underwent allogeneic SCT between 2007 and 2012 were retrospectively evaluated regarding EBV-viral loads, clinical signs of EBV-disease and treatment with rituximab.
Results: EBV-reactivation (defined as > 1000 genome equivalents (gEq)/ml in at least one test) was found in 39/222 (18%) of all patients. All patients with EBV-viremia had received ATG- containing conditioning (39/149, 26%), 26/101 myeloablative conditioning and 13/48 reduced intensity conditioning regimens. No patient without ATG as part of the conditioning was diagnosed with EBV-viremia (0/73).
Of 39 patients with EBV-viremia, 26 patients received treatment with rituximab (median 3 doses, range 1-6). In 23 cases (88%) complete response was acquired. Of the 26 rituximab treated patients, 23 patients had clinical signs of EBV-associated disease as lymph node enlargement, fever, meningo-encephalitis or hemolysis, and in three cases biopsy proven PTLD were documented. Two patients with proven PTLD received additive treatment with chemotherapy, still one of them died. The 13 patients who did not receive treatment all resolved their EBV-viremia spontaneously.
Discussion: After the reduction of ATG dosage, the biopsy proven cases of PTLD have been less prevalent (in this study 3/149 cases of PTLD in the high risk group compared to 3/15 before reduction of ATG). However, the incidence of EBV-associated diseases remains high (26%) for patients who received ATG-containing conditioning. Weekly monitoring in the high-risk group of EBV-PCR and early pre-emptive therapy with rituximab seems to be feasible.
Disclosure of Interest: None Declared.

PH-P480

PRE-TRANSPLANT DONOR AND RECIPIENT INFLUENZA VACCINATION ENHANCES SERO-RESPONSE RATES IN THE FIRST SIX MONTHS AFTER HSCT

A. Ambati[1,], L. Testa[2], P. Ljungman[1], L. S. Vilas Boas[2], M. Aoun[3], M. Maeurer[3], C. Machado[2]*
[1]Medicine Huddinge, Karolinska Insitutet, Stockholm, Huddinge, Sweden, [2]Amaral Carvalho Foundation and Institute of Tropical Medicine -USP, Sao Paulo, Brazil, [3]Therapeutic Immunology, Karolinska Insitutet, Stockholm, Huddinge, Sweden

Introduction: The immune responses to pre-transplant vaccination of the donor or HSCT candidate was evaluated in a randomized study conducted at two transplant centers in Brazil from October 2007 to January 2010.
Materials (or patients) and Methods: 122 allogeneic transplant recipients and their related donors were included. The subjects were assigned to three randomization groups: No pre-transplant vaccination (group 1; *n*=38), donor pre-transplant vaccination (group 2; *n*=44) and recipient pre-transplant vaccination (group 3; *n*=40). On d. 180, all groups received one dose of trivalent influenza vaccine. Donor serum samples were taken at study admission (baseline) and around the day of donation. Recipient serum samples were taken at baseline and on days zero, d.30, d.60, d.100, d.180 and approximately one month after d.180 vaccination. Serum IgG was assessed by both Hemagglutinin inhibition (HI) and in a subgroup of 57 patients by indirect in-house influenza specific ELISA for H1, H3 and B antigens.
Results: The titers against all 3 antigens were the highest in the pre-transplant recipient vaccination group until d.180 after transplantation. There was a significant difference in the specific Ig levels against A/H1N1/California/2009 and A/Solomon/3/H1N1/2006 between the three study groups at 6 months after HSCT with the highest level seen in the pre-transplant recipient vaccination group (*P*=0.02). The mean IgG levels against pandemic H1N1, A/Solomon/H1N1/2006, A/Uruguay/H3N2/2008, were highest for the patient group vaccinated pre-transplant. After recipient vaccination at d.180, the group with vaccinated donors had significantly improved immune response measured as HI titers against H1N1 and H3N3. No effect was seen in HI titers against influenza B.
Discussion: We conclude that the recipient pre transplant vaccination improved the influenza specific sero-response rates while donor vaccination might improve the recall response. However, an earlier post-transplant vaccination might have yielded even stronger immune responses in the group with vaccinated donors.
Grant support (FAPESP 2008/00282-8).
Disclosure of Interest: None Declared.

PH-P481

EARLY AND AGGRESSIVE MANAGEMENT OF IFI IN ALLOTRANSPLANTED PATIENTS PRODUCES LOW MORBIDITY AND MORTALITY

V. Cancelli[1,], M. Malagola[1], A. Turra[1], C. Skert[1], C. Filì[1], C. Bergonzi[1], R. Ribolla[1], F. Cattina[1], D. Russo[1]*
[1]Chair of Hematology, Bone Marrow Transplant Unit, University of Brescia, AO Spedali Civili Brescia, Brescia, Italy

Introduction: Invasive fungal infections (IFIs) are one of the most important causes of morbidity and mortality in patients submitted to allogeneic stem cells transplantation (SCT). Their incidence is increasing and due to the use of more intensive conditioning regimens, Graft versus Host Disease (GVHD) prophylaxis, higher frequency of Matched Unrelated Donor (MUD), alternative hemopoietic stem cells sources and patients >55 years old. We here report on morbidity and mortality in allotransplanted patients who developed an IFI and who have been early and aggressive managed.
Materials (or patients) and Methods: This retrospective single center study includes 128 consecutive patients submitted to SCT from January 1, 2007 to December 31, 2012. 56/128 (44%) had an acute leukaemia, 25 (19%) had a lymphoma, 21 (16%) a multiple myeloma and the remaining had other onco-hematological disorders. The disease was advanced in 67/128 (52%) and patients conditioned with reduced intensity regimen (RIC) were 84 (66%). Thirteen patients (10%) had previous fungal infection before SCT. Antifungal prophylaxis was fluconazole in 41 patients (84%). Our approach was an aggressive monitoring of patients with febrile neutropenia, including microbiological (e.g. Aspergillus Antigen and culture on Broncho-Alveolar Lavage) and radiological (e.g. HR CT) examination.
Results: Forty-nine (38%) patients developed an IFI. Four IFIs (8%) were classified as proven, 3 (6%) as probable and 42 (86%) as possible. Proven IFIs were due to Aspergillus spp in 3 cases and Mucor in one patient. No Candida's IFI were documented. Forty-one (84%) IFIs were diagnosed within 6 months from SCT, indicating a clear relationship with conditioning regimens, neutropenia,

immunosoppression and acute GVHD. First line therapy of IFIs was amphotericin b liposomal in 20 patients (41%), voriconazole in 15 (31%) and caspofungin in 14 (28%). Only 6 patients (12%) died for progression of fungal infection, the fungal free survival and overall survival at 5 years was 88% and 55%, respectively.
Discussion: Between 2007 and 2012, the incidence of IFIs moved from 18% to 43% due to the increasing number of elderly patients, advanced status disease (44% in the 2007-2009 and 59% in 2011-2012) and the prevalence of unrelated donors' choice. The absence of Candida IFIs was probably due to fluconazole-based prophylaxis, that is used routinely in this setting. Overall, only 6 deaths related to fungal infection progression were observed. This low mortality rate could be achieved thanks to an aggressive monitoring of patients with febrile neutropenia, together with prompt initiation of antifungal therapy.
Acknowledgments: This work was supported in part by Lions Club Bassa Bresciana, Banca di Credito Cooperativo di Pompiano e Franciacorta.
Disclosure of Interest: None Declared.

PH-P482
BLOOD STREAM INFECTIONS: A MAJOR CAUSE OF EARLY MORTALITY IN HEMATOPOIETIC STEM CELL TRANSPLANT
P. Palomo[1,], M. Mateos[2], D. Martínez[1], A. J. González[1], V. Dueñas[1], J. Solano[1], A. P. González[1], S. González[1], C. Rayón[1], C. Vallejo[1]*
[1]Hematopoietic Transplant Unit, [2]Preventive Department, Hospital Universitario Central de Asturias, Oviedo, Spain

Introduction: Blood stream infections (BSI) are a well-known cause of morbidity and mortality in patients (pts) undergoing HSCT. BSI incidence varies from 22% to 55.8% in this population, and is particularly high during the pre-engraftment phase.
Materials (or patients) and Methods: The aim of this study was to determine prevalence, etiology, and antimicrobial sensitivity of BSI in 384 pts who underwent HSCT in our Hematopoietic Transplant Unit from January 2009 to October 2013. Two hundred and eighteen pts were male (56.8%) and 166 female (43.2%). Median age was 55.5 years old (range: 1–73). Baseline disease was: multiple myeloma (43.5%), lymphoproliferative disorder (31.3%), acute leukemia (16.9%), and others (8.3%). Two hundred and fifty three pts underwent autologous HSCT (65.9%), and the remainder (34.1%) allo-HSCT (55% from unrelated donor). Stem cell (SC) sources were peripheral blood (PBSC) (50.4%), bone marrow (BM) (40.5%), and umbilical cord blood (UCB) (9.2%). Thirty-four pts underwent a second HSCT (8.9%).
Results: There were 160 episodes of BSI in 121 pts (31.5%), and 187 pathogens were isolated. Although the majority of cases were monomicrobial (87.5%), there were 12.5% of polymicrobial BSI. Gram-positive bacteria (GPB) accounted for 62% of isolates, Gram-negative rods (GNR) for 37.4% and fungi for 0.6%. Coagulase negative *Staphylococci* and *Enterococci spp* accounted for 38.5% and 10.7% of GPB, respectively. *Escherichia coli* was the most common GNR isolated (18.7%), followed by *Pseudomonas aeruginosa* (8.5%) and *Klebsiella spp* (3.2%). Multi-drug resistant (MDR) represented 27.1% of all GNR. BSI was presented at day +8 as a median (range: -4 to +1154). Pts who underwent allo-HSCT were more likely to have BSI than auto-HSCT pts (P<0.0001). In the allo-HSCT setting, no significant differences in BSI incidence were found depending on the SC source employed: 27 out of 66 (40.9%) for PBSC, 28 out of 53 (52.8%) for BM, and 8 out of 12 (66.6%) for UCB. There were no significant differences between allo-HSCT from siblings and from unrelated donors. Conditioning regimens containing total body irradiation (TBI) were associated with 59.5% BSI (P<0.0001). Average hospitalization was higher in pts who developed BSI compared to pts who did not: 29 days (range: 9-88) *vs* 22 days (range: 9-107) (P<0.0001). Second HSCT was not associated with a higher incidence of BSI. Overall early mortality (within 30 days following the SC infusion) was 3.9% (15 out of 384 pts). BSI was responsible for 10 of those 15 deaths (2.6% of overall mortality). MDR BSI was significantly associated with early mortality (P<0.001). *Pseudomonas aeruginosa* was responsible for 26.6% of deaths caused by BSI.
Discussion: 1) Overall early mortality of HSCT was low (3.9%). 2) BSI was the major cause of early mortality in the HSCT setting (2.6%). 3) Increasing antibiotic resistance is a major health concern, and investigation on new antibiotics is warranted.
Disclosure of Interest: None Declared.

Gram -	N 70	% 37,4	MDR 19
E coli	35	18,7%	8
P. aeruginosa	16	8,5%	6
Klebsiella spp	6	3,2%	2
Enterobacter cloacae	4	2,1%	3
Others	9	4,8%	
Gram +	N 116	% 62,6	
Staphylococci spp (S. coag. -/S. aureus)	74 (72/2)	39,6%(38,5%/1,1%)	
Enterococci spp(E. faecalis/E. Faecium)	20 (10/10)	10,7%(5,3%/5,3%)	
Streptococci spp	15	8%	
Others	7	3,7%	

PH-P483
THE CLINICAL AND FINANCIAL BURDEN OF PREEMPTIVE MANAGEMENT OF CMV DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION
A. Barrett[1,], N. Jain[1], S. Ito[1], K. Lu[1], P. Muranski[1], R. Childs[1], C. Hourigan[1], J. Haggerty[1], C. Ramos[1], L. Cook[1], M. Battiwalla[1]*
[1]Hematology, National Heart Lung and Blood Institute, NIH, Bethesda, United States

Introduction: Although CMV infection only rarely leads to direct infectious mortality after stem cell transplantation, and early CMV reactivation may reduce relapse rates in patients with myeloid malignancies, the management of CMV by preemptive antivirals may itself induce toxicity and financial burden which need to be quantified to provide justification for antiviral cellular immunotherapy.
Materials (or patients) and Methods: In this study, we analyzed CMV reactivation in 134 patients undergoing allo-SCT at our institute between 2006 through 2012, comparing outcomes and cost after viral reactivation based upon the risk of CMV reactivation. 81 subjects received CD34+ selected myeloablative, 12 received UCBT, and 41 had T-replete nonmyeloablative transplant. 119 (88.8%) were at risk for CMV by virtue of either the donor or recipient being seropositive prior to transplant. Of these, 90 (75.6%) had CMV reactivation in the blood and all received antivirals.
Results: Patients reactivating CMV and requiring therapy had comparable survival to non-treated patients but CMV therapy incurred an additional cost of $55,000-71,000 per patient. However, in multivariable modeling of NRM, CMV reactivation >250 copies/ml (OR=3, P<0.048), total duration of inpatient IV antiviral therapy (OR = 1.04, P<0.001), and type of transplant (T-deplete vs. T-replete) (OR=4.65, P<0.017), were found to be significantly associated.
Discussion: Our findings suggest that prophylactic antiviral cellular therapy should be administered early post transplant, prior to day 14 to meaningfully impact reactivation and that cost savings exceeding $25,000 are possible with even moderate (50%) reduction in the rate of reactivation requiring antiviral therapy.
Disclosure of Interest: None Declared.

PH-P484
GENERATION OF CLINICAL-GRADE CMV-SPECIFIC T-CELLS: T-CELL DONOR SELECTION AND MANUFACTURING OF T CELLS
S. Tischer[1,2], C. Priesner[3], L. Arseniev[3], K. Aleksandrova[3], L. Goudeva[1], H.-G. Heuft[1], B. Maecker-Kolhoff[2,4], R. Blasczyk[1,2], U. Köhl[2,3], B. Eiz-Vesper[1,2,]*
[1]Institute for Transfusion Medicine, [2]Integrated Research and Treatment Center Transplantation (IFB-Tx), [3]Institute for Celltherapeutics, [4]Clinic for Paediatric Haematology and Oncology, Hannover Medical School, Hannover, Germany

Introduction: Adoptive transfer of allogeneic antiviral T cells is important to restore virusspecific immunity in patients after stem

cell (HSCT) or organ transplantation (SOT). Early or pre-emptive treatment interventions in high risk patients require both (i) improvement in manufacturing of virus-specific T cells without long-term *ex vivo* stimulation (e.g. by using IFN-γ cytokine capture system, CCS) maintaining antiviral CD4+ and CD8+ cells as well as (ii) quick recruitment of the respective T-cell donor including at least 3/6 HLA-matching.
Materials (or patients) and Methods: Frequency assessments of antiviral T cells in HLA-typed healthy as well as in HSCT donors were performed over 4 years at Hannover Medical School using so far 17 different overlapping peptide pools to detect CMV-, EBV-, ADV-, HHV6- and BK-specific T cells. Potential T-cell donors are first identified by IFN-g ELISPOT assay followed by a detailed phenotypical and functional analysis with multimer staining and cytokine secretion assay (CSA). Using CSA a donor was defined as eligible for donation of CMV-specific T cells (CMV-CTLs) if the number of $CD3^+IFN\text{-}g^+$ T cells was more than 0.03% of the $CD3^+$ cells after 4 hour incubation with the respective peptide pool. Additionally, the potential enrichment of antiviral T cells was tested to predict effective enrichment of T cells of interest (TOIs) and should result in a purity of >60% of $CD3^+IFN\text{-}g^+$ T cells. In order to obtain a manufacturing license according to the German Medicines Act (AMG), purification of clinical grade CMV-CTLs from 3 healthy CMV-seropositive donors (covering the broad spectrum from low to high CMV-CTL frequency) was performed aseptically under GMP conditions using the CCS and the CMVpp65 overlapping peptide pool.
Results: Frequency assessments of antiviral T cells in >400 HLA-typed healthy donors as well as in HSCT donors were performed over a period of 4 years at Hannover Medical School. Further quality control of three validation runs for CMV-CTLs starting with 0.05-1.7% IFN-g secreting $CD3^+$ T cells resulted in 19.2%>81.2% of $CD3^+IFN\text{-}g^+$ T cells at the end of the manufacturing process. We were able to enrich a total of $0.3\text{-}1.8x10^6$ $CD3^+CD56^-CD45^+$ T cells. The total amount of $CD3^+IFN\text{-}g^+$ T cells in the final product ranged from $0.54–14.2x10^5$ cells with a purity between 19.2%>81.2% of $CD3^+IFN\text{-}g^+$ T cells. Among $CD3^+$ T cells we found 11.5-68.0% $CD8^+IFN\text{-}g^+$ and 4.9-53.2% $CD4^+IFN\text{-}g^+$ cells, respectively. Despite this low purity of IFN-g secreting $CD3^+$ T cells the amount of contaminating $IFN\text{-}g^-$ T cells was acceptably low ($2.3\text{-}6.7x10^5$ T cells). In all preparations we found contaminating B cells (4-5%), granulocytes (5-27%), monocytes (16-30%), and NK cells (12-21%).
Discussion: The manufacturing of antiviral T cells in the clinical scale using the CCS was validated at Hannover Medical School. To allow for a timely donor evaluation and also for a future registry the acceptance criterion for donor eligibility was a 3/6 HLA-match or better. So far the results obtained in the donors' pre-testing indicate, that a starting frequency of ≥0.03% might be sufficient for a successful purification of TOIs. Unfortunately the results obtained in pre-testing are not predictive for the efficiency of antiviral T-cell isolation by CCS: It is likely, that a high proportion of antiviral CD4+ T cells negatively influence the purity and therefore the efficacy of the antiviral cellular preparation.
Disclosure of Interest: None Declared.

PH-P485

EPIDEMIOLOGY AND MANAGEMENT OF CARBAPENEMASE–PRODUCING KLEBSIELLA PNEUMONIA INFECTIONS IN ALLOGENIC HSCT RECIPIENTS: A SINGLE CENTER EXPERIENCE

M. Caira[1,], S. Giammarco[1], P. Chiusolo[1], L. Laurenti[1], F. Sorà[1], T. Spanu[2], M. Tumbarello[3], L. Pagano[1], S. Sica[1]*
[1]Hematology, [2]Microbiology, [3]Infectious Diseases, Catholic University of Sacred Heart, Rome, Italy

Introduction: During the last few years infections due to carbapenemase–producing *Klebsiella pneumonia* (KPc) have dramatically increased, particularly in immunocompromised patients (pts), and they now represent a critical challenge. Aim of the present study was to describe the current epidemiology of KPc infections in haematological pts undergoing allogenic HSCT (alloHSCT) and to analyze their impact on patients' outcome.
Materials (or patients) and Methods: From January 1st, 2010 to July 31th, 2013, all consecutive pts undergoing alloHSCT were prospectively monitoring to collect and analyze cases of KPc colonization and/or infection. As per local guidelines, systematic surveillance included peri-rectal swab at admission and weekly

[PH-P485]

	N° infections 16	N° deaths* ALL CAUSES	N° deaths* DUE TO/WITH KPc	N° deaths* DUE to KPc
Years, N° (incidence%)				
- 2010	4 (17%)	3 (75%)	2 (50%)	1 (25%)
-2011	1 (4.5%)	1 (100%)	1 (100%)	0
-2012	8 (30 %)	5 (62%)	3 (37.5%)	1 (12.5%)
-2013**	3 (16%)	2 (67%)	2 (67%)	0
Type of donor				
- MUD	8	5 (62.5%)	4 (50%)	1 (12.5%)
- Sibling	8	6 (75%)	4 (50%)	1 (12.5%)
- cord blood	0	0	0	0
Conditioning				
- myeloablative	3	1 (33%)	1 (33%)	1 (33%)
- reduced intensity	7	7 (100%)	2 (28%)	1 (14%)
- non myeloablative	6	3 (50%)	5 (83%)	0
Time from allo-HSCT				
- early	8	4 (50%)	3 (37.5%)	0
- late	4	4 (100%)	3 (75%)	1 (25%)
- Very late	4	3 (75%)	2 (50%)	1 (25%)
Site of infection				
-Blood	10	9 (90%)	6 (60%)	2 (20%)
-Urinary tract	2	0	0	0
-lung	4	2 (50%)	2 (50%)	0

[illegible] month from infection diagnosis
[illegible] last to July, 31st**

thereafter on all pts admitted to the division of Hematology. Infection control strategy also included contact precautions, pts cohorting, chlorhexidine bathing and empirically oriented antibiotics on the basis of the results of susceptibility antibiotics testing for febrile severely neutropenic pts colonized with KPc. Principal demographic and clinical data, as well as antibiotic treatments were collected. To evaluate outcome, 3-months KPC-attributable mortality and overall mortality were evaluated.
Results: During the 43-months period, 91 alloHSCT procedures were performed in 88 pts (47 MUD, 41 sibling, 3 cord blood). KPc colonization/infection prior to HSCT (at least six months prior to the procedure) had occurred in 14 cases (15.4%). Sixteen pts developed microbiologically documented KPc infection during different phases after transplantation (incidence 17.6%). Infections included 10 sepsis, 2 urinary tract infections and 2 pneumonia. Eight cases occurred within 100th day from transplant and were defined as "early" infections. Remaining cases were classified as "late" (4 cases, occurred <6th month) and "very late" (4 cases, >6th month). All colonized pts received KPc-oriented empiric antibiotics in case of fever. Eleven deaths were registered among cases (11/16, 69%). Of them, 2 deaths were considered *due to* KPc only (2/16, 12.5%), while 6 pts died *with* KPc infection. Remaining 3 deaths were due to other causes (2 GVHD, 1 cerebral hemorrhage). Notably the majority of KPc-related deaths occurred late. Main demographic and clinical data are reported in the table.
Discussion: The management of KPc positive pts undergoing alloHSCT is a crucial issue. In our experience mortality due to KPc was lower than expected, particularly in the early phase post HSCT. Systematic pts surveillance and prompt combined antibiotic therapy may be a possible explanation. Notably, KPc infections may also occur during the later phases, with a worse outcome. Prevention strategies at this time should be implemented, particularly in complicated pts.
Disclosure of Interest: None Declared.

PH-P486
ACTIVE SURVEILLANCE AND PROMPT ANTIMICROBIAL THERAPY ALLOW REDUCING INFECTIOUS MORTALITY DUE TO CARBAPENEMASE-PRODUCING KLEBSIELLA SSP IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

A. Forcina[1,], P. Cichero[2], E. Xue[1], R. Greco[1], F. Lorentino[1], L. Crucitti[1], A. Orsini[1], T. Perini[1], M. T. Lupo Stanghellini[1], S. Marktel[1], A. Assanelli[1], F. Giglio[1], M. Morelli[1], M. Marcatti[1], M. Bernardi[1], J. Peccatori[1], F. Ciceri[1], C. Corti[1]*
[1]Hematology and Bone Marrow Transplantation Unit, [2]Microbiology and Virology Unit, San Raffaele Scientific Institute, Milan, Italy

Introduction: Carbapenemase-producing *Klebsiella ssp* (CPKs) are considered emerging killers in profoundly immunocompromised patients, especially in those undergoing hematopoietic stem cell transplantation (HSCT). From literature data, CPK-related infectious mortality is estimated to be approximately 50%, one of the highest due to multi-drug resistant (MDR) pathogens. Such a gloomy picture is caused by CPK resistance to multiple antimicrobial classes, including all available β-lactams, fluoroquinolones and aminoglycosides.
Materials (or patients) and Methods: Following a CPK outbreak in our Center, from July 2011 to November 2013, we surveyed the epidemiology of Gram-negative MDR pathogens through active surveillance with rectal swabs in a series of patients suffering from hematological malignancies. To unveil asymptomatic carriers at risk of subsequent clinical disease, we performed a total of 1783 rectal swabs in 267 consecutive patients.
Results: Eighty-nine patients out of 267 were found colonized with Gram-negative MDR pathogens (64 after allogeneic and 12 after autologous HSCT, and 13 after chemotherapy only). Among these 89 patients, 27 harbored CPKs, 23 carbapenemase-producing *Pseudomonas aeruginosa*, 25 ESBL-producing *Escherichia coli*, 10 *Stenotrophomonas maltophilia*, 8 carbapenemase-producing *Enterobacter spp* and 13 other pathogens. Some heavily pretreated patients were positive for two or more pathogens. During a 3-year observation period, we documented 12 CPK-related sepsis (1 before and 10 after allogeneic HSCT, and 1 after autologous HSCT). Seven patients subsequently developed septic shock and 4 of them died of multi-organ failure, resulting in an overall CPK-related mortality of 33%. The risk of CPK sepsis did not correlate with age, disease type and stage at transplant or Sorror's comorbidity index. Seventy-five percent of isolated CPKs showed sensibility to colistin and gentamicin. To reduce mortality due to Gram-negative MDR pathogens, from June 2012 we modified our internal guidelines by early initiating a triple antimicrobial therapy with colistin, tigeciclin and meropenem in all febrile patients with hemodinamic instability. All CPK-colonized patients were additionally put into reverse isolation. Following these preventive measures, in 2013 CPK sepsis was documented only in two patients, both surviving without septic shock.
Discussion: In conclusion, the availability of rectal swabs, the prompt initiation of appropriate combined antimicrobial therapy in patients carrying Gram-negative MDR pathogens and the timely reverse isolation of all CPK-positive cases, may reduce infectious mortality and improve the overall outcome after HSCT.
Disclosure of Interest: None Declared.

PH-P487
HUMAN HERPES VIRUS 6 AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

R. Greco[1,], F. Lorentino[1], L. Crucitti[1], L. Vago[2], M. T. Lupo Stanghellini[1], A. Forcina[1], F. Giglio[1], M. Morelli[1], G. Levati[1], A. Orsini[1], M. R. Carbone[3], V. Valtolina[3], M. Noviello[3], S. Marktel[1], A. Assanelli[1], M. Carrabba[1], M. Marcatti[1], J. Peccatori[1], M. Bernardi[1], C. Bonini[3], C. Corti[1], F. Ciceri[1]*
[1]Hematology and Bone Marrow Transplantation Unit, [2]Hematology and Bone Marrow Transplantation Unit, Unit of Molecular and Functional Immunogenetics, [3]Experimental Hematology Unit, San Raffaele Scientific Institute, Milano, Italy

Introduction: Human herpesvirus type 6 (HHV-6) is increasingly recognized as an opportunistic and potentially life-threatening pathogen in recipients of allogeneic hematopoietic stem cell transplantation (AlloSCT). Reported clinical manifestations of HHV-6 infection in transplanted patients are skin rash, interstitial pneumonia, bone marrow suppression and encephalitis. Moreover, some clinical reports suggest that HHV-6 can facilitate the occurrence of severe clinical complications of alloSCT, increasing transplant-related mortality.
Materials (or patients) and Methods: From January 2009 to February 2013, we retrospectively evaluated 54 consecutive adult patients (median age 50 years) who developed positivity to HHV-6 after alloSCT for high-risk hematological malignancies. Stem cell donors were family haploidentical (37), HLA identical sibling (8), unrelated volunteer (6), cord blood (3). The viral load was determined by quantitative PCR (Nanogen Advanced Diagnostic S.r.L) in cell-free body fluids such as plasma, bronchoalveolar lavage (BAL), cerebrospinal fluid (CSF), bone marrow (BM) aspirates or in gastrointestinal biopsies.
Results: Median time from alloSCT to HHV-6 reactivation was 34 days (range: 0–705). Thirty-one patients presented HHV-6 positive in plasma, 9/54 in BM, 33/54 in gut biopsies or BAL, 7/54 in CSF. At the time of viral positivity all pts were receiving acyclovir as viral prophylaxis except five. Twenty-nine patients had acute graft versus host disease (GvHD). Twenty-two out of these twenty-nine patients experienced a grade III-IV acute GvHD, requiring high dose steroids in twenty-six cases. A concomitant CMV positivity was detected in 15/54 patients. The median absolute count of CD3+ lymphocytes was 262 cells/mcl. In 52/54 cases we reported HHV-6 clinical manifestations: fever (43), skin rash (22), hepatitis (19), diarrhoea (24), encephalitis (10), BM suppression (18), delayed engraftment (11). HHV-6 positivity led to antiviral pharmacological treatment in 37/54 cases, using as first choice therapy foscarnet. Amongst the total fifty-four patients with documented HHV-6 positivity thirty-one solved the clinical event. However the mortality rate was relatively high in this population (only 30% of patients were alive), mainly related to severe infections or GvHD. A better overall survival (OS) is significantly associated with CD3+ cells higher than 200/mcl (*P*-value 0.011) and time after allo-

SCT more than 2 months (*P*-value 0.035). The overall survival is not significantly modified when patients with a better immune reconstitution (defined as CD3+ cells higher than 200/mcl) were separated in groups according to the time after transplant. In this analysis the overall survival was not significantly influenced by steroids administration, presence of acute GvHD, plasma viral load and organ involvement.
Discussion: This retrospective study confirms a correlation of HHV-6 with high morbidity and mortality rates after alloSCT, underlying the importance of a regular HHV-6 monitoring in allo-SCT recipients. Despite HHV-6 detection typically occurred in the first month after alloSCT, a better immune reconstitution has the potential to improve clinical outcome.
Disclosure of Interest: None Declared.

PH-P488
FIDAXOMICIN TREATMENT FOR CLOSTRIDIUM DIFFICILE IN ALLOGENEIC TRANSPLANT RECIPIENTS
T. Salmi[1,], J. Oksi[1], U. Salmenniemi[1], M. Putkonen[1], M. Kauppila[1], M. Oksman[1], K. Remes[1], M. Itälä-Remes[1]*
[1]Turku University Hospital, Turku, Finland

Introduction: Clostridium difficile – associated disease (CDAD) in allo-SCT recipients are more likely to develop septic infections, and increase risk of graft-versus-host disease and nonrelapse mortality. Fidaxomicin has narrow spectrum antimicrobial activity and has recently been available for treatment of CDAD. It appears to have limited activity against normal bacterial flora of the gut. Vancomycin treatment has a higher risk to promote colonisation of VRE and overgrowth of Candida species than fidaxomicin. Aim of this study was to analyse the outcome of CDAD treatment with fidaxomicin and compare it to vancomycin in allotransplanted patients.
Materials (or patients) and Methods: Retrospective collection of data on patients treated at Turku University hospital between May 2011 and July 2013, with a follow-up of at least 120 days. The patients treated for C. difficile with fidaxomicin were compared to the patients treated with vancomycin.
Results: Among 93 recipients of allo-SCT patients 23 patients were identified. Twelve patients received 14 treatment with fidaxomicin and eleven patients received 12 treatment with vancomycin. All analysed cases had a positive C. difficile culture and carried toxin-producing genes, identified by PCR assay. None of them harboured a hypervirulent strain of C. difficile.
For fidaxomicin treatment, a relapsed CDAD was observed in 2 (13%) patients and for vancomycin treatment in 2 (17%) patients (*P*=ns). Grade II-IV acute GVHD was observed in total of 16 patients (70%). aGVHD coincided with CDAD in 8 patients (4 patients in both groups). aGVHD did not develop after CDAD in 5 patients (62.5%) and in 2 patients (33.3%). In fidaxomicin group 3 patients (37.5%) and in vancomycin group 4 patients (66.7%) developed aGVHD after CDAD. aGVHD of GI tract all grade III-IV affected 2 out of 3 and 3 out of 4, respectively. No significant difference was observed for age, sex, levels of neutrophils, creatinine or albumin, exposure of broad-spectrum antibiotics before or during CDAD treatment, or the use of corticosteroids. VRE surveillance cultures were in all patients negative.
Discussion: In this study population fidaxomicin appeared as effective as vancomicin for treatment of CDAD. We observed a lower rate of aGVHD in patients treated with fidaxomicin compared to those treated with vacomycin. Therefore, it may be beneficial to use fidaxomicin in treating CDAD in allogeneic transplant patient population. The number of patients is, however, small and larger studies are warranted.
Disclosure of Interest: None Declared.

PH-P489
RAPID MOLECULAR DETECTION OF PATHOGENS IN 1941 BLOOD SAMPLES FROM 516 CONSECUTIVE PATIENTS WITH FEBRILE NEUTROPENIA
R. Greco[1,], N. Mancini[2], F. Lorentino[1], L. Crucitti[1], M. C. Barbanti[1], A. Forcina[1], A. Orsini[1], L. Vago[3], F. Giglio[1], M. Morelli[1], G. Levati[1], E. Sala[1], D. Clerici[1], M. T. Lupo Stanghellini[1], M. Carrabba[1], S. Marktel[1], A. Assanelli[1], M. Marcatti[1], M. Bernardi[1], J. Peccatori[1], C. Corti[1], M. Clementi[2], F. Ciceri[1]*
[1]Hematology and Bone Marrow Transplantation Unit, [2]Laboratory of Microbiology and Virology, Vita-Salute San Raffaele University, [3]Hematology and Bone Marrow Transplantation Unit, Unit of Molecular and Functional Immunogenetics, San Raffaele Scientific Institute, Milano, Italy

Introduction: Febrile neutropenia and sepsis are frequent and life-threatening complications in patients with haematological malignancies. Although the proportion of infectious deaths in haematological patients has decreased over the last two decades, much remains to be done to further reduce these events. Blood cultures (BC) identify a pathogen in only 20 to 30% of febrile episodes, the culturing and pathogen identification process is lengthy, postponing the start of a pathogen-targeted treatment. Thereby, a sensitive tool to promptly recognize pathogens causing sepsis is of high clinical relevance.
Materials (or patients) and Methods: We assessed the diagnostic usefulness of the LightCycler Septi*Fast* test (SF; Roche Molecular Systems), a PCR-based multiplex assay performed on peripheral blood and capable of detecting 25 among the most common species isolated in sepsis. In this study, blood samples from febrile haematological patients were concomitantly tested by traditional blood culture (BacT/Alert 3D; bioMerieux).
Results: A total of 1941 blood samples were collected from 516 consecutive patients treated for febrile neutropenia at the San Raffaele Haematology and Bone Marrow Transplantation Unit, from 2009 to 2013. Out of the total 1941 episodes examined, positive results were detected in 537 samples by SF (28%), and in 263 by BC (14%). Together, the two methods identified a total of 586 microorganisms in 509 (26%) episodes: Gram-positive bacterial species (80%), Gram-negative bacterial species (17%), and fungal species (3%). Concordance between the two methods was 77%, with most of the discordant samples that tested negative by culture but positive using the molecular approach (50% of the total positive samples). The cases positive by SF alone were mostly samples from patients already receiving antimicrobial therapy, or, importantly, sample positive for fungal pathogens such as *Aspergillus fumigatus*, which is hard to detect by the traditional approaches.
Discussion: This analysis demonstrates a significant correlation between the molecular test and the standard BC in haematological patients with febrile neutropenia. A molecular test such as SF, in combination with traditional assays, can play an important role in the diagnosis of sepsis, particularly in persistent fever despite antibacterial therapy, when a non-responding bacterial infection or an invasive fungal infection is suspected, therefore leading to a rapid diagnosis and an earlier targeted antimicrobial therapy.
Disclosure of Interest: None Declared.

PH-P490
EBV-REACTIVATION AFTER ALLOGENEIC HSCT – A SINGLE CENTER SCREENING-EXPERIENCE
M. Wachsmuth-Melm[1,], A. Naomi Lay[1], M. Theobald[1], R. Georg Meyer[1], E. Maria Wagner[1]*
[1]Haematology, Oncology and Pneumology, University Medical Center, Mainz, Germany

Introduction: EBV-reactivation and post-transplant lymphoproliferative disorder (PTLD) are severe complications after allogeneic hematopoietic stem cell transplantation (HSCT) with an incidence of up to 30%. Screening-procedures for all patients or patients at risk, as well as preemptive vs. prophylactic treatment are under

discussion. Here, we provide clinical data of EBV-screening for all patients after allogeneic HSCT in our outpatient clinic.
Materials (or patients) and Methods: All patients after allogeneic HSCT were seen at least once a year in the outpatient unit. Within a screening period of 2 years (2011/12), 532 patients were screened for EBV-DNA using real-time at every single visit, regardless of time after transplantation or risk factors. Of these, 145 were assessed during their first year after HSCT. If EBV-DNA-levels were positive but $<10^3$cp/ml, the analysis was repeated and the patients only clinically assessed for signs of lymphoproliferation. For patients with $>10^3$cp/ml or with clinical symptoms (fever, lymphadenopathy, other inflammatory symptoms), CT-scans and FACS-analyses for monoclonal B-cells were implemented. Treatment was initialized if patients developed clinical PTLD, monoclonal B-cells were confirmed, or the viral load (vl) further increased. Rituximab alone was used in most patients; In fast growing lymphoma, Rituximab was combined with CHOP.
Results: Of 102 (19.1%) patients with positive EBV-PCR, 11 had clinical symptoms (10.7% of EBV-reactivated, 2.0% of all). PTLD was present in 5 patients (4.9% of EBV-reactivated). All patients resolved their EBV-disease either after treatment (*n*=9) or reduction of immunosuppression (*n*=2). EBV-reactivation occurred any time after HSCT but patients without immunosuppression never developed significant increase in their vl ($>10^3$cp/ml) or EBV-related symptoms. PTLD or EBV-related symptoms were seen in patients with high vl ($>10^4$cp/ml). Clinically relevant EBV-reactivation occurred only in the first 2 years after HSCT. Other potential risk factors are currently under investigation: in first analysis T-cell-depletion and ongoing immunosuppressive medication are independent risk factors, whereas GVHD without systemic treatment or donor type did not significantly increase the risk of clinical relevant EBV reactivation in our patient cohort.
Discussion: EBV-reactivation is common after allogeneic HSCT at any time. A real-time PCR-based screening for all patients is feasible. There is no evidence for clinical relevance of positive EBV-vl in patients without active GVHD or immunosuppressive medication later than 2 years after HSCT in our cohort. We recommend the establishment of EBV-PCR-screening for all patients after allogeneic HSCT for the first 2 years and for patients at higher risk.
Disclosure of Interest: None Declared.

PH-P491

ADENOVIRUS TYPE A31 AS A CAUSE OF AN UNEXPECTED PERSISTENT INCREASED INCIDENCE OF ADENOVIRAEMIA ON A STEM CELL TRANSPLANTATION UNIT

P. Sivaprakasam[1,], B. Torres[1], L. Alonso[1], J. Moppett[1], M. Cummins[1], J. Cornish[1], P. Muir[2], C. Steward[1]*
[1]Paediatric Stem Cell Transplantation, [2]Department of Virology, Bristol Royal Hospital for Children, Bristol, United Kingdom

Introduction: A retrospective analysis of incidence of adenoviraemia (AdVM) was performed between January 2007 and December 2013 on all paediatric allogenic haematopoietic stem cell transplant (HSCT) patients admitted to the Bone Marrow Transplant Unit at Bristol Royal Hospital for Children. A high frequency of adenoviral infection had been demonstrated in a 2002-5 study, where 36% of patients had infection at one or more body sites and dissemination occurred in up to 53% of these patients. Despite more stringent infection control measures introduced following this, a significant increase in the frequency of AdVM occurred during 2012-13, leading to this investigation.
Materials (or patients) and Methods: For many years (1) patients with viral reactivation have been isolated and barrier nursed to prevent cross infection and (2) regular surveillance of adenovirus in blood by polymerase chain reaction (PCR) has been performed twice weekly for all inpatients (and at least weekly until six months post-transplant following discharge). In 2006 the chlorinated detergent, Actichlor, was introduced for double cleaning of individual cubicles due to its adenovirocidal properties and in order to minimise any potential for nosocomial spread. In this analysis, patient details and viral PCR titres were obtained retrospectively from our electronic database.Patients were classified as having AdVM if they had >2 positive blood PCR with >95 copies/ml. Patients with isolated adenoviral infection in other organs without AdVM were excluded from analysis.
Results: A total of 205 HSCT were performed on 201 paediatric patients during this study period. 31 patients (15%) developed AdVM. Of these, 13 (50%) occurred as a cluster between July 2012 and October 2013 and required prolonged admissions (median 98 days, range 40-175). 5 patients died (2-disseminated AdVM; 1-primary graft failure; 1-relapse; 1-RSV pneumonitis); 3 had post-transplant complications which adenoviral infection may have contributed to (cholangiohepatitis, severe gut graft versus host disease and secondary graft rejection respectively). Strain identification was attempted by PCR and sequencing analysis on 8 consecutive paediatric patients. This was successful in 6 and revealed A31 (three patients), A2 (2pts) and A1 (1pt) respectively. Two of the patients with A31 AdVM rejected their grafts and a third developed idiopathic leukoencephalopathy although adenovirus was not detected in CSF. Extended serotyping is awaited on the remaining patients. Interestingly, serotyping revealed that 3 adult Bristol HSCT patients developed A31 related AdVM during the same period. Simultaneous testing of community adenoviral infections showed A31 to be the cause of 1/7 patients investigated.
Discussion: From both published and unpublished findings, we are aware of five other units that have experienced outbreaks of adenoviral infection well above baseline frequency with A31 serotype contributing a significant proportion (*Leruez-Ville et al, Legrand F et al*). These observations suggest that type A31 adenovirus has a unique epidemiology and should always be considered when a sudden surge of AdVM develops in an otherwise well-controlled environment. It appears that A31 adenovirus is an increasing problem for transplant units and better understanding of this virus is urgently required in order to identify and restrict its transmission most effectively.
Disclosure of Interest: None Declared.

PH-P492

ASSESSMENT OF BACTERIAL GUT COLONIZATION PRIOR TO ALLOGENEIC STEM CELL TRANSPLANTATION AS A TOOL FOR PREDICTION OF SYSTEMIC INFECTIONS DURING EARLY POSTHSCT PERIOD

J. Bilinski[1], P. Rusicka[1], H. Marchel[2], M. Tormanowska[1], K. Halaburda[3], P. Boguradzki[3], M. Paluszewska[3], D. Bieńko[2], E. Swoboda-Kopeć[2], W. Wiktor-Jedrzejczak[3], G. Wladyslaw Basak[3,]*
[1]Hematology, Oncology and Internal Diseases, Central Public Clinical Hospital, [2]Medical Microbiology, [3]Hematology, Oncology and Internal Diseases, Medical University of Warsaw, Warsaw, Poland

Introduction: Patients after alloSCT are at risk of developing systemic infection with multidrug resistant (MDR) pathogens colonizing GI tract. We hypothesized that evaluation of gut colonization status prior alloSCT might allow to predict the etiology of bacteremia and guide antibiotic treatment.
Materials (or patients) and Methods: A routine stool culture of MDR bacteria (ESBL+, KPC+, VRE or MBL+) was carried out in our transplant center since 2010. At each febrile episode blood culture has been performed. This has allowed analyzing the correlation between gut colonization before alloSCT and the incidence of positive blood cultures within 30 days post alloSCT.
Results: The data were available for 112 patients. AlloSCT was performed following MAC (60%) or RIC (40%) conditioning. 35 patients (31%) were colonized with at least one MDR pathogen: *Enterococcus faecium* VRE (EfVRE, *N*=22, 63%); *E. coli* ESBL+ (EcESBL, *N*=9, 26%); *Klebsiella pneumoniae* ESBL+ (KpESBL, *N*=9, 26%); *Pseudomonas aeruginosa* MBL+ (PaMBL, *N*=4, 11%); *Enterobacter cloacae* ESBL+ (EclESBL, *N*=3, 9%); *Enterobacter cloacae* KPC+ (EclKPC, *N*=2, 6%); *Klebsiella oxytoca* ESBL+ (KoESBL, *N*=1, 3%); *Enterococcus faecalis* VRE (EfsVRE, *N*=1, 3%). Within the first 30 days postSCT, blood cultures have been performed for 81 patients (72%): more frequently from colonized (*N*=29, 83%) than from not colonized (*N*=52, 67%) (*P*=0.93). The median number of blood collections from a single patient was 2 (1-16). At least one positive blood culture was observed in 31patients (28%) including

14 colonized (40%) and 17 not colonized (22%, *P*=0.049). The frequency of blood pathogens was as follows: Coagulase-negative *Staphylococci* (*N*=19, 23,5%); KpESBL (*N*=7, 9%); EcESBL (*N*=4, 5%); EfVRE (*N*=4, 5%); EfsVRE (*N*=4, 5%); EclESBL ESBL+ (*N*=2, 2,5%); PaMBL (*N*=1, 1%); *Acinetobacter junii* (*N*=1, 1%); *Ochrobactrum spp*. (*N*=1, 1%); *Pseudomonas aeruginosa* ESBL(-) (*N*=1, 1%); KoESBL+ (*N*=1, 1%); *Klebsiella pneumoniae* KPC+ (*N*=1, 1%). Among patients with positive blood cultures, the comparable proportion developed bacteremia with MDR organism (57% colonized vs. 47% not colonized, NS). None of the patients colonized with EcESBL, PaMBL, EclESBL, EfsVRE or KoESBL developed positive blood culture with the corresponding pathogen. Three patients (33%) colonized with KpESBL and 2 patients (9%) colonized with EfVRE developed systemic infection with corresponding bacteria. In contrast, among patients not colonized with these pathogens, 4 (4%) had positive blood cultures with EcESBL, 2 (2%) with EfVRE, 1 (1%) with PaMBL; 2 (2%) with EclESBL, 4 (4%) with EfsVRE, and 1 (1%) with KoESBL+ (NS). Significantly lower proportion of patients not colonized with KpESBL developed bacteremia of this origin compared to patients colonized with this bacterium (4 vs. 33%, *P*<0.001). Overall, only 5 colonized patients (14%) experienced systemic infection with at least one pathogen with which they have been colonized.
Discussion: Evaluation of gut colonization before alloHSCT does not allow predicting the etiology of bacteremia, however, the culture results might have been influenced by the subsequent treatment with empirical antibiotics. Based on our results it seems that gut colonization with *Klebsiella pneumoniae* ESBL+ poses a significant risk of systemic infection with this microbe, however this requires confirmation in larger cohort of patients.
Disclosure of Interest: None Declared.

PH-P493
PROSPECTIVE STUDY OF ANTI-CMV SPECIFIC IMMUNE RECONSTITUTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION WITH THE CMV QUANTIFERON METHOD. PRELIMINARY RESULTS

I. Grispou[1,], S. Gigantes[2], I. Baltadakis[2], E. Xenou[2], A. Xirokosta[2], P. Vlachos[2], I. Apostolidis[2], D. Karakasis[2], K. Foundoulis[1], N. Harhalakis[2]*
[1]Microbiology Laboratory, [2]BMT Unit, Evangelismos Hospital, Athens, Greece

Introduction: Long-term management of CMV infection after allogeneic stem cell transplantation (SCT) is based on the reconstitution of the specific T-cell immunity against the virus. In the clinical setting there are no well-established methods for the measurement of the CMV-specific immunity. Recently, a standardized quantitated assay was developed, which measures responses to CMV peptide antigens targeting mainly CD8+ cells HLA class I haplotypes, thus covering >98% of human population (Quantiferon-CMV, Cellestis). The aim of the study is to evaluate the levels and time of CMV-specific immunity reconstitution after SCT, in order to establish criteria for the management of CMV infection.
Materials (or patients) and Methods: 26 adults (age 18-61) who underwent SCT from identical sibling (*n*=6), haploidentical sibling (*n*=1), unrelated donor (*n*=13) and dual cord blood (*n*=6) were enrolled in the study. Each recipient was examined before transplant and then monthly for the first trimester post transplant. All patients were monitored for CMV replication with RealTime PCR once or twice per week. The pre-transplant CMV serostatus was as follows: D+/R+ (*n*=8), D+/R- (*n*=3), D-/R+ (*n*=11) and D-/R- (*n*=4). The latter group was excluded from the study.
Results: All seropositive recipients tested positive prior to transplantation with the Quantiferon CMV, with a median value of >10 IU/mL. Nine recipients with related (*n*=4) or unrelated donor (*n*=5) reconstituted their cell-mediated immunity against CMV early in the post transplant period (<3 months) and never experienced CMV infection. In some cases, low transient viral loads were detected that did not demand specific antiviral treatment. On the contrary, 8 out of 19 seropositive recipients or recipients with seropositive donor who did not reach levels of CMV Quantiferon >1 IU/mL during the follow-up period had multiple episodes of CMV reactivation with high viral loads, and even CMV-disease in one patient. Among the cord blood recipients, none reconstituted CMV-specific immunity during follow-up period, but one who developed limited immunity (0.67 IU/mL) after a year.
Discussion: Evaluation of the level of CMV-specific immunity with the Quantiferon-CMV method seems to offer the ability to discern patients at high risk for developing CMV disease after SCT, as well as those who are capable to manage CMV reactivation on their own without specific antiviral treatment. Additionally, the use of the assay in everyday practice may contribute to the decision making for the follow-up schedule of the patients, thus probably resulting in more cost-effective strategies.
Disclosure of Interest: None Declared.

PH-P494
EFFICACY AND SAFETY OF CMV-SPECIFIC T CELL LINE (CMV-TCL) FOR CMV REACTIVATIONS IN HIGH RISK ADULT PATIENTS (PTS) AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

S. Bramanti[1,], P. Comoli[2], A. Vai[1], R. Crocchiolo[1], B. Sarina[1], S. Basso[2], R. Mineri[1], A. Santoro[1], L. Castagna[1]*
[1]Humanitas Cancer Center, Rozzano, [2]Policlinico S Matteo, Pavia, Italy

Introduction: CMV reactivation due to delayed specific immunological reconstitution causes significant morbidity and mortality after HSCT. The use of adoptive CMV-specific T cell line (CMV-TCL) therapy is able to control CMV reactivation. The inclusion of adoptive therapy into routine clinical care requires efficient methods for TCL generation. We investigated the efficacy of CMV-TCL for the treatment of refractory high risk pts.
Materials (or patients) and Methods: We performed a single center retrospective analysis of consecutive pts treated with allogeneic HSCT between February 2010 and October 2013. CMV reactivation was monitored using biweekly quantitative blood PCR starting from day +15 until day +100 and then weekly until day +180. A preemptive therapy with gancyclovir or foscarnet was started for number of ≥ 3,000 copies/mL. Pts receiving a haplo or cord blood unit (CBU) HSCT with at least 1 CMV reactivation, or any HSCT recipient experiencing at least 2 CMV reactivations, were considered at high risk. CMV-TCL were produced from the HSCT or from third-party family donors, according to a GMP protocol, by stimulation with a CMV peptide pool and culture for 24 days. Pts were treated on a compassionate use basis and an informed consent was obtained.
Results: 118 allogeneic transplantations were performed: 47 from HLA-identical donor, 65 from haploidentical donor and 6 from CBUs. 76 pts were considered at high risk for CMV reactivation (71 because of donor type and 5 because of more than 2 reactivations). 35 out of 71 (49%) pts did not reactivate CMV; 23 pts (30%) had one reactivation, 18 (24%) more than one and 9 (12%) more than 2. TCL expansion was attempted in 24 donors (59%); in a third donor was CMV-seronegative. Target CMV-TCL dose was obtained from 22 donors (92%), in 2 cases TCL expansion failed to reach the target. Eight pts (20%) were infused with CMV-TCL. In those pts, 30 CMV reactivations were diagnosed. The median day of first CMV reactivation and of first infusion were respectively +40 (33-62) and +141 (77-511). 5 out of 8 pts were experiencing GVHD. Mean number of reactivations before receiving TCL was 3 (1-6). The mean total dose of infused cells was 3 x 10^5/kg body weight. At the time of first infusion, mean absolute lymphocyte count was 1.23 x10^3/mL and PCR CMV was negative in all but one pt. After TCL infusion, 6 out of 8 pts had no more CMV reactivations, and 1 pt had 1 reactivation and 1 pt 2 other reactivations. After TCL infusion, one pt developed cGVHD that did not require treatment, and 2 pts experienced a late acute grade II GVHD at the time of discontinuation of immunosuppressive therapy. No pts died of CMV infection, at a median follow up of 288 days.
Discussion: Adoptive T cell therapy with CMV–specific T cells is safe and effective in the management of refractory CMV infection, in adult patients after HSCT. However, only a small percentage of patients candidate for immunotherapy can be effectively treated.
Disclosure of Interest: None Declared.

PH-P495

HUMAN HERPESVIRUS 6 REACTIVATION ASSOCIATED WITH DELAYED ENGRAFTMENT AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN HEMATOLOGICAL PATIENTS

S. Bramanti[1], A. Vai[1], R. Crocchiolo[1], B. Sarina[1], F. Tordato[1], R. Mineri[1], A. Santoro[1], L. Castagna[1,]*
[1]Humanitas Cancer Center, Rozzano, Italy

Introduction: Human herpersvirus type 6 (HHV6) is a member of the β herpesvirus subfamily. More than 90% of the general population has been infected by HHV6. In immunocompromised patients, viral reactivation has been associated with fever, cutaneous rash, interstitial pneumonitis, encephalitis, and myelosuppression. The aim of this study was to evaluate the incidence of HHV6 reactivation in patients receiving ASCT with delayed hematopoietic reconstitution

Materials (or patients) and Methods: We performed a single center retrospective analysis of 117 consecutive hematological patients treated with ASCT between February 2011 and October 2013. Patients with a delayed engraftment (defined as ANC<500/μL at day +14 after HSCT) were tested for quantitative blood PCR HHV6, CMV, and EBV. Conditioning regimen used was carmustine, etoposide, cytarabine, and melphalan (BEAM) in 45% and high-dose melphalan in 55% of cases.

Results: 117 ASCT were performed and 62 of them presented a delay in engraftment. Among this group, 34 (55%) were tested and 7 out of 34 (21%) turned out to be HHV6+. Characteristics of the patients with engraftment delay are presented in Table. In 48% of cases, pre-transplant disease status was complete remission. In multivariate analysis, patients with POEMS syndrome reactivated HHV6 more than other diseases (50% vs. 21%, OR=5.00 (95% CI: 0.56-44.34, *P*=0.15); same trends were observed also with higher age (*P*=0.14) and lower CD34+ cells infused (*P*=0.13). Interestingly, no patient with Hodgkin's lymphoma reactivated the virus. Median time of HHV6 reactivation was day +20 (14-27) and 100% of patients experienced fever but no one had clinical HHV6-related disease. 4 patients were treated with foscarnet and 3 with ganciclovir. Virus DNA integration was searched only in 3 cases and all resulted negative. Neutrophil engraftment (ANC>500/μL) occurred in 100% of HHV6+ patients after a median of 29 days and in 87.5% (95% CI: 74.5-100, *P*=0.73) of HHV6- patients after a median of 24 days; platelet engraftment (PLT>20,000/μL) occurred in 57% (95% CI: 20-94) of HHV6+ vs. 72.5% (95% CI: 53.5-90.5) of HHV6- (*P*=0.48). 1 (14%) HHV6+ and 6 (22%) HHV6- patients needed a second CD34+ cells reinfusion. Median discharge day after transplant was 41 (range: 20-56) for HHV6+ and 27 (15-54) for HHV6- patients. CMV reactivations were diagnosed in 14% of HHV6+ and 7% of HHV6-; 29% among HHV6+ had other viral infections vs. 7% among HHV6- patients. After a median follow-up of 246 (27-994) days, 86% of HHV6+ and 67% of HHV6- patients are alive and the most frequent (62%) cause of death was relapse.

Discussion: In this retrospective study, we found that 21% of patients undergoing to ASCT with delayed engraftment had HHV6 reactivation. In multivariate analysis POEMS syndrome, older age, and low number of CD34+ cells infused were found as risk factors for HHV6 reactivation. HHV6+ pts have a longer hospital stay. However, these results should be mitigated by several selection biases: reactivated patients were not systematically evaluated for DNA integration, only half of patients with delayed reconstitution have been tested for HHV6.

Disclosure of Interest: None Declared.

[PH-P495]

Characteristics	HHV-6+ (n=7)	HHV-6- (n=27)
Age		
Median, in years (range)	64 (41-70)	50 (23-71)
Sex, n (%)		
Male	5 (71%)	18 (67%)
Female	2 (29%)	9 (33%)
Diagnosis		
Multiple myeloma	2 (29%)	8 (30%)
Non-Hodgkin's lymphoma	3 (43%)	11 (41%)
Hodgkin's lymphoma	0	6 (22%)
POEMS syndrome	2 (29%)	2 (7%)
Disease status at HCT		
CR-PR-VGPR	3 (43%)	19 (70%)
SD-PD	4 (57%)	8 (30%)
Conditioning regimen		
BEAM	2 (29%)	12 (44%)
Melphalan	5 (71%)	15 (56%)

PH-P496

GAMMA-HERPESVIRUS LOAD AS SURROGATE MARKER OF PRECOCIOUS DEATH IN HIV-POSITIVE LYMPHOMA PATIENTS SUBMITTED TO HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

C. Pratesi[1], S. Zanussi[1], R. Tedeschi[1], M. T. Bortolin[1], R. Talamini[2], M. Rupolo[3], C. Scaini[1], G. Basaglia[1], M. Mazzucato[4], L. Abruzzese[4], U. Tirelli[5], E. Zanet[3], M. Michieli[3,], P. De Paoli[6]*

[1]Microbiology, Immunology and Virology Unit, [2]Epidemiology and Biostatistics Unit, [3]Cellular Therapy and High-Dose Chemotherapy Unit, [4]Stem Cell Collection and Processing Unit, [5]Division of Medical Oncology A, [6]Scientific Directorate, CRO Aviano National Cancer Institute, Aviano (Pordenone), Italy

Introduction: In one of the largest mono-institutional series of HIV-1 positive (HIV+) relapse/refractoring lymphoma patients (pts) submitted to Autologous Stem Cell Transplantation (ASCT), EBV and KSHV-DNA loads were evaluated in order to assess the frequency of γ-herpesviruses reactivation and their prognostic and predictive value during the ASCT procedure and follow-up.

Materials (or patients) and Methods: 22 relapse/refractory HIV+ lymphoma pts who underwent ASCT at the National Cancer Institute (Aviano, Italy) and with at least 2 months (mo) follow-up after ASCT were included in this retrospective immunovirological study. EBV- and KSHV-DNA were measured by RT TaqMan PCR using the ABI PRISM 7900 HT SDS (Applied Biosystems). The HIV RNA level was quantified by using the Versant HIV-1 RNA 3.0 assay kit (bDNA; Bayer Diagnostics). CD4+ T, CD8+ T, CD56+ and CD19+ lymphocyte subsets were evaluated by flow cytometry (EPICS XL – Beckman - Coulter). Immunovirological parameters were evaluated before and after debulking chemotherapy (DCT), and at mo 0.5, 1, 3, 6, 12 after ASCT. The median follow-up of the pts after ASCT was 49.75 mo (range 2-108.91 mo).

Results: Pre-treatment plasma and PBMCs EBV-DNA were detectable in 12 (median 12135 copies/mL) and 18 pts (median 417 copies/10^6 PBMCs). After DCT, EBV-DNA viremia in both compartments predicted overall survival (HR, 5.88, 95% CI, 1.40-24.66, P=0.02; HR, 5.52, 95% CI, 1.08-28.16, P=0.04, respectively). Moreover, response <75% to DCT was associated to persistent EBV-DNA detection in plasma (P=0.03). A positive significant correlation between EBV-DNA in PBMCs and residual post-DCT B cell counts and percentage was found (r=0.69, P=0.002; r=0.6 P=0.009) and a negative correlation between plasma EBV-DNA and CD4+ T cell percentage and CD4/CD8 T cell ratio (r=-0.55, P=0.01; r=-0.45 P=0.04) was also observed.

After ASCT, plasma EBV-DNA was detectable in 5/10 pts who died very early after transplantation. No occurrence of lymphoproliferations or of life threatening clinical complications was diagnosed for the remaining 12 pts during year 1 after transplantation and afterward. In these pts, EBV-DNA in plasma was always negative, except for 2 sporadic increase, and EBV-DNA in PBMCS was detected with increasing frequency from mo 0.5 to 12 after ASCT (4/12 pts at mo 0.5, 11/12 pts at mo 12, P=0.01). At this time point, median EBV-DNA in PBMCs was 316 copies/10^6 PBMCs (range: undetectable-2083 copies/10^6 PBMCs) and no correlation to B cell counts was found (r=0.07, P=0.83); the inverse correlation with CD4/CD8 T cell ratio was kept like after DCT (r=-0.74 P=0.006).

KSHV-DNA load was detected in plasma samples of 3/16 pts before DCT (18.8%) and 2 of them showed detectable KSHV-DNA also in PBMCs. After DCT, a decrease in plasma KSHV load, ranging from 83 to 100%, was observed in all 3 pts, while KSHV-DNA in PBMCs became undetectable. After ASCT, KSHV-DNA was positive only in 1 patient who died for evolution of the initially diagnosed plasmablastic NHL to Primary Effusion Lymphoma.

Discussion: The presented findings underline that γ-Herpesvirus load is associated with deep immune deficiency and with precocious mortality after ASCT in HIV+ pts. This suggests the utility of EBV- and KSHV-DNA monitoring as a simple complementary tool for the management of HIV+ lymphoma pts submitted to ASCT.

Disclosure of Interest: None Declared.

PH-P497

DAY 30 MORTALITY OF ASYMPTOMATIC HSCT RECIPIENTS WITH RESPIRATORY VIRUS INFECTION DETECTED BY MULTIPLEX PCR IN RESPIRATORY SAMPLES TAKEN IMMEDIATELY BEFORE TRANSPLANTATION

M. Oliveira Souza [1], L. Perilio Zanetti[1], P. Moreira Silva[1], A. C. Ferrari dos Santos[1], A. C. Mamana Souza[2], V. Rensi Colturato[1], C. Machado[1,2,]*

[1]HSCT Program, Amaral Carvalho Foundation, Jahu, [2]Virology Laboratory - Institute of Tropical Medicine, University of São Paulo, São Paulo, Brazil

Introduction: Respiratory virus (RV) can cause great morbidity in HSCT recipients. Previous studies have shown that patients with upper respiratory infection caused by respiratory syncytial virus (RSV) have a greater risk of progression to pneumonia especially if infection occurs in the pre-engraftment period. To avoid this risk, transplant delay has been recommended if RSV is diagnosed during this period. No information is available concerning to other respiratory viruses.

Materials (or patients) and Methods: From January 2009 to June 2012, nasal wash (NW) samples were routinely taken from HSCT candidates before admission and RV detection was performed by DFA (Millipore). Aliquots of NW were stored at –80°C for future processing by PCR to assess the increased positivity using a more sensitive technique and the impact of pre-HSCT RV infection on day 30 mortality.

Results: 237 patients (pts) tested negative by DFA. Median time of NW sampling was day -8, ranging from day -14 to day zero. In 182 pts, pre-transplant samples were available for PCR processing (Seeplex RV 15, Seegene). RVs were detected in 33 of them (18.1%): 14 rhinovirus (7.7%), 7 coronavirus (3.8%), 6 parainfluenza (3.3%), 4 influenza A (2.2%), 1 RSV B (0.5%) and 1 adenovirus (0.5%). No difference was observed on day 30 mortality. One of the 33 RV infected pts (3%) died up to day 30, in comparison to 7 of the 149 pts (4.7%) not infected (P=0.67).

Discussion: Conclusions: The use of multiplex PCR increased the detection of RV in asymptomatic patients in about 20%. The detection of RV in nasal washes immediately before HSCT did not increase mortality rates in the first month of HSCT.

Disclosure of Interest: None Declared.

PH-P498

POOR PROGNOSIS OF INVASIVE ASPERGILLOSIS IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION COMPARED TO THOSE TREATED FOR LEUKEMIA

S. Bonnet[1,], R. Dulery[1], K. Regany[2], M. Bouketouche[3], S. Alfandari[4], C. Berthon[1], J. Gauthier[1], B. Carpentier[1], Z. Van de Wyngaert[1], L. Magro[1], I. Yakoub-Agha[1]*

[1]CHRU Claude Huriez Lille, [2]Hopital Saint Vincent de Paul, [3]CH Saint Quentin, Lille, [4]CH Tourcoing, Tourcoing, France

Introduction: Invasive aspergillosis (IA) remains a major concern among patients with hematological malignancies who receive cytotoxic chemotherapy or allogeneic stem cell transplantation (allo-SCT). With a view toward better understanding the differences in terms of clinical significance and outcomes of IA between allo-SCT recipients and patients treated for leukemia, we report a single-center study of 739 unselected consecutive patients treated in our unit between August 2000 and February 2004. IA episodes were classified according to the 2008 EORTC/MSG criteria.

Materials (or patients) and Methods: Patients were hospitalized either for allo-SCT (n=135), or for the treatment of acute leukemia (n=378), chronic lymphocytic leukemia (n=116), or myelodysplasic syndrome (n=105). Probable or confirmed IA were observed in 29 patients, among whom 7 were undergoing allo-SCT (5.2%), 20 were treated for acute leukemia (5.3%), 1 for chronic lymphocytic leukemia (0.8%), and 1 for myelodysplastic syndrome (0.95%). Most of the clinical signs were mild and nonspecific, especially in allo-SCT recipients.

Results: Comparison of patients undergoing allo-SCT to the others revealed significant differences. In allo-SCT recipients, the onset of IA occurred later than in the other patients, after the neutropenic

period. The median time between the last hematologic treatment and the diagnosis of IA were 231 days (range, 68-341) in allo-SCT recipients and 17 days (6-57) in the other patients (P<0.001). At the time of AI onset, the median absolute neutrophil count were 1.8x10^9 cells/L (0.7-5) and 0 x 10^9 cells/L (0-2.1) in allo-SCT patients and the others, respectively (P<0.0001). In addition, the median C-reactive protein level was lower in allo-SCT recipients (53 mg/L, 13-146) compared to the other patients (143.5 mg/L, 22.5-285), P=0.023. It is noteworthy that the 7 cases of IA in allo-SCT recipients occurred only in patients treated with corticosteroids for graft-versus-host disease (GVHD) (n=42).
First line therapy was liposomal amphotericin B (n=22), caspofungin (n=8), voriconazole (n=6), or itraconazole (n=1), either alone (n=21) or in combination (n=8). Complete remission of IA on CT was observed in 12 cases, stable lesions in 8 cases and progression in 9 cases. Mortality directly related to IA was 24% in our study and occurred early after the onset of infection. The 100-day, 1-year, 2-year and 10-year overall survival (OS) were 42.9%, 28.6%, 0%, 0% for allo-SCT recipient, respectively, compared to 68.1%, 40.9%, 18.2%, 13.6% for the other patients, respectively (P-values ≥ 0.05). These poor outcomes were mainly attributable to the non-relapse mortality (NRM), especially in allo-SCT recipients, who had a 2-year NRM of 100% compared to 54.6% in the other patients, P=0.034 (2-year cumulative incidence of relapse were 0% and 70.2%, respectively).
Discussion: In conclusion, our data seem to distinguish 2 types of IA. One has an early onset in neutropenic leukemia patients. The second, observed in allo-SCT recipients, occurs later, after GVHD development and is associated with a very high NRM incidence, suggesting that those patients remain fragile. Thus, systematic broad-spectrum anti-fungal prophylaxis is highly recommended in patients undergoing allo-SCT who develop GVHD.
Disclosure of Interest: None Declared.

PH-P499
PROFILE OF TOLL-LIKE RECEPTORS ON MONOCYTES AND LYMPHOCYTES IN RELATION TO INFECTIOUS COMPLICATIONS AND OUTCOME IN ALLOGENEIC STEM CELL TRANSPLANTATION

C. Skert[1,], M. Fogli[2], E. Garrafa[2], S. Perucca[1], S. Fiorentini[2], A. Turra[1], R. Ribolla[1], C. Filì[1], C. Bergonzi[1], S. Bernardi[1], M. Malagola[1], V. Cancelli[1], F. Cattina[1], E. Alghisi[1], A. Caruso[2], D. Russo[1]*
[1]Chair of Hematology, Bone Marrow Transplant Unit, University of Brescia, AO Spedali Civili Brescia, [2]Section of Microbiology, Department of Experimental and Applied Medicine, University of Brescia, Brescia, Italy

Introduction: Despite anti-infective prophylaxis, bacterial, fungal and viral infections remain an important complication after allogeneic stem cell transplantation (SCT). Recovery from infections depends on the complete integration and balance of innate and adaptive immune responses, which may affect survival. In this complex interplay, Toll-like receptors (TLRs) play a key role and recognize pathogen-associated molecular patterns (PAMPs), such as common protein, carbohydrate or DNA/RNA pattern motifs. Extracellular PAMPs, especially of bacteria and fungi, are recognized by surface TLRs (TLR-1,TLR-2,TLR-4,TLR-5, and TLR-6). Intracellular TLRs (TLR-3,TLR-7,TLR-8 and TLR-9) bind mainly to foreign nucleic acids. To our knowledge, no studies deal with expression and function of all human TLRs together in relation to infectious complications in the setting of allogeneic SCT. In this study we analyse 9 TLRs on T- lymphocytes and monocytes of transplanted patients in relation to bacterial, fungal, viral infections and outcome.
Materials (or patients) and Methods: Bacterial infections included all blood stream infections with or without organ localization. Fungal infections were evaluated and defined according to the revised criteria of EORTC/MSG Consensus Group. CMV, EBV and HHV6 reactivations/infections were monitored weekly by quantitative real-time PCR until the third month after SCT. The expression of TLRs on T-lymphocytes and monocytes was analysed in 35 patients by flow cytometry as mean fluorescence intensity at day +30. Functional data were obtained by ELISA assay after TLRs activation. The cell supernatants were collected and assayed for TNF-alpha, IL-4, IFN-gamma, and MCP-1. Lymphocyte subsets were evaluated by flow cytometry.
Results: Clinical/transplant characteristics and infections before day +30 did not influence levels of TLRs (multifactor analysis of variance). In multivariate Cox regression analysis, levels of TLR-9 expression on T-lymphocytes (HR 0.97; P=0.01) and values of NK cells (HR 0.95; P=0.01) correlated negatively with bacterial infections after day +30. We observed a trend for negative correlation between TLR-7 levels on T-lymphocytes and fungal infections after day +30 (HR 0.36; P=0.07). Values of monocytes were negatively associated with CMV reactivation (HR 0.99; P=0.03), whereas levels of TLR-5 on T lymphocytes were positive predictors (HR 1,2; P=0.01). Age and bacterial infections negatively influenced overall survival (HR 1.06 P=0.03; HR 10 P=0.006). Monocyte values were positive predictors of survival (HR 0.96 P=0.003).
Discussion: Bacterial, fungal and CMV infections were associated with a different expression of some TLRs. The protective role of TLR-7 and TLR-9 against fungal and bacterial infections, respectively, seems to be dominant in comparison to other known TLRs, which recognize these pathogens. The atypical involvement of TLR-5 in the response against CMV may suggest more complex and pleiotropic functions of TLR-5 in the immune reactions. A specific TLR profile and an adequate recovery of monocytes could positively influence the outcome of allogeneic SCT by promoting an effective control of infections and inflammatory responses.
Disclosure of Interest: None Declared.

PH-P500
CONSISTENT HHV-6 DNAEMIA POST-ALLO TRANSPLANTATION IS CHARACTERIZED BY HIGHER LEVELS OF HHV-6 IN THE BLOOD AND SIGNIFICANTLY HIGHER INCIDENCE OF GVHD AS COMPARED WITH PATIENTS WITH OCCASIONAL HHV-6 DNAEMIA

L. Francois Span[1,], C. Van Leer Buter[2], A. Riezebos-Brilman[2], H. G Niesters[2]*
[1]Department of Hematology, [2]Department of Medical Microbiology, University Medical Center Groningen (UMCG), Groningen, Netherlands

Introduction: Several recent studies have shown that HHV-6 DNA is frequently found in blood (HHV-6 DNAemia) of hematological stem cell transplantation (SCT) recipients. Reactivations of HHV-6 are associated with the development of acute Graft versus Host Disease (GvHD), CMV reactivations, poor engraftment and higher overall mortality. Ongoing discussions revolve around the nature of the association, i.e. whether HHV-6 triggers the other complications, whether HHV-6 DNAemia is caused by the above mentioned complications, or whether HHV-6 DNAemia has no significance at all. In this observational study we investigate not only the presence of HHV-6 DNA in blood of SCT recipients, but also the duration and the height of the HHV-6 DNAemia, and how these factors relate to the occurrence of GvHD.
Materials (or patients) and Methods: Frozen whole blood samples from 50 consecutive SCT patients were tested for HHV-6 by quantitative PCR. All samples taken until one year after transplantation were analyzed retrospectively (total 961 samples). Patient data about the underlying hematological disease, type of transplantation (myelo-ablative, reduced intensity, cord blood, sibling, or matched unrelated donations) were recorded from existing medical files, as well as the occurrence of acute or chronic GvHD and overall outcome.
Results: Forty-six of 50 patients had at least one blood sample which tested positive for HHV-6 DNA. 25 patients had occasional samples with low level HHV-6 DNAemia (<4 consecutive positive samples with HHV-6 DNA <1000 copies/ml). 21 patients had consistent HHV-6 DNAemia (≥4 consecutive HHV-6-DNA positive samples). Eighteen out of these 21 patients had DNA levels >1000 copies/ml for at least 4 consecutive blood samples. In the group of patients with consistent HHV-6 DNAemia, 13 out of 21 (62%) were diagnosed with acute GvHD, and 8 of these patients had clinical

presentations compatible with viral illness or GvHD at the time of the first blood sample with detectable HHV-6 DNA. In the group of patients with occasional HHV-6 DNAemia, 6 out of 25 (24%) were diagnosed with acute GvHD ($P<0.05$).
Discussion: While HHV-6 DNAemia is a frequent finding following hematological SCT, the pattern of HHV-6 DNAemia (either occasional or consistent) may be the most important predictor of complications. Routine monitoring of HHV-6 DNAemia is necessary to distinguish patients with occasional HHV-6 DNAemia form those with consistent HHV-6 DNAemia.
Disclosure of Interest: None Declared.

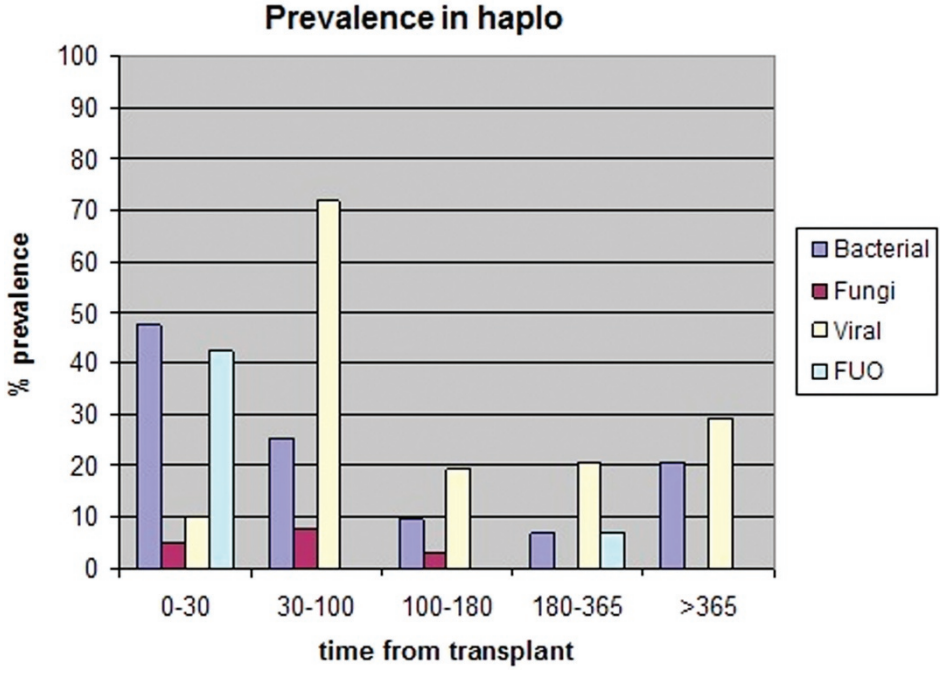
PH-P501
LOW INCIDENCE OF LATE INFECTIONS AFTER STEM CELL TRANSPLANTATION USING T-CELL REPLETE GRAFT FROM HAPLOIDENTICAL DONOR AND POST-TRANSPLANT HIGH-DOSE CYCLOPHOSPHAMIDE SUGGESTS A SATISFACTORY IMMUNE RECONSTITUTION AFTER TRANSPLANTATION
R. Crocchiolo[1,], S. Bramanti[1], A. Vai[1], B. Sarina[1], E. Mauro[1], C. Carlo-Stella[1], A. Santoro[1], L. Castagna[1]*
[1]Humanitas Cancer Center, Rozzano, Italy

Introduction: Thanks to recent advances, a platform of T-cell replete haploidentical hematopoietic stem cell transplantation (HSCT) using post-transplant cyclophosphamide (as a part of graft-versus-host disease prophylaxis) is now available, and it is characterized by a high reproducibility and an acceptable safety profile. However, detailed data on the timing and etiology of infections occurring after such a transplant approach are still limited.
Materials (or patients) and Methods: Present analysis reported data on 40 consecutive haplo-HSCT and compared them with a cohort of 72 consecutive HSCTs from a HLA-identical donor treated at the same center after a reduced-intensity or nonmyeloablative conditioning, with the aim of reporting etiology and timing of infections occurring after HSCT in both groups. For haplo-HSCT, antimicrobial prophylaxis consisted of acyclovir, levofloxacine, caspofungin followed by itraconazole, and cotrimoxazole until day -2. In HLA-identical group acyclovir, levofloxacin, fluconazole and cotrimoxazole were administered.
Results: In haplo cohort, a total of 38 patients presented at least one infectious episode: 22 had bacterial (55%), 28 had viral (70%) and 5 (12.5%) had at least one fungal infection. In particular, we found a 72% prevalence of viral infections/reactivations between day+30 and +100 with a median of viral events per patient of 2 (range 1-4). Twenty episodes of CMV reactivation occurred in 17 patients (42%), BK-virus-associated cystitis was observed in six patients (15%). Interestingly, beyond day +365 only five bacterial events were observed out of 24 patients at risk (21%) and no fungal infections were detected. Incidence of viral infections or reactivations was higher in haplo group compared with HLA-identical one, while late bacterial events occurred less frequently (prevalence 21% vs. 55% beyond day +365), probably due at least in part to low rate of chronic GVHD among haplo-HSCTs (10% vs. 51%, $P<0.0001$). Two-year NRM was 10% (3-17) and 25% (12-38) in HLA-id and haplo cohort respectively ($P=0.02$).
Discussion: Despite a quite high rate of viral infections in the early period, present data suggest a satisfactory immunological recovery after T-cell replete haplo-HSCT using post-transplant CTX, in particular with few late bacterial events and no fungal infections beyond one year after HSCT.
Disclosure of Interest: None Declared.

PH-P502
UTILITY OF POSACONAZOLE MONITORING AND FACTORS AFFECTING ITS LEVELS IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION:SINGLE CENTER EXPERIENCE FROM INDIA
K. himmat Doshi[1,], M. Gurjar[1], K. Pathak[1], N. Shetty[2], B. Bagal[2], V. Gota[1], N. Khattry[2]*
[1]Clinical Pharmacology, [2]BMT Unit, ACTREC, Navi Mumbai, India

Introduction: Posaconazole (posa) is used for prophylaxis against invasive fungal infections in BMT. There is large interpatient variability in posaconazole kinetics as well as an established concentration-effect relationship, which makes it an ideal candidate for therapeutic drug monitoring (TDM). It has been recommended to maintain trough concentration >0.7 µg/ml for prophylaxis against invasive mycosis. We report our experience.

[PH-P501]



Materials (or patients) and Methods: All patients who received posa prophylaxis during peritransplant period between September 2013 and December 2013 were included. Posa prophylaxis was started at least 3 days prior to start of conditioning regimen in all patients. All patients received standard oral dose of 200 mg TDS. Posa levels were measured on day 7 of treatment by HPLC assay. Subsequent levels were done at physician's discretion or a week after dose modification, if any. If Cmin was <0.7 µg/ml, frequency of dosing was increased as per standard therapeutic guidelines for posa. Posa was continued for at least 100 days for all patients undergoing transplant and longer if patients were on steroids due to engraftment fever or GVHD. Breakthrough fungal infection was documented as definite, probable or possible as per standard guidelines. Influence of covariates included age, sex, height, weight, body mass index (BMI), diarrhea, liver and renal function parameters and concomitant drugs were analyzed by linear regression with backward elimination.
Results: Twenty-nine patients were monitored during this period The median age was 33 (10-57) years. The median number of TDM was 3 (1-8) per patient. High interpatient variability was observed (CV=97%). Seven patients (24.13%) had trough levels < 0.7 µg/mL after the first monitoring. Six patients had their dose modified after first monitoring due to low levels. All of them achieved target trough concentration after dose modification. Six patients who had adequate levels at first TDM had fall in Cmin below 0.7 µg/mL during subsequent TDMs. Introduction of steroids significantly reduced posa trough levels [*N*=4; (presteroid median Cmin- 2.035 µg/mL (1.12-2.78); post steroid median- 0.94µg/mL (0.5-1.59); *P*=0.06 - Wilcoxon sign rank test)]. None of the demographic or laboratory parameters influenced posa levels. Breakthrough fungal infections were seen in 2 patients (definite-1, possible-1). One of them had undetectable levels and the other had adequate but decreasing trend in previous Cmin levels.
Discussion: About one in five patients required dose modification after first TDM. The incidence of breakthrough fungal infections was approximately 7% which is comparable with other centres in the West. Introduction of steroid may lead to fall in Cmin levels. Such patients would require a repeat TDM and appropriate dose correction, if the levels are low. Interestingly, none of the baseline characteristics and laboratory parameters influenced posa level. Other studies have shown diarrhea and concomitant medication (PPIs) to have an impact on posa levels. This needs to be confirmed in our setting in larger sample size. Pharmacoeconomic analysis showed that as little as Rs. 2,500 (approximately 30 euros) spent on TDM would benefit one patient. Thus TDM could be adopted even in resource limited settings to reduce breakthrough fungal infection in BMT patients on posa prophylaxis.
Disclosure of Interest: None Declared.

PH-P503
SERIAL MONITORING OF BLOOD CONCENTRATION OF VORICONAZOLE IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

T. Mori[1,], J. Kato[1], Y. Koda[1], S. Kohashi[1], S. Okamoto[1]*
[1]Division of Hematology, Keio University School of Medicine, Tokyo, Japan

Introduction: Most of the therapeutic drug monitoring (TDM) studies of voriconazole applied the single blood concentration of each patient. The aim of this study is to elucidate the dynamics of blood concentrations of voriconazole by serial monitoring in hematopoietic stem cell transplantation (HSCT) recipients.
Materials (or patients) and Methods: Thirty-eight patients who received voriconazole (400 mg/day) orally after allogeneic HSCT for hematological diseases with weekly monitoring of plasma voriconazole concentration were identified by using the institutional database and enrolled into the analysis. Data, including patient demographics and voriconazole concentration, were retrospectively collected from the medical records. Plasma trough concentrations of VCZ were measured by high-performance liquid chromatography weekly starting 5-10 days after initiating voriconazole administration.
Results: Median age and body weight of the patients were 44.5 years (range: 22-66) and 51.7 kg (range; 43.1-71.5), respectively. The plasma voriconazole concentration initially measured 5-10 days after starting voriconazole was 1.69+1.10 µg/ml, which was less than 1.0 µg/ml, defined as a low concentration, in 12 patients (32%, Group A), and 1.0-5.0 µg/ml, defined as an optimal concentration, in 26 patients (68%, Group B). In Group A, 6 patients subsequently achieved an optimal concentration 2-4 weeks after initiating voriconazole without an adjustment of the voriconazole dose. No patients showed voriconazole concentration greater than 5 µg/ml at any time points in Group A. In Group B, voriconazole concentration decreased to a low concentration in 4 patients at the second week and exceeded 5.0 µg/ml in 2 patients 4-5 weeks after initiating voriconazole. In 6 patients whose voriconazole concentration was monitored longer than 8 weeks, the concentration was at a steady state and no accumulation was observed.
Discussion: These results suggest that the voriconazole concentration measured 5-10 days after initiating voriconazole could predict its subsequent dynamics. However, since a proportion of patients attain a steady state at later than 4-5 weeks, a serial monitoring of voriconazole concentration is required to elucidate the optimal dose in each patient to prevent toxicities and maximize its efficacy.
Disclosure of Interest: None Declared.

PH-P504
FUNCTIONAL PATTERNS OF CYTOMEGALOVIRUS (CMV)-SPECIFIC CD8+ T CELLS THAT ASSOCIATE WITH PROTECTION FROM AND CONTROL OF CMV DNAEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (ALLOSCT)

J. Montoro[1,], E. Giménez[2], P. Amat[1], B. Muñoz-Cobo[2], R. de la Cámara[3], J. Nieto[4], J. López[5], D. Bravo[2], M. J. Remigia[1], C. Arbona[1], J. L. Piñana[1], D. Navarro[2], C. Solano[1]*
[1]Hematology and Medical Oncology, [2]Microbiology Service, Hospital Clinico Universitario-INCLIVA, Valencia, [3]Hematology Service, Hospital La Princesa, Madrid, [4]Hematology Service, Hospital Morales Meseguer, Murcia, [5]Hematology Service, Hospital Ramon y Cajal, Madrid, Spain

Introduction: Though end-organ disease has radically reduced, CMV DNAemia continues to be a frequent event after Allo-SCT with potential deleterious effects. Experimental evidence suggests that polyfunctional $CD8^+$ T cells are critical in the control of some chronic viral infections.
Aims: 1. To determine the functional profile of CMV-specific $CD8^+$ T cells that associate with protection from and control of CMV DNAemia after Allo-SCT, 2. To investigate whether $NKG2C^+$ NK cells contribute to affording protection.
Materials (or patients) and Methods: We prospectively analysed 108 non-consecutive pts from Apr-2010 to May-2012. We enumerated pp65 and IE-1-specific CD8+ T cells expressing IFN-γ, TNF-α and CD107a, and NKG2C+ NK cells, at days +30 and +60 after transplant. CMV DNAemia was quantitated once or twice weekly by CMV RT-PCR, (Abbott Molecular or LightCycler CMV Quant Kit, Roche). Quantitation of monofunctional (IFN-γ, TNF-α, and CD107a), bifunctional (IFN-γ/ TNF-α, IFN-γ/CD107a, and TNF-α/CD107a) and trifunctional (IFN-γ/TNF-α/CD107a) CMV-specific CD8+ T cells was performed by ICS using whole blood. Specific responses were considered as those that were >0.1% for IFN-γ, TNF-α, and CD107a-expressing CD8+ T-cell populations. Polyfunctional CD8+ T cells those positive for 2-3 markers. NK cell immunophenotyping was performed in EDTA WB samples in a FACScant II flow cytometer (BD Biosciences). Patients with active CMV infection were pre-emptively treated with valganciclovir (900 mg/12 h), ganciclovir (5 mg/kg/12 h) or foscarnet (90 mg/kg/12 h) upon detection of >500 CMV DNA copies/mL. Antiviral therapy was discontinued after two consecutive negative PCR results.
Results: Fifty-nine out of 108 pts (54.6%) did develop CMV DNAemia within the study period. CMV-specific CD8+ T-cell responses (of any functional type) were more likely to be detected in patients not developing CMV DNAemia (*P*=0.04). Qualitatively, no major

differences in the functional signature of CMV-specific CD8+ T cells were noted between patents who had or had not CMV DNAemia. However, the level of polyfunctional CD8+ T cells at day +30 in patients who had a subsequent episode of CMV DNAemia was lower than in patients who did not, though not achieving statistical significance (P=0.32). The presence of bifunctional and trifunctional CD8+ T cells was associated with lower levels of CMV replication, and higher frequency of self-resolved episodes. The levels of NKG2C+ NK cells were comparable in patients with or without CMV DNAemia (P>0.1), and in those with or without reconstituted polyfunctional CD8+ T-cell responses (P=0.4).
Discussion: Our data indicate that the enumeration of IFN-γ-producing CD8+ T cells permits an estimation of the polyfunctionality of the CD8+ T cell population, and should therefore be considered a reliable surrogate marker for predicting protection against the occurrence of CMV DNAemia. Our results do not confirm a direct role of NKG2C+ NK cells in CMV control or in promoting the reconstitution of CMV-specific polyfunctional CD8+ T-cell responses. The data reported further clarify the immune mechanisms involved in preventing the occurrence and in control of CMV DNAemia in Allo-SCT recipients, and may be helpful in the design of therapeutic strategies aimed at optimizing the management of CMV infection in the clinical setting
Disclosure of Interest: None Declared.

PH-P505
HIGHER RISK OF HHV6 REACTIVATION AMONG PATIENTS UNDERGOING UMBILICAL CORD BLOOD TRANSPLANTATION

P. G. Alvarenga Oliveira[1,], M. Ueda[2], J. Monte Real[3], E. Sa Moreira[4], M. Vescovi Goncalves[1], T. X. Carneiro[1], F. Rodrigues Kerbauy[1], N. Hamerschlack[5], A. Seber[6], J. S. Oliveira[1], Y. Novis[3], C. Granato[2], C. Arrais Rodrigues[1]*
[1]Hematology and Tranfusion Medicine, [2]Laboratory of Virology, UNIFESP, [3]Bone Marrow Transplantation, Hospital Sirio Libanes, [4]Dendrix Research, [5]Bone Marrow Transplantation, Hospital Israelita Albert Einstein, [6]Bone Marrow Transplantation, Instituto de Oncologia Pediatrica - GRAACC - UNIFESP, Sao Paulo, Brazil

Introduction: Hematopoietic stem cell transplantation (HSCT) is a potentially curative procedure to many hematological diseases. Human herpesviruses may cause severe complications after HSCT such as interstitial pneumonia, encephalitis and post-transplant lymphoproliferative disease (PTLD). A prospective survey on the incidence of primary infection or reactivation and clinical features of herpesvirus infections after HSCT has not yet been performed in Brazilian patients. Additionally, the impact of most of these infections on the HSCT outcome is still unclear. This study aimed to develop a test to screen and quantify all known human herpesviruses (HSV1, HSV2, VZV, EBV, CMV, HHV6, HHV7 and HHV8) in plasma samples from patients undergoing a HSCT and evaluate the impact of these infections on hematopoietic transplantations outcomes.
Materials (or patients) and Methods: Between August 2010 and December 2012, peripheral blood samples from 98 allogeneic HSCT recipients were collected weekly after transplant until day +100, from four transplant centers in Sao Paulo (Brazil), totalizing 1045 samples.Median age was 16 years (range: 2-73), 61% were male, and acute leukemias were the most frequent diagnosis (55%). Stem cell sources were bone marrow in 54%, umbilical cord blood in 25% and mobilized peripheral blood in 21%. 40% percent of donors were related identical. In a semi-automated workflow, the DNA was extracted from plasma in the QIAcube robot. A test based on quantitative real-time PCR (Taqman®) was optimized to screen and quantify all known human herpesviruses. Infected cell cultures and plasma specimens with a known viral load/amplicon copy number have been used as controls. For all the viruses the lower limit of detection (LOD) was around 5 copies of target per reaction, representing 250 copies/ml of plasma. No cross-reaction or false positive results were detected and within-run and between-run precision estimates are equal or higher than 95%.
Results: The frequencies of herpesviruses reactivation or primo-infection were: CMV=43%, HHV6=18%, HHV8=6%, EBV=3%, HSV1=3%, VZV=3%, HHV7=2%, and HSV2=1%. CMV reactivation was significantly more frequent in adults (71% vs. 26% for children, P<0.0001). HHV6 reactivation was significantly more frequent after umbilical cord blood transplant than after transplant from other sources (41% vs. 6%, respectively, P<0.0001). CMV reactivation was associated with a higher risk of acute GVHD, with a cumulative incidence at 100 days of 35% vs. 16% (P=0.04), but had no impact on the other outcomes. HHV6 reactivation also had no significant impact on outcomes. HHV8 reactivation was associated with an increased risk of chronic GVHD (83% vs. 48%, P=0.001).
Discussion: HHV6 primo-infection or reactivation is more frequent after transplant from umbilical cord blood than from adult donor grafts. CMV and HHV6 reactivation are frequent after HSCT, but had no significantly impact on the transplant outcomes, possibly due to monitoring and preemptive measures. Monitoring these viruses constitute an essential measure to improve outcomes. Additional prospective studies are required to confirm these findings and determine whether therapy influences survival.
Disclosure of Interest: None Declared.

PH-P506
EBV DETECTED VIRAL LOAD IN PERIPHERAL BLOOD OF DONORS AT THE TIME OF TRANSPLANT HAS NO IMPACT ON EBV-LYMPHOPROLIFERATIVE DISEASE DEVELOPMENT IN RECIPIENTS

I. Sakellari[1,], A. Papalexandri[1], I. Batsis[1], D. Mallouri[1], A. Marvaki[1], A. Touloumenidou[1], A. Xocheli[1], A. Afisidis[1], P. Zerva[1], E. Tsorlini[2], E. Yannaki[1], A. Anagnostopoulos[1]*
[1]Haematology department and BMT unit, [2]Microbiology laboratory, George Papanicolaou Hospital, Thessaloniki, Greece

Introduction: EBV-associated lympoproliferative disorders (EBV-LPD) in the setting of allogeneic haematopoetic transplantation (alloHCT) occur with an increasing incidence due to innovations in transplant techniques. Several risk factors have been correlated with a higher probability of EBV-LPD. Serial monitoring and pre-emptive treatment with monoclonal anti-CD20 antibody in patients with elevated EBV viral load has proven effective in reducing the risk of EBV-PTLD in transplanted patients. Although a donor sero-positivity has been recognized as a risk factor for EBV-LPD in sero-negative recipients, the role of EBV DNA in whole peripheral blood (PB) of donors has not yet been elucidated and measurement of EBV DNA in donors is not a standard part of donor evaluation. EBV DNA in whole blood represents both the EBV genomes from mononuclear cells (mainly B cells reflecting latency) and EBV DNA released in plasma due to infection. Thus, a positive result from donor's blood could harbor the risk of transmitting active EBV infection.
Materials (or patients) and Methods: In our study, we retrospectively analyzed the role of EBV DNA in PB of donors at the time of transplant in developing EBV-LPD. Forty-two patients (29 male/13 female) with a median age of 41 (13-61) who underwent alloHCT for hematologic malignancies and aplastic anemia were evaluated. Forty out of 42 received graft from a matched unrelated donor, and almost all (41/42) received ATG. Only five patients underwent transplantation after reduced intensity conditioning. EBV DNA was measured in whole blood with real-time PCR. Donors were evaluated at the time of transplant and patients were monitored every 15 days.
Results: Thirteen donors (13/42, 31%) had detectable EBV DNA [24-2030 genomic copies (GC)/ml, mean 462]. During follow up 15/42 patients (36%) presented with EBV DNA greater than 10.000 GC/ml (10.020-311.000, median 21400) in a median of 74 (20-1114) days and one patient developed EBV-LPD. Four out of these 15 patients had no detectable IgG antibodies for EBV at the time of transplant; two seronegative patients received graft from seropositive donors. Among the patients that developed EBV DNA greater than 10.000 GC/ml, eleven received graft from a donor with non detectable EBV DNA. However, in statistical analysis donor EBV DNA was not associated with EBV reactivation and no significant cut off value of donor viral load was found to indicate transmission to the recipient.

In our population other consistent risk factors (EBV seronegativity, aGvHD, RIC) were not significantly correlated with EBV reactivation. Moreover, none of these donors developed an EBV overt infection or any other EBV-related disorder.
Discussion: Our results indicate that even in a high risk population measurement of EBV DNA in whole blood of donors does not predict EBV-LPD and should not be done routinely. Since there is still no evidence to suggest that a healthy donor with high copy number is of greater risk than a healthy donor with an undetectable copy number, these donors should not be excluded as haematopoietic stem cell donors.
Disclosure of Interest: None Declared.

PH-P507

CIPROFLOXACIN PROPHYLAXIS REDUCES THE INCIDENCE OF SEVERE BK HEMORRHAGIC CYSTITIS IN CORD BLOOD PLUS THIRD-PARTY DONOR DUAL TRANSPLANT RECIPIENTS. A SINGLE-CENTRE EXPERIENCE

I. Sánchez-Ortega[1,], B. Patiño[1], M. Arnan[1], A. Fernández de Sevilla[1], C. Gudiol[1], R. F. Duarte[1]*
[1]Institut Català d'Oncologia - Hospital Duran i Reynals, Barcelona, Spain

Introduction: BK virus-associated hemorrhagic cystitis (BKHC) is a potential serious complication of allogeneic hematopoietic cell transplantation (alloHCT). Ciprofloxacin prophylaxis has been shown to reduce the incidence of severe (grades III-IV) BKHC in HLA matched sibling and unrelated volunteer donor alloHCT recipients. Whether these results would translate onto other populations of patients at high risk of severe BKHC remains unknown. Here, we present our experience on the effectiveness and safety of ciprofloxacin for the prevention of severe BKHC in high-risk cord blood plus third-party donor (CB-TP) dual transplant recipients.
Materials (or patients) and Methods: Nineteen consecutive alloHCT candidates who in the absence of compatible related or unrelated volunteer donors received a myeloablative (fludarabine, cyclophosphamide, busulphan, ATG) CB-TP dual transplant in our center from feb/2009 to sept/2013, were included in this study (11 AML/MDS, 3 ALL, 3 CLPD, 2 CML; 11 men; median age 44 [20-64]; 10 refractory to ≥1 chemotherapy lines, 4 relapsed after previous BMT, 5 with active disease at time of transplantation; 02/2009–09/2013). In this series, patients received no formal antibacterial prophylaxis. Eight patients receiving no BKHC ciprofloxacin prophylaxis were compared to 11 patients receiving 60 days of post-transplant ciprofloxacin for BKHC prophylaxis (500mg po bid or 400 mg IV bid) regardless of IV broad-spectrum antibiotics. GVHD prophylaxis (cyclosporine A and corticosteroids), antiviral (acyclovir), anti *pneumocystis jirovecii* (pentamidine or trimethoprim-sulfamethoxazole) and antifungal (posaconazole) prophylaxis strategies and supportive therapy were identical in both groups.
Results: Three patients in the non-ciprofloxacin group developed severe BKHC (one grade III and two grade IV) after neutrophil engraftment (25-35 days after alloSCT) compared with no cases in the ciprofloxacin group (37.5% vs 0, *P*=0.027). BKHC episodes increased the hospital stay a median of 11 weeks (8-12) during which patients required intense transfusion support (median 30 [29-77] packed red blood cells, median 41 [22-56] pool platelets) and up to four lines of treatment; two patients achieved complete response and one, died in refractoriness 8 weeks after BKHC diagnosis. All eleven patients in the ciprofloxacin group completed the prophylaxis as initially planned and no ciprofloxacin-related severe adverse events were reported. Time to neutrophil engraftment (mean 12.5 +/- 2.9 vs 12.8 +/-4.16, *P*=n.s.) and 100-day survival after CB-TP dual transplantation (87.5% vs 82%, *P*= n.s.) were comparable between ciprofloxacin and non-ciprofloxacin groups.
Discussion: Our results suggest that ciprofloxacin prophylaxis may reduce the incidence of severe BKHC in high-risk cord blood transplant recipients, and the morbidity and resource use associated with it. They also confirm that the maintenance of the prophylaxis during 60 days after transplantation regardless of IV broad-spectrum antibiotics is safe and well tolerated. Further studies with larger series of patients are warranted.
Disclosure of Interest: None Declared.

PH-P508

NON-ASPERGILLUS (NA), NON-MUCORALES (NM) INVASIVE MOLD INFECTIONS IMI IN RECIPIENTS OF HEMATOPOIETIC CELL TRANSPLANTATION (HCT)

S. Singh Dadwal[1,], B. R. Tegtmeier[1], J. Kriengkauykiat[1], J. I. Ito[1]*
[1]Infectious Disease, City of Hope National Medical Center, Duarte, United States

Introduction: Fungal infections are common after HCT that lead to significant morbidity and mortality with aspergillus and mucorales being the most common encountered molds. The frequency of other emerging mold infections is not well known. The purpose of this study was to review IMI caused by NA/NM molds (including dimorphic fungi).
Materials (or patients) and Methods: Retrospective review study. Patients who had HCT between 1998 and 2009 (autologous and allogeneic) diagnosed with IMI related to NA/NM molds were identified from the microbiology database. Only culture positive cases that met the criteria of proven and probable NA/NM IMI were included (according to EORTC and MSG). Response to therapy at 4 weeks [complete response (CR), partial response (PR), stable, failed therapy and deaths] and 90-day mortality was reviewed.
Results: Total of 5346 HCT performed [2848 autologous, 1375 matched related donor (MRD), 1030 matched unrelated donor (MUD) and 93 cord blood (CB)]. 24 culture positive cases of proven (11)/ probable (13) NA/NM IMI were identified; 4 *Fusarium spp.*, 11 *Scedosporium spp.*, (6 S. *apiospermum*, 5 *S. prolificans*), 2 *Paecilomyces spp.*, 1 *Acremonium spp.*, 1 *Chaetomium spp.*, 1 *Alternaria spp.*, and 4 *Coccidioides immitis*. 20 had allogeneic (8 MUD, 9 MRD, and 3 CB) and 4 autologous HCT. Mean age was 44 years and mean time to diagnosis of IMI was 575 days post HCT; although 10/24 had IMI diagnosed within first 90 days of HCT. 12/20 allogeneic HCT had active graft versus host disease (acute or chronic GvHD) and 4 had recent resolution of GvHD. Only 5 patients with active GvHD were on steroids at dose of 1-2 mg/kg/day and 10/20 allogeneic HCT were on steroids but at a dose lower than 1-2 mg/kg/day. 11/24 had pulmonary involvement. Fungemia was notable with *S. prolificans* (3/5) and 4/4 of *Coccidioides immitis* had pulmonary involvement (2 had disseminated infection). 17/24 received antifungal prophylaxis in the 2 weeks preceding the diagnosis of IMI. Response to antifungal therapy at 4 weeks: none of the patients had CR, 6 had PR, 4 had stable disease and 14 died. At 90 days after diagnosis 14/24 had died (58%). No difference in mortality noted in those diagnosed early after HCT (within 90 days of HCT) versus those with late onset disease (6/10 vs. 8/14). 10/16 (62.5%) who had active GvHD or recent resolution of GvHD died vs. 4/8 (50%) with no GvHD. Mortality by fungal pathogen at 90 days; 3/4 with *Fusarium* died (75%), 7/11 with *Scedosporium spp* died (63%), 2/2 with *Paecilomyces spp* died, 1 each with *Chaetomium spp* and *Alternaria spp* died, while none of the patients with *Coccidioides immitis* died.
Discussion: Although NA/NM IMI is infrequent after HCT, the response to antifungal therapy is sub-optimal and the mortality associated with these infections is significantly high.
Disclosure of Interest: None Declared.

PH-P509

ADENOVIRUS INFECTION IN PEDIATRIC ALLOGENEIC HSCT: IMPACT OF A VIRO-IMMUNOLOGICAL MONITORING AND PREEMPTIVE TREATMENT STRATEGY ON OUTCOME

L. Rubert[1,], F. Introzzi[1], A. Tolva[1], S. Basso[1], I. Guido[1], G. Quartuccio[1], T. Mina[1], A. Pellerano[1], M. A. Grignano[1], L. Calafiore[1], C. Cugno[1], F. Baldanti[2], M. Zecca[1], P. Comoli[1]*
[1]Pediatric Hematology/Oncology, [2]Virology Service, Fondazione iRCCS Policlinico San Matteo, Pavia, Italy

Introduction: Adenovirus (HAdV) is a significant cause of morbidity and mortality in pediatric hematopoietic stem cell transplant recipients. As yet, HAdV DNA detection and quantification in plasma is the only objective parameter for identifying HAdV-infected patients at high risk for disseminated disease. However, viral monitoring has not been widely adopted in clinical practice,

owing mainly to the relatively low frequency of HAdV disease and to the lack of safe and effective treatment. Pediatric HSCT recipients could benefit from HAdV surveillance, provided that cost-effectiveness of the procedure be enhanced by a center-focused determination of the subgroups of patients at high risk of developing HAdV infection. Aim of this study was to assess risk factors for HAdV infection in a single-center cohort of pediatric HSCT recipients monitored prospectively, and evaluate efficacy of a viro-immunological surveillance program and preemptive treatment approach on infection and patient outcome
Materials (or patients) and Methods: 104 recipients of HSCT from matched related (*n*=18), matched-unrelated (*n*=41), or haploidentical family donors, transplanted at our center between 03/2010 and 04/2013 for malignant (*n*=70) or non-malignant (*n*=34) disease, were monitored weekly for HAdV DNA by real time polymerase chain reaction in blood, and for immune reconstitution. In case of persistently positive HAdV DNA >10.000 copies/ml and/or HAdV disease, patients received cidofovir treatment. Donor HAdV-specific T cells were employed as rescue therapy in unresponsive patients.
Results: HAdV viremia occurred in 22/104 HSCT recipients (cumulative incidence, CI, 21%) at a median time of 27 days from transplant (range 6-124 days). Peak HAdV DNA was 1900 copies/ml (range, 100 – 4.123.100). Fourteen of the 22 HSCT recipients experienced self-limiting viremia without signs of viral disease, while in 8 patients, an increase in HAdV DNA from a median value of 6700 copies/ml up to a median peak of 1.65 x 105/ml was observed. Factors significantly associated with HAdV viremia were a diagnosis of malignant disease, peripheral blood as stem cell source, development of grade II-IV acute GVHD, and failure to reconstitute CD4+ T cell subset and HAdV-specific T cell frequency. Although the rate of HAdV infection was double in recipients of haplo-HSCT compared with MUD HSCT, this difference was not statistically significant.
Patients with HAdV DNA >10.000 copies/ml were treated with cidofovir; in 4 of the 7 evaluable cases, viraemia further increased, and HAdV disease developed, that required a rescue with HAdV-specific T cell therapy. Viremia and clinical symptoms cleared in all patients, and no death due to HAdV disease progression was observed. Despite the good HAdV infection outcome, cumulative incidence of transplant-related mortality in the HAdV group was 30%, compared to 10% in the patients that remained HAdV DNA-negative (*P*<0.01).
Discussion: There seems to be a benefit for prospective monitoring and preemptive therapy in pediatric HSCT recipients with HAdV viral load >10,000 copies/ml in terms of HAdV disease prevention. However, HAdV-associated morbidity exerts a toll on TRM, that may possibly be prevented by a prompter intervention with HAdV-specific T cells in patients with aGVHD and delayed immune reconstitution.
Disclosure of Interest: None Declared.

PH-P510

SURVEY OF CURRENT PRACTICES USED FOR PREVENTION OF CENTRAL VENOUS CATHETER ASSOCIATED INFECTION (CLABSI) IN HEMATOPOIETIC STEM CELL TRANSPLANTATION RECIPIENTS

E. Snarski[1,], A. Mank[2], S. Iacobelli[3], J. Hoek[4], J. Styczyński[5], S. Cesaro[6], E. Johansson[7] and Infectious Diseases Working Party of EBMT*
[1]Hematology, Oncology and Internal Diseases, Medical University of Warsaw, Warszawa, Poland, [2]Department of Oncology/Hematology, Academic Medical Center, Amsterda, Belgium, [3]Dipartimento di Medicina dei sistemi, Università degli Studi di Roma "Tor Vergata", Roma, Italy, [4]Data Office, EBMT, Leiden, Belgium, [5]Department of Paediatric Haematology and Oncology, Medical University of Bydgoszcz, Bydgoszcz, Poland, [6]Pediatric Hematology Oncology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy, [7]Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden

Introduction: In 2009 Infectious Diseases Working Party of EBMT with other international bodies introduced updated evidence based guidelines for placement and management of central venous catheters in patients undergoing hematopoietic stem cell transplantation. The central venous catheter infection prevention bundle consists of recommendations regarding hand hygiene, full barrier precautions, cleaning the insertion site with chlorhexidine, avoiding femoral sites for insertion, and removing unnecessary catheters. The aim of this study was to survey recommendations included in standard operating procedures (SOP) and current clinical practice regarding CLABSI prevention, infection monitoring and education on CVCs at hematologic and oncologic EBMT centers performing HSCT.
Materials (or patients) and Methods: Setting and sample This cross sectional study was performed during January to June 2013 among European centers performing HSCT and being a member of EBMT. Among the 545 EBMT transplant centers worldwide 103 (19%) participated. The questionnaire A questionnaire concerning recommendations included in standard operating procedures (SOP) of the center for management of CVCs to prevent CLABSI (Part A) and current practice regarding management of CVCs (Part B) was specifically developed for this study by the authors.
Results: CLABSI prevention bundle The recommended CLABSI prevention bundle consists of hand hygiene, full barrier precautions, cleaning the insertion site with chlorhexidine, avoiding femoral sites for insertion, and removing unnecessary catheters. In 33% of the centers all parts of the bundle were included in SOP. This corresponded to 27% of centers that fully incorporated recommended CLABSI prevention bundle into current clinical practice. The most common unfulfilled parts of CLABSI prevention bandle were lack of complete full barrier precautions and lack of use of chlorhexidine in clinical practice. Full barrier precautions All five parameters of full barrier precaution (use of cap, mask, sterile gloves, sterile gown, full size body drape: 60 x 60 cm) were fulfilled in 57% of the centers SOP and 36% reported these procedures being used in clinical practice. The most common unfulfilled criteria for full barrier precautions was lack of use of full size body drapes during CVC insertion. In clinical practice 35% of the centers used a full size body drape, 48% used a larger drape and 17% used a smaller drape than 60 x 60 cm.
Discussion: This is the first report on the current practice on CLABSI prevention among the EBMT centers. Among the surveyed centers only minority report implementation of the full recommended practices. The most common recommendations missing in clinical practice are use of all full barrier precautions and use of chlorhexidine containing solutions. Also considerable variation exists regarding preferred site of the central line insertion and every day care. The results of the study show that prevention of CLABSI is still a challenge for many centers and there is a need for improvement. The further study is needed to assess if observed differences between the centers could influence HSCT outcome at the centers.
Disclosure of Interest: None Declared.

PH-P511

BLOOD STREAM INFECTIONS CAUSED BY MULTI-DRUG-RESISTANT BACTERIA IN HEMATOLOGIC AND HSCT PATIENTS

M. E. Zannier[1,], A. Candoni[1], E. Simeone[1], D. Lazzarotto[1], F. Zanini[1], C. Scarparo[2], R. Fanin[1]*
[1]University of Udine and AOUD, [2]Division of Microbiology, Santa Maria della Misericordia Hospital, Udine, Italy

Introduction: We evaluate the incidence and outcome of blood stream infections (BSI) caused by multi-drug resistant (MDR) bacteria in patients (pts) with hematologic diseases and in hematopoietic stem cell transplantation (HSCT) recipients.
Materials (or patients) and Methods: 256 BSI have been recorded between october 2008 and october 2013 in 187 pts. The characteristics and outcome of MDR-BSIs have been evaluated. MDR-BSIs and multi-drug-sensible bacteria-BSI (MDS-BSI) have been compared. Moreover the results were evaluated according to the status of underling hematologic disease and the HSCT.
Results: 316 isolates have been documented in 256 BSIs. Among these, 55 (17%) bacteria were MDR and 261 (83%) were MDS.

MDR isolates were: MDR Pseudomonas aureginosa, 28/55 (51%); Escherichia coli ESBL, 16/55 (29%); Stenotrophomonas maltophilia, 6/55 (11%); Klebsiella pneumoniae-KPC, 4/55 (7%); Enterococcus sp-VRE, 1/55 (2%). A progressive increase of MDR-BSI was documented in the last two years (with 1,55 episodes/month in 2013).Septic shock and infection related mortality were significantly higher in the MDR-BSI group compared to MDS-BSI (36% vs 12% and 35% vs 11%, respectively – P less than 0,001). Occurrence of septic shock was particularly elevated in KPC-BSI (75%) and in MDR-P. aureginosa BSI (50%). Pts with refractory/relapsed hematologic disease had higher infection mortality rate (70%). In the MDR-BSI group, a higher mortality rate was observed in HSCT pts, (52% in HSCT vs 20% in No-HSCT, P less than 0,05).
Discussion: MDR-BSI (particularly MDR-*Pseudomonas aureginosa* and *Klebsiellae*-KPC) are an emerging and serious problem in oncohematologic pts. The onset of this MDR-BSI is often associated with septic shock and with high mortality rate, despite a target and combined antibiotic therapy. Mortality MDR-BSI related is particularly elevated in HSCT recipients (52%) and in pts with refractory/relapsed hematologic disease (70%). These results underline the urgent need of guidelines for surveillance and treatment of hematologic and HSCT pts with MDR bacteria colonization or/and active infection.
Disclosure of Interest: None Declared.

PH-P512
RELIABLE ASSAY FOR MONITORING CMV-SPECIFIC T CELL IMMUNITY FOLLOWING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

L. Brix[1,], D. Pan[2], C. Halgreen[1], T. Hahn[2], H. Pedersen[1], P. Wallace[2] and Philip Mcarthy, Dalin Pan, Kivin Jacobsen, Yali Zhang, Joseph Tario*
[1]Immudex, Copenhagen, Denmark, [2]Roswell Park Cancer Institute, Buffalo, United States

Introduction: Cytomegalovirus (CMV) infects and establishes persistent lifelong infections in 50-85% of adults. Reactivation of the virus is a frequently occurring complication of immunosuppression following transplantation and can significantly contribute to morbidity and mortality in such patients.
Reconstitution of CMV-specific T cell immunity after allogeneic hematopoietic cell transplantation (alloHCT) has previously been quantified using CMV tetramers, and shown to be a valuable aid in predicting patients at risk of developing CMV reactivation.
Materials (or patients) and Methods: We have developed an assay for quantifying CMV-specific CD8+ T cells using CMV-specific Dextramers. Dextramers are MHC multimer reagents that are used in flow cytometry to detect antigen-specific T cells in the blood. Dextramers have much higher resolution than conventional MHC multimers like Tetramers, and thus provide a more reliable means for identification of antigen-specific T cells.
Results: We here show that the CMV Dextramer assay including 7 alleles (HLA-A*01,-A*02, A*03, A*24, B*07, B*08 and B*35) has high specificity and sensitivity and accurately enumerates CMV-specific T cells in both healthy donors and alloHCT patients, with a lower detection limit of 0.08 cells/ul. The assay is highly reproducible with low intra and inter assay variation.
Using the CMV Dextramer assay we were able to quantify reconstitution of CMV T cell immunity post transplantation at day 30, 100, and 365 in 89 patients. Furthermore, in some recipients receiving transplants from HLA-mismatched donors we could measure CMV-specific T cells restricted by donors HLA and not recipients HLA, indicating that adoptive transfer of CMV-specific T cells can occur with alloHCT.
Discussion: This study demonstrates that CMV Dextramers are reliable reagents that can be used to monitor reconstitution of CMV immunity post-alloHCT, and shows that adoptive transfer of anti-CMV immunity can be quantified.
Disclosure of Interest: None Declared.

Multiple Myeloma

PH-P513
NON-MYELOABLATIVE ALLOGRAFTS IN MULTIPLE MYELOMA: TIME FOR A RE-LOOK?

S. D. J. Gibbs[1,], M. James Shipton[1], N. Bodagh[1], O. Rafael Rodriguez[1], F. Dignan[1], M. Waller[1], J. Woodward[1], E. Tholouli[1]*
[1]Clinical Haematology, Manchester Royal Infirmary, Manchester, United Kingdom

Introduction: Non-myeloablative allografts (NMA) remain contentious in the treatment of multiple myeloma (MM). We report our experience in 21 patients from 2005-2013 using a fludarabine and low-dose TBI regimen for NMA within 6 months of autologous stem cell transplantation (the Seattle regimen, see Rotta *et al*, Blood, 2010).
Materials (or patients) and Methods: We reviewed our transplant database medical files of all patients who had undergone NMA for MM since 2005.
Results: The median age was 56 (range 40-62). Males accounted for 15 out of 21 patients.
Five patients had sibling donors. All patients had a creatinine clearance of 45ml/min or greater at time of NMA.
13 patients were treated in first clonal remission, 7 in second remission and one in third remission. 16 had measured cytogenetic abnormalities, and the majority of patients had either poor risk cytogenetics and/or were ISS Stage III. 15 patients had their NMA in partial remission, three in very good partial remission and three in complete remission.
Median patient follow-up from NMA was 13.5 months (range 1-49). Estimated median overall survival was 28 months. Five patients have died since NMA. Transplant-related mortality at 2 years was 10%. No patients died as a consequence of the Seattle conditioning and the majority died from progressive disease. The patient who underwent NMA in third remission died within two months of progressive disease.
Median estimated progression-free survival was 21 months. Five patients had evidence of clonal relapse within a year of NMA. Five patients received DLI and six patients received lenalidomide-based salvage treatment, with good clonal responses observed in the majority.
Acute graft-versus-host disease (GVHD) was observed in 7 patients: 4 skin, 2 oral and 2 gut, Grades 1-3. Chronic GVHD was observed in 12 patients: 6 oral, 5 skin, 2 gut and 2 liver. 3 mild, 5 moderate and 4 severe.
Discussion: The role of NMA in the treatment for MM remains undefined. The Seattle regimen is easy to administer and well tolerated, even in poor risk and heavily pre-treated patients. GVHD was common but there was a trend that this was associated with improved survival. Clonal relapses were not uncommon in this particularly poor-risk population but salvageable in the majority. Larger numbers and further clinical trials are still required to determine the optimal place for NMA in the treatment of myeloma, and the optimum conditioning regimen.
Disclosure of Interest: None Declared.

PH-P514
HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA WITH RENAL FAILURE

O. Pokrovskaya[1,], L. Mendeleeva[1], E. Urnova[1], M. Firsova[1], M. Nareyko[1], L. Kuzmina[1], I. Rekhtina[1], I. Tauson[1], E. Varlamova[1], E. Parovichnikova[1], V. Savchenko[1]*
[1]National Research Center for Hematology, Moscow, Russian Federation

Introduction: Renal failure (RF) is a common complication in multiple myeloma (MM). It has been associated with inferior survival in patients (pts) treated with conventional regimens. The impact

of renal failure on prognosis of multiple myeloma patients treated with high-dose chemotherapy and autologous stem cell transplantation (HDT/ASCT) is incompletely studied as those pts are usually not included in clinical trials. A retrospective study was performed to evaluate the efficacy and long-term outcomes in a cohort of MM pts with RF, especially in pts with end-stage RF on dialysis.
Materials (or patients) and Methods: From September 2001 to August 2013, 164 patients with MM were transplanted in the department of Bone Marrow Transplantation. Within this group 32 (19.5%) had RF at diagnosis, 6 (4.5%) were dialysis-dependent. Among them there were 12 (37.5%) pts with myeloma G, 7 (21.9) with myeloma A, 11 (34.4%) with light-chain myeloma, 1 (3.1%) with myeloma D and 1 (3.1%) with plasma-cell leukemia. In 132 (80.5%) pts renal function was normal. In this group 76 (57.6%) pts had myeloma G, 27 (20,4%) – myeloma A, 23 (17.5) - light-chain myeloma, 3 (2.25%) - non-secretory myeloma, 1 (0.75%) – myeloma D and 1 (0.75%) – plasma-cell leukemia. Pts received VAD chemotherapy and/or bortezomib + dexamethasone +/– doxorubicin +/– cyclophosphamide as induction therapy. The main mobilization regimen was cyclophosphamide (4-6 g/m^2) followed by daily administration of G-CSF, pts with end-stage RF received G-CSF only. HDT/ASCT entailed conditioning with melphalan 140-200 mg/m^2. Response was defined according to IMWG criteria.
Results: After the induction the overall response (CR+VGPR+PR) was 90,1% with 34,8% of CR and 31,8% of VGPR in the group of pts with normal renal function. Among pts with RF overall response was achieved in 93,7% pts with 53,1% of CR and 21,9% of VGPR. Renal response was developed in 24/32 (75%) pts after induction therapy. An improvement in glomerular filtration rate to >15 ml/min/1,73 m^2 was noted in 2 more cases post HDT/ASCT. 1 pt became dialysis-independent after induction, another 2 pts – after ASCT. The treatment-related toxicity was slightly higher in pts with end-stage RF after ASCT, all of them required i.v. antibiotics due to neutropenic fever, while 30% pts with normal renal function do not undergo any infectious complications. The median time of follow-up after ASCT was 30 months (2,5-127). In all 164 pts the median of OS was not reached, the probability of PFS at 5-y was 45,3%. Transplant-related mortality was 0,6%. 138 pts (84,1%) are still alive. There was no significant difference in PFS and OS in pts with or without RF at the time of diagnosis and ASCT.
Discussion: In pts with myeloma and RF, despite little differences in early post-transplant period, the hematological outcome after HDT/ASCT is similar to those with normal renal function at the time of diagnosis and autografting. RF may be partially reversible following treatment with HDT/ASCT and some pts become dialysis-independent.
Disclosure of Interest: None Declared.

PH-P515

PERSISTENCE OF EXTRAMEDULLARY DISEASE BY PET/CT IS AN INDEPENDENT PREDICTOR OF ADVERSE OUTCOME AFTER ALLOGENEIC TRANSPLANT IN MULTIPLE MYELOMA

F. Patriarca[1,], F. Carobolante[1], E. Zamagni[2], V. Montefusco [3], B. Bruno[4], E. Englaro[5], M. Isola[6], S. Buttignol[1], R. Stocchi[1], P. Corradini[3], M. Cavo[2], R. Fanin[1]*
[1]Division of Hematology, University of Udine, Udine, [2]Istituto Seragnoli, Università di Bologna, Bologna, [3]Istituto Tumori, Milano, [4]Università di Torino, Torino, [5]Nuclear Medicine Institute, Udine University Hospital, [6]Statistics Institute, University of Udine, Udine, Italy

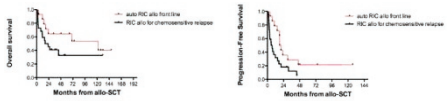
Introduction: Positron emission tomography integrated with computed tomography (PET/CT) has been reported to be useful for screening of myelomatous lesions in multiple myeloma (MM) patients at diagnosis and for monitoring response to therapy and prognosis after autologous stem cell transplantation (auto-SCT). Aim of the study was to prospectively evaluate the prognostic significance of PET/CT in MM patients who received allogeneic stem cell transplantation (allo-SCT).
Materials (or patients) and Methods: Patients were studied with PET/CT before and within 6 months after allo-SCT. The number, the maximum standard uptake value (SUV) and the location (medullary or extramedullary) of focal lesions (FL) were recorded and PET/CT was considered positive if it showed at least 1 FL. The outcome of PET/CT positive and negative patients was compared in term of PFS, TTP and OS after allo- SCT. Multivariate Cox regression analyses were performed to identify pre transplant and post treatment prognostic factors significantly affecting PFS, TTP and OS.
Results: A total of 67 patients, median age of 51 years were analyzed. High risk MM features (ISS stage 3 or unfavourable FISH karyotype or CNS localization or plasma cell leukemia) were present in 51% of patients before allo-SCT. All pts received upfront auto-SCT, followed by allo-SCT within 3 months in 27 cases, while other 40 cases performed allo-SCT after failure of previous auto-SCT. Median time between diagnosis and allo-SCT was 36 months (8-128). Conditioning was at reduced intensity in 88%. Fifty-five per cent were transplanted from unrelated donors. Two-year NRM, PFS and OS were 23%, 44% and 67%, respectively. Before allo-SCT, 34/54 patients (63%) had a positive PET/CT, 21/54 (39%) had SUV > 4.2 and 6 patients (11%) had extramedullary disease (EMD). Rate of PET negativity was significantly higher in patients with the deepest response before transplant: in fact, 8/13 (61%) patients in CR were PET negative, in comparison with 14/40 (35%) patients in VGPR or less (P= 0.04). On univariate analysis, persistence of at least 1 FL, SUV > 4.2 and EMD before allo-SCT adversely affected PFS , TTP,and OS. In a Cox regression analysis of prognostic factors before allo-SCT, persistence of EMD at transplant was an independent predictor of worst TTP and PFS. OS was negatively influenced by unrelated donor and SUV higher than 4.2. Six months after allo-SCT, PET-CT was negative in 27/59 pts (46%), whose 2-year rate of OS was superior to that of PET-positive pts (80% vs 63%, P=0.01). Multivariate analysis of pre transplant and post treatment variables showed that persistence of EMD and failure to obtain CR or VGPR after allo-SCT were strongly associated with shorter PFS, TTP and OS. Moreover, allo-SCT at relapse was an additional independent predictor of worse OS.
Discussion: Our study shows that PET-CT adds information of prognostic significance to conventional evaluation of clinical and biochemical response before and after allo-SCT in MM. Persistence of EMD by PET-CT before and after allo-SCT was an independent prognostic factor for poor PFS,TTP and OS. Moreover, persistence of high tumor metabolism before transplant was associated with worse OS. These data suggest that PET/CT scanning can be an useful tool for MM restaging before allo-SCT and for monitoring response after transplant.
Disclosure of Interest: None Declared.

PH-P516

OUTCOME AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION WITH REDUCED-INTENSITY CONDITIONING IN PATIENTS WITH MULTIPLE MYELOMA.

J. Bourcier[1,], F. Malard[1], P. Chevallier[1], T. Guillaume[1], J. Delaunay[1], P. Peterlin[1], M. Mohty[2], P. Moreau[1]*
[1]CHU Nantes, Nantes, [2]CHU de l'AP-HP, Paris, France

Introduction: The use of new agents has considerably improved the survival of patients with multiple myeloma (MM). Nevertheless, MM remains incurable with conventional therapy and curative strategies are needed, especially in young patients. In this setting, allogeneic stem cell transplantation (allo-SCT) could be the only curative treatment, through graft-versus-myeloma effect. However, high non relapse mortality (NRM) remains a concern, especially with conventional myeloablative conditioning regimens. Reduced intensity conditioning (RIC) have been developed with the aim to decrease NRM, despite the risk to increase relapse. With this background, this study aims at evaluating patient outcome after RIC allo-SCT for MM.
Materials (or patients) and Methods: In this single center retrospective trial, we have analyzed the outcome of 36 consecutive patients who received a RIC allo-SCT for MM between 1998



and 2011 at Nantes. The cohort included 23 males (64%) and 13 females (36%) with a median age at time of allo-SCT of 55 (range, 37-65) years. Patients received a median of 2 lines of treatment (ranges, 1-4) before allo-SCT. 14 patients (39%) with high-risk MM (del13 by FISH + high beta2-microglobuline at diagnosis) received allo as part of frontline therapy, in a tandem auto-RIC allo program (protocol IFM99-03, Moreau *et al*, Blood 2008;112:3914-3915) and 22 patients (61%) received allo as part of salvage therapy and all but 1 had chemosensitive disease at the time of allo. PBSC were used as stem cell source in 35 patients (97%), while one patient received BM. A MRD was used in 24 cases (67%) and an UD in 12 cases (33%). The conditioning regimen was based on the combination of fludarabine and busulfan and high-dose ATG in patients who received frontline RIC allo (14 cases), while the others received either fludarabine and 2 Gy TBI (2 patients) or fludarabine, melphalan and bortezomib (20 patients) as part of conditioning prior to RIC allo.

Results: The median time between MM diagnosis and allo-SCT was 66 months. The median follow-up among surviving patients was 48 months (range, 25-132). Overall, the cumulative incidence of acute GVHD grade III-IV was 25% and the cumulative incidence of extensive chronic GVHD at 1 year and 5 years were 30% and 30%, respectively. At 3 months, 43% of patients were in CR. Relapse or disease progression occurred in 22 patients at a median of 11 months (ranges, 2-46) after allo-SCT, the cumulative incidence of relapse/progression was 33% at 1 year and 63% at 5 years. The cumulative incidence of NRM was 19% at 1 year and 34% at 5 years. For the whole group of 36 patients, the KM estimates of OS and PFS at 5 years after allo-SCT were 46% and 12%, respectively. When comparing the group of 14 high-risk patients treated with the tandem auto/RIC allo program as part of front treatment versus the group of 22 patients treated by RIC for chemosensitive relapse later in the course of the disease, the 5-years PFS and OS estimates were 21.4% vs 6.1% (P = .02), and 64.3% vs 32.7% (P =0.12).

Discussion: A frontline tandem allo-RIC program may induce durable responses (and cure?) in one fifth of the patients. PFS of patients treated with RIC as salvage therapy is dramatically poor, indicating the low probability for cure. Nevertheless, OS in both groups of patients is high, indicating that patients can be salvaged by novel-agent based therapies following relapses after RIC-allo.

Disclosure of Interest: None Declared.

PH-P517
BEAM VS MELPHALAN BASED CONDITIONING THERAPY FOR SECOND AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) FOR MULTIPLE MYELOMA (MM)

M. Veeraputhiran[1,], T. Jain[2], A. Deol[1,2,3], S. Kim[4], G. Dyson[4], J. Uberti[1,2,3], M. Abidi[1,2,3]*

[1]Hematology/Oncology, Karmanos Cancer Institute, [2]Internal Medicine, Wayne State University, [3]Blood and Marrow Stem Cell Transplant Program, [4]Biostatistics Core, Karmanos Cancer Institute, Detroit, United States

Introduction: Salvage ASCT is increasingly utilized for eligible MM patients (pts). Despite concerns for resistance, high dose melphalan (HDM) is predominantly used as conditioning regimen for 2nd /or salvage ASCT. BEAM (Carmustine, etoposide, Ara-C and Melphalan) may have superior outcomes compared to HDM during 1st ASCT for MM pts as shown in a single institution study. We conducted a retrospective analysis at our institution to evaluate toxicity and response with BEAM conditioning used as salvage therapy after HDM ASCT.

Materials (or patients) and Methods: Thirty eight pts who received 2nd ASCT for MM (2008-2013) were identified. Nineteen pts received HDM (140-200mg/m^2; Group A) and an equal number received BEAM (carmustine 300 mg/m^2, etoposide 100 mg/m^2, cytarabine 100 mg/m^2, and melphalan 140 mg/m^2; Group B). Patient characteristics, toxicity and response at day 100, progression free survival (PFS) were collected and compared between the 2 groups. Fischer's exact, Cochran-Mantel-Haenszel tests and log rank tests were used to compare responses, toxicities and PFS.

Results: The median age of the patients was 59 yrs (range: 39-72) in Group A and 58 yrs (range: 44-68) in Group B. Group A had more female pts (53% vs 32%) and a decreased baseline renal function (median creatinine clearance 84 vs 108 ml/min) compared to Group B. All patients in both groups received salvage therapy prior to 2nd ASCT and received stem cells which were collected/cryopreserved prior to the 1st ASCT. The median time between 1st and 2nd ASCTs were 47 (range: 17-68) vs 34 (range: 18-59) months in group A and B respectively. There was no significant difference in disease status pre-2nd ASCT between the 2 groups. In group A, eight pts (50%) received reduced dose melphalan (140mg/m^2) due to renal insufficiency while the remaining eight pts received HDM (Median 200mg/m^2, range 180-240mg/m^2). At day +100 post 2nd ASCT nine patients in each group had CR (Table 1). The median time of follow-up after 2nd ASCT was 9 and 5 months for groups A and B respectively. The median PFS were 12.9 vs 7.7 months (P=0.62) for Group A and B respectively (Figure-1). Group B had a higher incidence of febrile neutropenia (19 vs 13 pts, P=0.02) and longer hospitalization (22 vs 15 days, P <0.0001). Other toxicities were not significantly different between these groups.

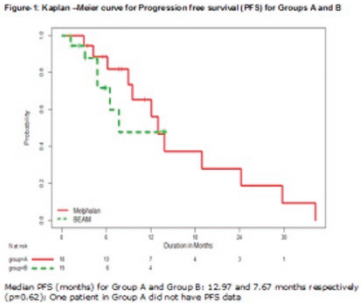
Discussion: BEAM seems to be a reasonable alternative conditioning regimen for 2nd ASCT with comparable safety and efficacy to HDM, however requires prolonged hospitalization and has increased incidence of febrile neutropenia. Longer follow-up

[PH-P517]

Table-1: Data Summary

Variables	Group A, N=19	Group B, N=19
Age* (yrs)	59 (39-72)	58 (44-68)
KPS* (%)	70 (60-80)	70 (60-80)
Creatinine Clearance pre 2nd ASCT* (ml/min)	84 (42-179)	108 (44-218)
CR pre- 2nd ASCT [n (%)]	5 (26)	4 (21)
CD34+ cells infused for 2nd ASCT* (x 10^6 cells/kg)	3.37 (1.69-47.1)	3.48 (2.79-12.04)
Febrile Neutropenia [n (%)]	13 (68)	19 (100)
In-patient stay for 2nd ASCT* (days)	15 (12-24)	22 (19-51)
CR at Day +100 post 2nd ASCT [n (%)]	9¶ (47)	9¶ (47)

KPS- Karnofsky's Performance score; CR- Complete response; ASCT- Autologous Stem cell transplant; * Values are median with range in parenthesis; ¶ Day +100 response data not available for 2 patients in Group A; ℓ Day +100 response data not available for 3 patients in Group B



is needed to determine whether there is any significant difference in PFS and or OS between the two groups.
Disclosure of Interest: None Declared.

PH-P518

VERY LOW RATE OF RE-ADMISSION AFTER AN EARLY DISCHARGE OUTPATIENT MODEL FOR AUTOGRAFTING IN MULTIPLE MYELOMA PATIENTS: AN ITALIAN MULTI-CENTER RETROSPECTIVE STUDY

M. Martino[1,], M. Montanari[2], F. Ferrara[3], F. Ciceri[4], I. Scortechini[2], G. Console[5], G. Messina[1], G. Irrera[1], S. Palmieri[3], S. Marktel[4], M. Cimminiello[6], L. Castagna[7], G. Milone [8], M. Offidani[2], G. Tripepi[9], B. Bruno[10], R. Massimo Lemoli[11], A. Rambaldi[12], A. Olivieri[2] and on behalf of "Gruppo Italiano per il Trapianto di Midollo Osseo, Cellule staminali emopoietiche e terapia cellulare" (GITMO) – Sezione Trapianto Autologo*

[1]Azienda Ospedaliera BMM, Reggio Calabria, Hematology and Bone Marrow Transplant Unit, Reggio Calabria, [2]Clinica di Ematologia, Azienda Ospedaliero-Universitaria Ospedali Riuniti, Ancona, [3]Ematologia con Trapianto di Midollo Osseo, Azienda Ospedaliera "A. Cardarelli", Napoli, [4]Ematologia con Trapianto di Midollo Osseo IRCCS Ospedale "San Raffaele", Milan, [5]Hematology and Bone Marrow Transplant Unit, Reggio Calabria, [6]Ematologia con Trapianto di Midollo Osseo, Azienda Ospedaliera "San Carlo", Potenza, [7]Humanitas Cancer Center, Rozzano (Milano), [8]Unita` Trapianto di Midollo Osseo, Dipartimento di Ematologia, Azienda Ospedaliera "Policlinico Vittorio Emanuele", Catania, [9]Consiglio Nazionale delle Ricerche, Istituto di Biomedicina ed Immunologia Molecolare, Reggio Calabria, [10]Divisione Universitaria di Ematologia, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Torino, [11]Cattedra di Ematologia, Dipartimento di Medicina Interna e Specialità Mediche, Università di Genova, Genova, [12]Ematologia con Trapianto di Midollo Osseo, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy

Introduction: We analyzed main modalities and clinical outcomes of the early discharge outpatient model- autologous stem cell transplantation (EDOM-ASCT) for multiple myeloma in Italy.
Materials (or patients) and Methods: This retrospective study has been conducted through the GITMO trial office, which promotes independent clinical research studies in the setting of both autologous and allogeneic transplantation in Italy. A first questionnaire was mailed to 75 GITMO Centers accredited for autologous transplant to evaluate how many had been involved in outpatient transplant models for MM patients between 1998 and 2012. If a given Center was involved, further specific queries included patient selection criteria, infectious prophylaxis, supportive care, criteria for hospital re-admission, management of febrile neutropenia, and clinical outcomes. Overall, 55/75 (73.3%) answered the first questionnaire, and among these 6 had been involved in outpatient transplant programs according to "EDOM".
Results: EDOM-ASCT was applied in 382 patients, for a total of 522 procedures, between 1998 and 2012. Our study showed high homogeneity among Centers in terms of inclusion criteria and supportive care, and in-hospital re-admission criteria. Overall, re-admissions during the aplastic phase occurred in 98 of 522 transplants (18.8%). Major extra-hematological complication was neutropenic fever in 161 cases (30.8%) that required re-admission in 76 cases. Incidence of severe WHO 3-4 mucositis was 9.6%. By univariate analysis, fever, mucositis, renal function at diagnosis, second transplant, transplant performed late in the course of the disease were significantly correlated with re-admission, whereas fever, mucositis, renal function and timing of transplant were the only independent predictors by multivariate analysis. Overall, transplant related mortality was 1.0%. No center effect was observed in this study (P=0.36).
Discussion: The low rate of re-admission and the safety of this EDOM-ASCT in myeloma indicates that this strategy could be extended to other transplant centers if a stringent policy for patient selection and management is applied.
Disclosure of Interest: None Declared.

PH-P519

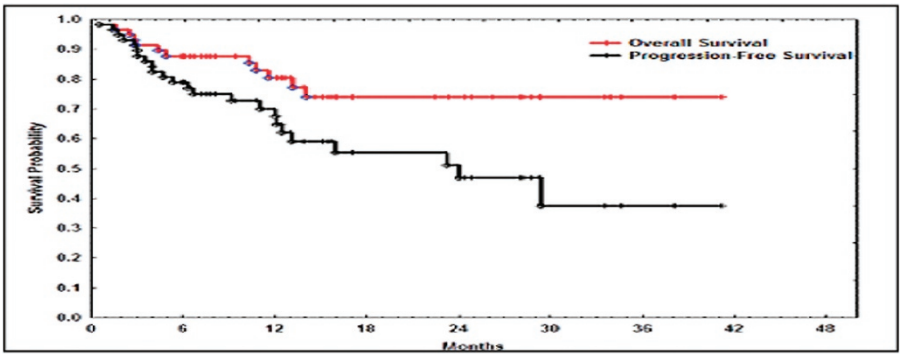
PHASE I/II TRIAL OF LENALIDOMIDE AND HIGH-DOSE MELPHALAN AS PREPARATIVE REGIMEN FOR RELAPSED MYELOMA

M. Qazilbash[1,], P. Thall[2], P. Fox[2], N. Shah[1], Q. Bashir[1], J. Shah[3], S. Parmar[1], P. Kebriaei[1], Y. Nieto[1], Y. Dinh[1], U. Popat[1], C. Hosing[1], P. Lin[4], A. Afrough[1], E. Shpall[1], R. Orlowski[3], R. Champlin[1]*

[1]Stem Cell Transplantation and Cellular Therapy, [2]Biostatistics, [3]Lymphoma/Myeloma, [4]Hematopathology Adm, U.T. MD ANDERSON CANCER CENTER, Houston, United States

Introduction: Lenalidomide (LEN) in combination with standard-dose melphalan has shown significant anti-multiple myeloma (MM) activity. In this phase I/II trial we evaluated the safety and efficacy of combining escalating doses of LEN and high-dose melphalan (HDM) for patients undergoing autologous hematopoietic stem cell transplantation (auto-HCT) for relapsed MM.
Materials (or patients) and Methods: Doses were chosen adaptively for successive cohorts of size 3 using an extension of the safety-efficacy trade-off design. Each cohort received melphalan 200 mg/m^2 plus LEN at one of the four dose levels orally, for 7 days. Primary endpoints were dose-limiting toxicity (DLT), defined as regimen-related death, graft failure, grade 3 or 4 atrial fibrillation, grade 4 deep venous thrombosis, or pulmonary embolism occurring within 30 days post auto-HCT, and efficacy, defined as complete response (CR) at day +90.
Results: Fifty-seven patients were enrolled between March 2010 and April 2013. After safely escalating to 100 mg of LEN, patients were adaptively randomized among the 4 LEN dose levels. Three, 5, 24 and 25 patients were treated at 25, 50, 75 or 100 mg respectively. Median age at auto-HCT was 60 (34-72) years, and median time from diagnosis to auto-HCT was 33 (5-214) months. Sixteen (28%) patients had high-risk abnormalities on conventional cytogenetic or fluorescent in-situ hybridization (FISH) studies. Median prior lines of treatment were 3 (1-11). Twenty-two (39%) patients were LEN-refractory, 30 (53%) bortezomib-refractory and 13 (23%) refractory to both. Eighteen patients (32%) had a prior auto-HCT. DLT was not seen at any of the 4 dose levels prior to adaptive randomization. Grade 3-4 non-hematologic toxicity was seen in 40 (70%) patients, with no significant differences between the 4 dose levels. Two patients died of nonrelapse causes (viral infection 1, cardiac failure 1) with a TRM of 3%. Median time to both neutrophil and platelet engraftment was 11 days. At day 90, 8 (14%) patients achieved a CR, 25 (44%) achieved CR or very good partial response (VGPR), and 42 (74%) achieved CR, VGPR or partial response (PR), with no significant differences in response rates among the 4 LEN dose levels. By day 180 and post auto-HCT, 12 patients (21%) had achieved a CR. Thirty-six (63%) patients received post auto-HCT maintenance therapy (lenalidomide 32, bortezomib 2, and pomalidomide 2). With a median follow up of 12.3 months (range 0.5-41), median progression-free (PFS) was 24 months and median overall survival (OS) has not yet been reached. Two-year PFS and OS were 48% and 72%, respectively. These compare favorably with our previous experience of 2-year PFS and OS of 24% and 59%, respectively, after salvage auto-HCT with melphalan alone. There was no significant difference in median PFS (13 months) and OS (not reached yet) in patients undergoing a 2nd auto-HCT, compared to patients undergoing first auto-HCT (P=0.47 and 0.85, respectively). No second primary malignancy (SPM) has been seen so far.
Discussion: LEN up to 100 mg daily x 7 days can be safely combined with high-dose melphalan for relapsed/refractory myeloma. The regimen is associated with a high overall response rate, 2 year PFS and OS of 48% and 72% in relapsed and heavily pretreated patients, and no SPM after a follow up extending to 41 months.
Disclosure of Interest: None Declared.

[PH-P519]



PH-P520

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN LIGHT CHAIN AMYLOIDOSIS (AL) WITH RENAL INVOLVEMENT

A. M. Cornelison[1,], R. M. Saliba[1], Y. Dinh[1], S. Ahmed[1], Y. Nieto[1], Q. Bashir[1], N. Shah[1], J. Howell[1], S. Parmar[1], C. Hosing[1], U. Popat[1], E. Shpall[1], R. Champlin[1], M. Qazilbash[1]*

[1]Stem Cell Transplantation and Cellular Therapy, University of Texas MD Anderson Cancer Center, Houston, United States

Introduction: Immunoglobulin light chain amyloidosis (AL) is a plasma cell proliferative disorder characterized by deposition of insoluble fibrils composed of immunoglobulin light chains, causing progressive organ dysfunction. In 50% of cases of AL, there is documented renal involvement, which, if left untreated, progresses to end-stage renal disease and is often associated with significant morbidity.

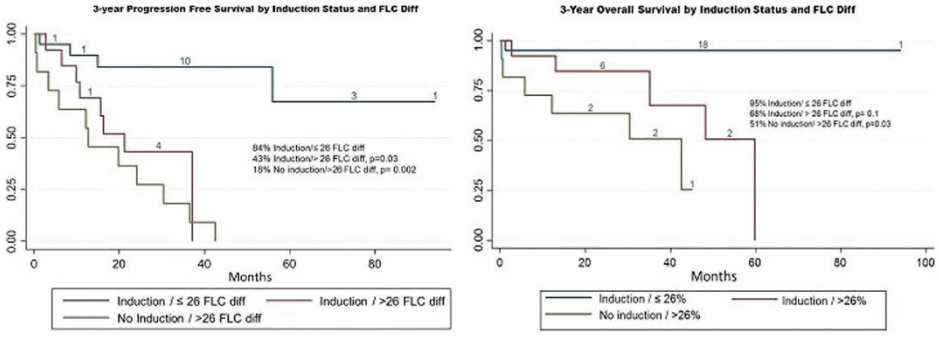
Materials (or patients) and Methods: We performed a retrospective analysis in 72 patients (pts) with AL who underwent high-dose chemotherapy and autologous hematopoietic stem cell transplantation (auto-HCT) at our institution between 1999 and 2011. Fifty-five of these AL pts had renal involvement, as defined by the International Consensus Criteria. Primary objectives were to assess hematologic and organ response, progression free survival (PFS), overall survival (OS) and correlation between hematologic and organ response.

Results: Median age at auto-HCT was 59 years (range, 41-74). Median time from diagnosis to auto-HCT was 6.6 months (range, 2.2-121.9). Forty-one pts (75%) had lambda light chain disease. Additional visceral organs involved were: heart in 5 pts (9%), liver in 5 pts (9%), GI tract in 3 pts (5%), and peripheral nerves in 1 pt (2%). Median baseline proteinuria was 5.055 grams (range, 0.174-27.854 grams) in a 24 hour urine specimen. Median baseline serum creatinine was 1.20 mg/dL (range, 0.53-9.28). All pts received melphalan or melphalan-based combinations as their preparative regimen. Median time to neutrophil engraftment was 10 days (range, 7-15). Median follow up from auto-HCT was 37 months (range, 4-126). Six pts died of non-relapse causes with a treatment-related mortality (TRM) at both 100 days and 1 year of 11% (95% CI 5-23). Fifty pts were evaluable for a hematologic response, while 5 pts were inevaluable due to early death. Eight (16%) pts achieved complete remission (CR), 10 (20%) achieved a very good partial remission (VGPR) and 21 (42%) achieved a partial remission (PR), with an overall response rate of 78%. Organ response was evaluated at 6, 12, and 24 months, and was defined as a >50% decrease in total proteinuria over 24 hours without an increase of >25% of serum creatinine. Organ response was demonstrated in 8 (16%) of 50 evaluable pts at 6 months, in 13 (31%) of 42 evaluable pts at 12 months, and in 17 (49%) of 35 evaluable pts at 24 months, with a median decrease in proteinuria of 1.21 grams, 1.24 grams, and 2.86 grams at 6, 12, and 24 months, respectively. Organ responses at 2-years post auto-HCT were seen in 15 of 28 (54%) pts with > PR and 2 of 7 pts (29%) with <PR (P=0.2). Kaplan-Meier estimates of 3-year PFS and OS were 54% and 73%, respectively. In a multivariate model, induction prior to auto-HCT and a low absolute difference between involved and uninvolved serum free light chains (FLC diff ≤ 26 mg/dL) were associated with longer PFS and OS. At the time of last follow-up, 24 pts (44%) were alive and in remission.

Discussion: High-dose melphalan and auto-HCT is associated with durable hematologic and organ response and prolonged survival in patients with AL and renal involvement. Induction therapy prior to auto-HCT and absolute FLC diff ≤ 26 mg/dL predicted longer PFS and OS.

Disclosure of Interest: None Declared.

[PH-P520]



PH-P521

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AUTO-HCT) FOR MULTIPLE MYELOMA (MM) IN THE INPATIENT (INPT) VS. OUTPATIENT (OUTPT) SETTINGS

A. M. Cornelison[1,], R. M. Saliba[1], Y. Dinh[1], S. Ahmed[1], Y. Nieto[1], Q. Bashir[1], N. Shah[1], S. Parmar[1], C. Hosing[1], U. Popat[1], E. Shpall[1], R. Champlin[1], M. Qazilbash[1]*

[1]Stem Cell Transplantation and Cellular Therapy, University of Texas MD Anderson Cancer Center, Houston, United States

Introduction: High-dose chemotherapy followed by auto-HCT is the standard of care for pts with MM. Auto-HCT can be performed in the outpt setting in appropriately selected pts and may be as safe and more cost effective than in the inpt setting. We compared the characteristics and outcomes of pts with MM who underwent auto-HCT in both inpt and outpt settings between January 2008 and December 2012 at our institution.

Materials (or patients) and Methods: We identified a total of 1046 pts, with 669 transplanted as inpts and 377 as outpts. Primary endpoints were time to neutrophil and platelet engraftment, 100-day treatment-related mortality (TRM), grade 2-4 adverse events (AEs), defined by the National Cancer Institute's Common Terminology Criteria, version 4, incidence of unscheduled admissions, and average cost of auto-HCT in both inpt and outpt groups. Secondary endpoints were overall response (OR) rates, progression-free survival (PFS), and overall survival (OS).

Results: Median age at auto-HCT was 62 (range, 31-82) in inpts and 58 (range, 34-78) years in outpts, (P<0.001). Median Karnofsky performance score in both groups was 90. There was no significant difference in baseline cytogenetic abnormalities, median bone marrow plasma cell percentage, serum LDH or ISS stage II/III between the 2 groups. Forty-four (6.6%) inpts and 7 (2%) outpts had serum creatinine of ≥2 mg/dL (P=<0.001). Twenty-nine percent of inpts vs. 21% of outpts had relapsed disease at auto-HCT (P=0.02). Melphalan alone was used as the preparative regimen in 81% of inpts vs. 95% of outpts (P<0.002). There was no significant difference in the median CD34 cell dose infused (P=0.4). Median follow up in inpts vs. outpts was 14 and 15 months, respectively. Subsequent unscheduled hospitalizations were required in 208 (55%) outpts, with neutropenic or non-neutropenic fever being the most common reason (157 pts: 42%). Median time to neutrophil and platelet engraftment in both groups was 11 days. One-hundred day TRM was 1.5% in inpts vs. 0.3% in outpts (P=0.09). Grade 2-4 AEs were seen in 552 (83%) inpts vs. 277 (73%) outpts, (P=0.001); grade 3-4 in 359 (54%) inpts vs. 163 (43%) outpts, (P=0.001); and grade 4 in 19 (3%) inpts vs. 6 (2%) outpts, (P=0.2). OR rates were significantly better in outpts, with 364 (96.5%) outpts with ≥PR vs. 599 (89%) inpts (P<0.001). Outpts had significantly longer 2-year PFS (60% vs. 50%, P=0.005, HR 0.7, 95% CI 0.6-0.9) (Figure 1) and OS (83% vs. 77%, P=0.01, HR 0.6, 95% CI 04-0.9) (Figure 2). The average cost of auto-HCT from the date of original consult to day +30 was $416,154 for inpts and $292,572 for outpts.

Discussion: In this study, pts selected for auto-HCT in the outpt setting were younger, with significantly better renal function at diagnosis, and were more likely to receive conditioning with single agent melphalan. Outpt auto-HCTs were associated with fewer grade 2/3 AEs, significantly better OR rates, and improved PFS and OS compared to inpts, which may reflect healthier pts transplanted as outpts. Fifty-five percent of outpts required unscheduled hospitalization, most commonly for fever, with a cost savings of approximately $125,000 for those who received auto-HCT in the outpt setting. Appropriately selected pts with MM can safely undergo auto-HCT in the outpt setting without any significant increase in toxicity or TRM.

Disclosure of Interest: None Declared.

PH-P522

REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION IN MULTIPLE MYELOMA

S. Hassan[1,], J. Matthews[2], A. Guy[1], M. Farrell[1], D. Taussig[2], M. Smith[1], H. Oakervee[1], J. Cavenagh[1], J. Gribben[2]*

[1]Haemato-oncology, St Bartholomew's Hospital, [2]Haemato-oncology, Barts Cancer Institute, London, United Kingdom

Introduction: Allogeneic transplantation in myeloma remains a controversial area. Following unacceptable non-relapse mortality (NRM) with myeloablative conditioning, the advent of reduced-intensity conditioning allogeneic stem cell transplantation (RIC-allo-SCT) has reintroduced this treatment modality, although its role remains unclear. This study aims to analyse outcomes using this approach and identify which patients may benefit from it most.

Materials (or patients) and Methods: A retrospective analysis of 35 RIC-allo-SCTs performed in patients with myeloma between 1999 and 2011 was carried out. 71% had a sibling donor and the remainder had matched unrelated donors. Fludarabine and cyclophosphamide conditioning was used in 34 cases with HLA matched donors and 1 case where the donor was a 1X mismatch received Alemtuzumab in addition. Peripheral blood was the stem cell source in all cases.

Results: 82% patients were male and the median age at the time of transplant was 52 years. 11, 6 and 10 patients had an ISS of 1, 2 and 3 respectively at diagnosis (data was unavailable on the remainder). 2 patients failed to harvest stem cells and had a RIC allo-SCT in first response. 10 patients had a tandem autologous-allogeneic approach having achieved a CR following autograft. 11 patients received maintenance or further chemotherapy following their initial autograft to improve response, prior to proceeding with RIC-allo-SCT. 4 patients had their RIC-allo-SCT following relapse and a second autograft and 2 had it following relapse but failure to harvest cells for a second autograft. There was no data on the remainder. Methotrexate and cyclosoporin were used for GVHD prophylaxis, apart from in 2 cases where cyclosporin was not tolerated. There were no cases of engraftment failure. GVHD occurred in 81% of cases (20% acute, 80% chronic). 57% were

[PH-P521]

Figure 1. 2-Year Progression Free Survival

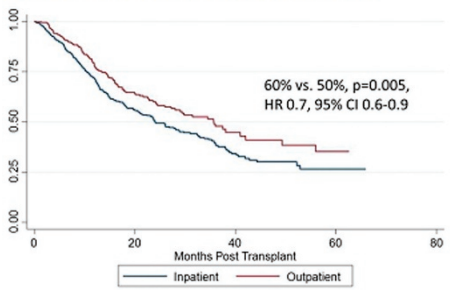
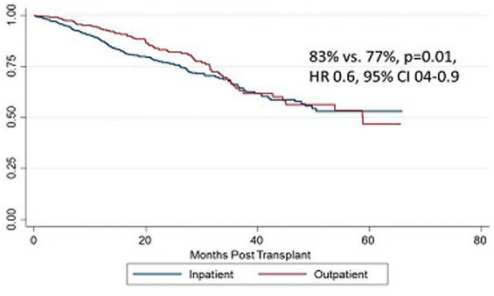


Figure 2. 2-Year Overall Survival



Grade II-IV. 4 patients received DLI; 3 for disease progression all of whom died within the following 12 months. 1 received DLI for low T-cell chimerism. This was followed by the onset of GVHD 1 month later and the patient remains alive 6 years later. 100 day NRM was 5% (*n*=2). Cumulative 1 year NRM was 11% with 1 patient having a GVHD-related death. 3 year NRM mortality remained at 11%. Best responses post allograft were 72% CR and 28% PR from all evaluable cases (17% data unavailable). Median overall survival (OS) from RIC allo-SCT for all patients was 5.2 years. Median progression free survival (PFS) was 2.4 years. ISS and age at time of transplant had no effect on PFS or OS. Achieving a CR vs PR pre-allo SCT had no significant effect on OS, but resulted in a significant difference in median PFS (63% vs 23% at 60 months; *P*=0.03). A significant difference in PFS was seen in patients who received ≤3 vs 4 lines of treatment prior to allo-SCT (median PFS 2.15yrs vs 0.54yrs; *P*=0.0003), and demonstrated a trend towards an effect on median OS (5.6yrs vs 3.2yrs; *P*= 0.06).
Discussion: RIC-allo-SCT in myeloma using fludarabine/ cyclophosphamide conditioning is an effective treatment strategy with an acceptable NRM. PFS is significantly increased if a CR is achieved prior to allo-SCT, and if the patient has received ≤3 lines of treatment.
Disclosure of Interest: None Declared.

PH-P523
THE ASSOCIATION BETWEEN COX-2 EXPRESSION AND SURVIVAL IN MYELOMA PATIENTS
F. Vural[1,], N. Soyer[1], M. Hekimgil[2], S. Cagirgan[1], A. Donmez[1], M. Tombuloglu[1]*
[1]Department of Hematology, [2]Department of pathology, Ege University Medical Faculty, Izmir, Turkey

Introduction: Increased cyclooxygenase-2 (COX-2) expression has been associated with poor prognosis in multiple myeloma (MM). This study examined retrospectively the relationship between COX-2 expression in bone marrow and prognosis in MM patients.
Materials (or patients) and Methods: Bone marrow biopsy samples of 67 newly diagnosed MM patients were examined immunohistochemically for COX-2 expression. Mean age of the patients was 52.69 years (52.69±9.17) and median follow-up time was 99.5 months (range: 6- 170 months).
Results: Of all patients, 30 (44.8%) were COX-2 positive and 37 (55.2%) were COX-2 negative. Median overall survival (OS) was 78 months (range: 54.07-101.92 months) among all patients, 75 months (range: 45.61-104.38 months) in COX-2-positive patients, and 98 months (range: 50.36-145.63 months) in COX-2-negative patients. Median progression-free survival (PFS) was 30 months (range: 3- 134 months) in all, 29.5 months (range: 3- 68 months) in COX-2-positive and 35 months (range: 3-134 months) in COX-2-negative patients. Statistically significant differences in OS and PFS between COX-2-positive and COX-2-negative patients were not observed (*P*=0.84 and *P*=0.22, respectively). Differences between the COX-2-positive and COX-2-negative patients in gender, hemoglobin, β2-microglobulin (β2M), creatinine, albumin, and disease stage were not statistically significant.
Discussion: COX-2 expression neither had a role in prognosis nor significantly affected OS and PFS. We conclude that stem cell transplantation might eliminate the detrimental effects of COX-2 positivity. Larger series of patients are needed to investigate this observation.
Disclosure of Interest: None Declared.

PH-P524
ALLOGENEIC TRANSPLANTATION AFTER 8 GY TBI/ CYCLOPHOSPHAMIDE CONDITIONING IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA – A RETROSPECTIVE SINGLE CENTER ANALYSIS.
T. Schmitt[1], S. Schoenland[1,], A. Bondong[1], H. Goldschmidt[1], K. Neben[2], K. Herfarth[3], A. D Ho[1], P. Dreger[1], U. Hegenbart[1]*
[1]Department of Hematology, Oncology, and Rheumatology, Heidelberg University Hospital, Heidelberg, [2]Department of Internal Medicine II, Stadtklinik Baden-Baden, Baden-Baden, [3]Department of Radiation Oncology, Heidelberg University Hospital, Heidelberg, Germany

Introduction: Despite recent advances in treatment of multiple myeloma (MM), the disease is still incurable and prognosis for high-risk patients is dubious. Long-term remissions have been achieved after allogeneic transplantation (alloSCT). However, relapse rate and treatment-related mortality (TRM) can be substantial. Here we report on our single center experience with alloSCT using 8 Gy total body irradiation/cyclophosphamide conditioning (TBI8/CY).
Materials (or patients) and Methods: Eligible for this retrospective analysis were patients with advanced MM who were considered to have poor prognostic factors and were allografted after TBI8/CY between 09/09 and 06/13. Patients received 2x2 Gy TBI days –5 and –4, cyclophosphamide 40 mg/kg BW days –3 and –2, and ATG in case of unrelated donors (10 mg/kg BW in matched unrelated donors, MUD, and 20 mg/kg BW in mismatch donors, MMD) days -3 to -1. Ciclosporine, tacrolimus or sirolimus (in case of renal insufficiency) combined with mycophenolat mofetil (MMF) were used for GVHD prophylaxis.
Results: Twenty-four patients referred for allografting met the criteria (early relapse after autoSCT, *n*=16; adverse karyotype, *n*=12; chemotherapy refractory disease, *n*=4; extramedullary disease, *n*=2; PCL, *n*=2; relapse after 1st alloSCT, *n*=1) and were thus conditioned with TBI8/CY. Median age and follow-up were 53 years [range 38 – 65] and 24.1 months, respectively. Remission status prior to alloSCT was at least VGPR in 8 patients, PR in 11 patients and non-response in 5 patients. Donors were matched siblings (*n*=9); MUD (*n*=11); and MMD (*n*=4). Median time to leukocyte and platelet engraftment were 14 and 14 days, respectively. Major toxicities (grade ≥3) included FUO (*n*=22), nausea/vomiting (*n*=7), pneumonia (*n*=5), catheter/soft tissue infections (*n*=7) and mucositis (*n*=4). Only one patient required parenteral nutrition. Overall, acute GVHD (grade II – IV) and chronic GVHD occurred in 10 and 12 subjects. Median overall (OS), progression-free and event-free survival (EFS) were 36.8, 11.0 und 10.5 months, respectively. TRM occurred in 2 patients after 2.2 and 11.0 months, respectively, due to acute GVHD grade IV and their complications. Patients with chronic GVHD (*n*=12) had a significantly prolonged PFS and EFS (median 12 vs 4 months, *P*<0.01), and a trend to an improved OS (median 37 vs. 8 months, *P*=0.1) in comparison to patients without cGVHD (*n*=9).
Discussion: TBI 8/CY is a feasible new conditioning regimen for advanced myeloma patients with a low TRM and no undue toxicities. Overall efficacy is moderate. However, a longer follow-up will be needed to assess long-term benefit. Post-transplant immune interventions might further enhance myeloma disease control.
Disclosure of Interest: None Declared.

PH-P525
INDUCTION OF PROLIFERATION AND SURVIVAL OF MULTIPLE MYELOMA CELLS BY CD137 LIGAND REVERSE SIGNALING
C. Fu[1], H. Liu[1], J. Wang[2], L. Ma[1], S. Ju[3], D. Wu[1,]*
[1]The First Affiliated Hospital of Soochow University, [2]The JiuLong Hospital, [3]Biotechnology Institute, Soochow University, Suzhou, China

Introduction: CD137L, in addition to its ability to costimulate T cells by triggering CD137 receptor, also signals back into antigen presenting cells inducing proliferation, prolonging survival and enhancing secretion of proinflammatory cytokines. While,

the new studies have testified that the CD137 ligand (CD137L) is expressed by various carcinoma cells too. However, the expression of CD137L and its function on multiple myeloma cells is unknown.
Materials (or patients) and Methods: We identified the constitutive expression of CD137L by flow cytometry on U266, RPMI 8226, LP1, MY5 and KMS-11 of Multiple myeloma (MM) cell lines as high as 96%, 97.5%, 89%, 93% and 94%.But, CD137 expressed on the cell surface was low as 4%, 5%, 1%, 2%, 5% respectively. We investigated CD137L expression of MM cells from 85 BM samples of patients diagnosed with active multiple MM, CD137L protein was expressed by a select group of CD45-CD38++CD138+cells as higher than 95%, the same, CD38 and CD138 are expressed more than 90% of the cells of CD45-CD137L+.There were 22 samples from 11 cases collected before and after treatment and this was further evidence that CD137L molecule was consistently expressed on the MM cell surface. However, CD137L expression was not or hardly detectable on normal plasma cells confirmed by CD45+CD38++CD138+ CD56- CD19+, indicating that CD137L was ectopically expressed by MM cells and probably a specific marker of MM cells.
Results: We selected U266-a MM cell line to explore the biological effect of CD137L reverse signaling and its underlying mechanism. The proliferation and survival of U266 was enhanced by stimulating- CD137L mAb (1F1) than those induced by control mouse IgG by cell counting (4.2 $X10^5$/ml VS 3.3 $X10^5$/ml, the average of 6-well plates), WST-8(1.15 VS 0.81, the average of 6-well plates absorbance) and CFSE assay (930 VS 991, fluorescence measured by flow cytometer) at incubation for 48h. In addition, the cell cycle analysis showed that CD137L induces proliferation and increases the number of cells in the S phase from 36.1% to 42.5% after 72h incubation. The percentage of apoptosis cells was 19.6% VS 21.2% with no statistical significance.
Intracellular cytokine staining showed that treatment of cells with 1F1 increased the production of IL-6 from 3.8% to 63.9% by Flow cytometry. When neutralizing anti-IL-6 mAb (5 μg/ml) was added to the culture medium, the cells ($2X10^5$/ml) were cultured for 48 h in pure medium or plus 10 ng/ml Fc or CD137–Fc protein and the cell proliferation measured by WST-8 was 0.79 VS 0.80 VS 0.72, the average of 6-well plates absorbance.1F1-induced cell proliferation was effectively inhibited. IL-6 can promote cell proliferation and survival of MM. An increase of these cytokines might explain why CD137L expression could stimulate the proliferation of U266. Finally, the U266 cells were treated with bortezomib and the growth of cells was analyzed by WST-8 assay. It demonstrated that bortezomib could inhibit the function of 1F1 and the inhibition ratio of bortezomib was 22%, 51% and 58% at 24h, 48h and 72h.
Discussion: In our study, CD137L is not only a novel ectopic constitutive marker of MM, but also a promoting proliferation factor. This suggests the possibility that its expression on MM cells may be directly target for immunomodulatory therapy for MM.
Disclosure of Interest: None Declared.

PH-P526
T CELL REPLETE HAPLOIDENTICAL TRANSPLANTATION FOR REFRACTORY MULTIPLE MYELOMA USING NONMYELOABLATIVE CONDITIONING AND HIGH-DOSE POST TRANSPLANT CYCLOPHOSPHAMIDE

S. Bramanti[1], A. Vai[1], J. El Cheikh[2], S. Furst[2], R. Crocchiolo[1], B. Sarina[1], S. Harbi[2], A. Granata[2], E. Mauro[1], C. Carlo-Stella[1], A. Santoro[1], A. M. Stoppa[2], D. Blaise[2], L. Castagna[1,]*
[1]Humanitas Cancer Center, Rozzano, Italy, [2]Institut Paoli Calmettes, Marseille, France

Introduction: Post transplant Cyclophosphamide (PT-Cy) after nonmyeloablative conditioning regimen (NMAC) after T-cell replete haploidentical transplantation reduces graft-versus-host disease (GVHD) and graft rejection. This major HLA incompatibility can be supposed to harness an important anti-tumor effect, in several haematological malignancies. In this retrospective study, we evaluated the safety and efficacy of haplo and PT-Cy in refractory multiple myeloma (MM) patients.
Materials (or patients) and Methods: Between April 2009 and November 2013, 124 patients with hematological malignancies were treated in two different centers. 9 refractory multiple myeloma patients were retrieved. Conditioning regimen consisted of Cy 14.5mg/kg/day i.v. on days −6 and −5, fludarabine 30 mg/m^2/day i.v. on days −6 to −2, and 200cGy of TBI on day −1. Cy was administered. Prophylaxis of GVHD consisted of PT-Cy (day +3 and +4), 50 mg/kg tacrolimus or cyclosporine and mycophenolate mofetil (MMF). Unmanipulated haplo marrow or peripheral blood stem cells was infused on day 0.
Results: Disease status before transplant was PD for 4 patients (44%) and DRI was HR in 5 and INT in 4. All patients relapsed after 1 or 2 HDC and autologous stem cell support. HCT-CI was ≥ 2 in most patients (67%). Median quantity of CD34+ cells infused was $4.7x10^6$/kg. Only one patient (11%) developed a cutaneous grade II aGVHD; no cGVHD was recorded except one occurred after DLI infusion. The median times to neutrophil (>500/μL) and platelet recovery (>20,000/μL) were 20 and 24 days, respectively. No graft failure occurred. Bacterial, viral and fungal infections were diagnosed in 4, 3 and 2 patients, respectively. With a median follow-up of 7 months (1-35), the cumulative incidence of NRM was 11%. Current overall survival (OS) is 67% (see Table).

PMN engraftment (ANC>500) day	
Median (range)	20 (15-27)
PLT engraftment (PLT>20,000) day	
Median (range)	24 (10-52)
Infections, n (%)	
Bacterial	4 (44%)
Viral	3 (33%)
Fungal	2 (22%)
FUO	1 (11%)
Disease status at +100	
CR	3 (33%)
PR	1 (11%)
PD	3 (33%)
NA	2 (22%)
Patient status	
Alive	6 (67%)
Dead	3 (33%)
Causes of death	
Disease	2 (67%)
Toxicity	1 (33%)

Discussion: Despite short follow-up time, our multicentric experience suggests that in advanced multiple myeloma patients, NMAC haploidentical transplant with PT-Cy is well tolerated without an excessive GVHD and NRM, and it seems to afford an interesting anti-myeloma effect. More patients and longer follow-up are needed.
Disclosure of Interest: None Declared.

PH-P527

INFLUENCE OF ASCT ON DURATION OF RESPONSE (DOR) AND OVERALL SURVIVAL (OS) OF PATIENTS WITH REFRACTORY OR RELAPSED MULTIPLE MYELOMA TREATED BY CASE-ADJUSTED BORTEZOMIB-BASED SALVAGE REGIMENS – A REPORT BY THE POLISH MYELOMA STUDY GROUP

A. Walter-Croneck[1,] and Polish Myeloma Study Group*
[1]Haematooncology and Bone Marrow Transplantation, Medical University of Lublin, Lublin, Poland

Introduction: Multiple myeloma remains incurable, but new drugs offers a significant prolongation of survival in clinical trials. There is need to look for real-life effectiveness of new drugs and ASCT role in daily practise.
Materials (or patients) and Methods: The observational study was aimed to evaluate influence of ASCT consolidation on DOR and OS of 295 relapsed or refractory, bortezomib-naïve MM patients, responding to bortezomib-based salvage regimens, suitable for ASCT (≤65 yrs, Karnofsky index >60%). Bortezomib was given iv. alone, or combined with steroids or anthracyclines, Thal and alkylators. The drugs combination and ASCT were case-adjusted in order to balance antitumor potential and toxicity at physician discretion. The survival analyses were carried out using the Kaplan-Meier method and log-rank test. The multivariate Cox proportional hazard regression analysis was done for categorised variables (resistant/relapsed MM, sex, Ig isotype, D-S and ISS stage, number of previous treatment lines, usage of Thal before, undergone ASCT, response to therapy, B2microglobuline>2,5mg/dl; WBC<4,0G/L) and continous (age, time from dgn to bortezomib therapy, protein M, Hgb, PLT, creatinine, albumine and calcium). Factors of prognostic value p≤0.05 were included into final model.
Results: The therapy result in ORR 67.9% for refractory MM and 69.9% for relapsed MM. The median DOR and OS were 16 and 56 months resp. Bortezomib salvage+ASCT vs bortezomib salvage only resulted in better DOR (median 23 vs 8 months; *P*<0.000001) and OS (median not reached vs 8 months; *P*<0.000001). The significant predictors for DOR were: relapsed MM (HR=2.14 *P*<0.000001), ISS stage (HR=1.29 *P*=0.033), partial response to bortezomib (HR=1.19, *P*=0.046), age (HR=1.03 *P*<0.01) and female (HR=0.70 *P*=0.016), but for OS were: relapsed MM (HR= 1,85 *P*= 0.022), ISS stage (HR= 1,78 *P*= 0.004), partial response to bortezomib (HR= 1,44 *P*= 0.025), PLT prior bortezomib (HR= 1,02 *P*= 0.004), HGB prior bortezomib (HR= 0,79 *P*= 0.00014) and female (HR= 0,54 *P*= 0.023). No toxicity to bortezomib therapy was reported for 38.6% of patients. Severe toxic events were reported for 35.9% of patients: mainly neurotoxicity (16.0%), neutropenia (6.1%), thrombocytopenia (4.4%) and infections (5.4%) but there were no influence of toxicity on DOR and OS by multivariate analysis.
Discussion: The patients benefit strongly from ASCT consolidation after bortezomib-based salvage and there are identifiable survival predictors. Toxicity after bortezomib-based treatment was predictable and manageable. Bortezomib-based regimens are highly effective salvage MM and should be supported by ASCT consolidation in suitable patients.
Disclosure of Interest: None Declared.

PH-P528

CONTINUOUS MAINTENANCE THERAPY WITH ALTERNATE-DAY LOW DOSE LENALIDOMIDE IN MULTIPLE MYELOMA PATIENTS AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION INCREASES THE NUMBER OF CIRCULATING NATURAL KILLER CELLS

L. Pezzullo[1,], R. Fontana[1], B. Serio[1], S. Annunziata[1], M. Sessa[1], I. Ferrara[1], V. Giudice[1], B. Esposito[1], G. Villani[1], M. Rocco[1], C. Selleri[1]*
[1]Hematology and Hematopoietic Stem Cell Transplant Center, Department of Medicine and Surgery, University of Salerno, Salerno, Italy

Introduction: Maintenance therapy with immunomodulatory drugs has shown to improve responses and delay relapse and progression in Multiple Myeloma (MM) patients after autologous stem cell transplantation (ASCT). Although maintenance therapy with Thalidomide (T) after ASCT increases progression free survival (PFS) in MM patients, it is associated with dose-limiting multiple toxicities. Lenalidomide (revlimid, R) has been reported to be associated with lower rates of toxicities than thalidomide.
Materials (or patients) and Methods: We evaluated efficacy, safety and the effect on circulating Natural Killer (NK) cells of continuous maintenance therapy with alternate-day low dose R (LD-R, 10 mg/day), after high-dose melphalan (HD-MEL, 200 mg/mq) and ASCT, in 8 MM patients (6 male and 2 female) with median age of 68 years (range 60-73), receiving pre-ASCT induction treatment with 4 cycles of conventional bortezomib, Thalidomide and dexamethasone (VTD) regimen. Of these 8 MM patients, 2 and 6 patients were in complete remission (CR) and in very good partial remission (VgPR) after ASCT, respectively. The effects of lenalidomide maintenance therapy on NK cells were evaluated the numbers of circulating NK cells by flow cytometry assessing the expression of T-cell antigens (CD3, CD4, CD8), NK-cell antigen (CD56) and NK-cell–activation antigens (CD2, HLA DR). All antibodies were obtained from Beckman Coulter. Phenotypic analysis of all these T- and NK-cell antigens were performed before starting maintenance, after the 1st month, and then every 3 months during R therapy.
Results: After a median follow-up of 20 months (range 9-48) from the initiation of LD-R maintenance, patients in CR maintained their CR, and all patients in VgPR improved the depth of response except one who showed disease progression. In this LD-R group, PFS and overall survival (OS) at 24 months were 83% and 100%, respectively. Noteworthy, no significant specific R-related toxicity was encountered. All MM patients treated with continuous alternate-day lenalidomide showed progressive increase in the percentage of circulating CD56+ CD3- Natural Killer cell. The median percentage of NK cells was 4% before R maintenance versus 9%, 22%, 26% and 31% at +3, +6, +12 and +18 months, respectively.
Discussion: Our preliminary results provide evidence that continuous therapy with alternate-day LD-R is a feasible and effective maintenance treatment after ASCT for MM patients, enabling a long-lasting maintenance therapy, and that prolonged low-dose lenalidomide treatment increases circulating NK cells further supporting that this drug may mediate its anti-MM effect, at least in part, by modulating NK cell number and function. These results require further validation in prospective larger studies.
Disclosure of Interest: None Declared.

PH-P529

AUTOLOGOUS STEM CELL TRANSPLANTATION IN THE TREATMENT OF PATIENTS WITH MULTIPLE MYELOMA- OUR EXPERIENCE

S. Marjanovic[1,], D. Stamatovic[2], M. Elez[2], B. Balint[2], O. Tarabar[2], L. Madjaru[2], A. Zivanovic Ivic[2], Z. Tatomirovic[2], L. Tukic[2]*
[1]Clinic of hematology, [2]Military Medical Academy, Belgrade, Serbia

Introduction: In the last 10 years, the overall survival (OS) of patients (pts) with multiple myeloma (MM) has improved considerably. Transplant eligible pts have a 5-year survival rate of more than 70% with modern therapy. Autologous hematopoietic stem cell transplantation (auto-HSCT) is standard therapy for initial or treatment of relapse for MM pts younger than 65 years or even older, but fit pts in good clinical condition and it is connected with better OS and progression free survival (PFS) in comparison to conventional therapy. We retrospectively analyzed pts with MM treated with auto-HSCT in our center from 1998. till 2012.
Materials (or patients) and Methods: A total of 155 procedures of auto-HSCT in 120 *de novo* MM pts were performed after one or two lines of initial treatment. Male/ female ratio in our group was 72/28, average age was 52 years (37-64). Majority of pts, 72 (60%) were in III clinical stage. Pts have received 3-6 cycles of induction therapy (VAD, PAD, CTD, TAD or TD). Prior to auto-HSCT 43 pts (35,8%) were in complete remission (CR) + very good partial remission (VGPR), 35 pts (35.8%) in partial remission (PR), 7 pts (5.5%) were in less than PR and 4 (3.4%) had progression of disease (PD). Median time from diagnosis till auto-HSCT was 13,9 months (3-90)

and from those transplanted from 2004 till 2012. even shorter, 7 months (5-9). Single auto-HSCT was done in 120 pts (100%), tandem in 18 (15%) and secondary in 17 (14%) pts. Mobilization was combination of Cyclophosphamide (Cy) and Etopiside with G-CSF in 111 pts (92,5%) or Cy alone with G-CSF in 9 (7.5%) pts. 106 pts (88,3%) were conditioned with Melphalan (Mel) 200 mg/m^2, while 14 pts (11,7%) were conditioned with Busulphan (16 mg/kgBW), Cy (120mg/kgBW) and Mel (140 mg/m^2). MNC were collected mostly with one "large volume" apheresis and median number was 9.85x10^8/kg BW (range 3.8-28x10^8/kg BW). OS and duration of response were showed with Kaplan Meier method. Peripheral blood progenitor cells were the preferred source of stem cells. The definition of response is estimated by International Myeloma Working Group in 2006.
Results: In 112 pts engraftment was observed on +12 day after auto-HSCT (range 8-36, respectively), in 3 pts there were no reconstitution, in 3 pts it was delayed and 2 pts have died during the procedure. Overall response rate (ORR) is achieved in 72 (61%) pts. After auto-HSCT, 72 pts (61%) have achieved CR+VGPR, while 35 pts (29,7%) were in PR. 21 pts (17,5%) have died and 91 (75,8%) are still alive. 8 pts (6,7%) were lost from following . Median OS in 115 pts is 42 months with maximum follow up for 57 months. Median PFS for 67 pts after auto-HSCT was 13 months and 40 of them had progression. Longest time till progression was 49 months.
Discussion: Achievement of CR+VGPR before and after auto-HSCT is important predictive factor for PFS and OS. Better OS and PFS were achieved in those pts in whom auto-HSCT was performed earlier (99 pts transplanted from 2004. till 2012.). Transplant related mortality (TRM) is observed in heavily pretreated pts who were transplanted more than 12 months after diagnosis. TRM in our group was 2% from 2004. New agents and better supportive therapy together with auto-HSCT with well defined maintenance using either immunomodulatory drugs or bortezomib will improve treatment results even more.
Disclosure of Interest: None Declared.

PH-P530
SURVIVAL ANALYSIS IN PATIENTS WITH MULTIPLE MYELOMA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION, A SINGLE CENTER STUDY

S. Hofmann[1,], L. Müller[1], S. von Harsdorf[1], J. Greiner[2], P. Liebisch[3], C. Langer[1], H. Döhner[1], D. Bunjes[1]*
[1]Ulm University Hospital, Ulm, [2]Diakonieklinikum, Stuttgart, [3]Onkologische Praxis, Moers, Germany

Introduction: The role of allogeneic stem cell transplantation (allo-HSCT) in the treatment algorithms for patients with multiple myeloma remains controversial although it is the only potentially curative approach currently available.
Here we present the retrospective analysis of 88 allo-HSCT performed between 1994 and 2011 at Ulm University Hospital. We focused on the impact of cytogenetics, graft-versus-host disease (GvHD) and intensity of conditioning on overall survival (OS), progression- free survival (PFS), relapse and non-relapse mortality (NRM).
Materials (or patients) and Methods: Median age at initial diagnosis was 49 years (range 25-64), median age at time of allo-HSCT was 51 years (range 26-65). Median time from initial diagnosis to allo-HSCT was 14 months (range 3-106). Indications for allo-HSCT were 1) primary allo-HSCT after induction therapy (10 pts), 2) planned tandem auto-allo-HSCT (40 pts), 3) relapse after single allo-HSCT (24 pts), 4) relapse after tandem-auto-HSCT (14 pts). The conditioning regimen in 60 pts was a reduced intensity conditioning (RIC), 28 pts received myeloablative conditioning (MAC). In 58 pts cytogenetic data were available: 14 pts were stratified into standard-, 31 pts into the intermediate- and 13 pts into the high-risk group according to the mSMART recommendations.
Results: Median follow-up was 71 months (95% CI, 59,2-82,8). The estimate 1-, 2- and 5-year OS was 63,6 %, 58.7 % and 42,8 % with a median OS of 36 months (95 % CI, 19.9 -52.1).
For RIC versus MAC median OS was 35 months (95% CI, 21.4–48.6) versus 89 months (95% CI 0-193.2) but this difference was not statistically significant. The cumulative incidence of TRM was not different for RIC and MAC but there was a highly significant difference in relapse (*P*=0.011).
With respect to the indication for allo–HSCT outcomes were as follows: Median OS was 147 months for primary allo-HSCT, 47 months for tandem auto-allo-HSCT, 20 months for relapse after auto-HSCT and 15 months for relapse after double auto-HSCT. OS did not differ significantly.
Median OS in the standard risk group was 20 months (95% CI, 5.3-34.7), in the intermediate group 41 months (95% CI, 21.2-60.8) and in the high-risk group 7 months (95% CI, 0-14), showing no statistical significance. 1-year OS was 64,3% vs 67.7% vs 38.5%, and 2-year OS was 40.8% vs 67.7% vs 38.5% (standard vs intermediate vs high risk).
The median PFS was 12 months (95% CI 7.8-16.2). 1-year PFS was 49.9%, 2-year PFS 34.6% and 5-year PFS was 21.6%. PFS according to the cytogenetic aberration showed a median PFS of 12 vs 14 vs 5 months, 1-year PFS was 50% vs 54.5 % vs 30.8 %, 2-year PFS with 33.3% vs 37.5% vs 7.7% (standard vs intermediate vs high risk). These differences are not statistically significant.
Considering the impact of acute GvHD, OS significantly differed between the groups with no aGvHD or aGvHD grade I and aGvHD grade II-IV with inferior survival for patients suffering from aGvHD grade II-IV. Chronic GvHD had no impact on outcome.
Discussion: Our data of a 17 year experience in treatment of patients with advanced multiple myeloma with allo-HSCT showed an effective treatment option with a curative potential even for patients after intensive pretreatment including autologous stem cell transplantation. Patients who received MAC had a significantly lower cumulative incidence of relapse compared to RIC without increasing TRM in our study.
Disclosure of Interest: None Declared.

PH-P531
THIOTEPA-BASED CONDITIONING FOLLOWED BY ALLOGENEIC SCT IN PATIENTS WITH MULTIPLE MYELOMA A SURVEY OF THE CHRONIC MALIGNANCIES WORKING PARTY OF EBMT

N. Kröger[1,], S. Iacobelli[2], A. van Biezen[3], L. Garderet[4] and CMWP*
[1]University Hospital, Hamburg, Germany, [2]Universita tor Vergata, Rome, Italy, [3]University Medical Center, Leiden, Netherlands, [4]Hospital Saint Antoine, Paris, France

Introduction: To investigate the use and outcome of thiotepa in combination with fludarabine or other drugs as conditioning regimen followed by allogeneic stem cell transplantation for multiple myeloma we screened EBMT database.
Materials (or patients) and Methods: We found 45 patients with a median age of 53 years (range 37-69) who received thiotepa-based regimen. Allogeneic stem cell transplantation was performed either as first (*n*=21) or second transplant (*n*=24) Male/female distribution was 28/17. Most patients had advanced stage III (73%) or II (23%) myeloma, which was common type (IgG or IgA) in 58%, light chain in 33% or non-secretory MM in 9% of the patients. At transplantation remission status was evaluable in 40 pts: CR: 18%, PR: 37%, SD/MR: 15% and Relapse/PD: 30%.
In-vivo (29%) and *ex-vivo* (11%) or combined (9%) T-cell depletion was used in 49% of the patients. Donorwere: HLA-identical sibling (73%), matched unrelated (16%), and mismatched related or unrelated (7%) donor or other relatives (4%). Conditioning regimen was classified as reduced intensity (62%) or myeloablative (38%). Bone marrow or peripheral blood was used in 16% and 84%, respectively.
Results: The median time to leukocyte engraftment was 14 days. Acute GvHD grade II-IV was seen in 15% of the patients, severe grade III in only 1 patient (2%). The cumulative incidence of non-relapse mortality at 1 year was 24% (95% CI: 11-37%) and of relapse at 3 years was 43% (95% CI: 27-60%). The 3-year progression-free and overall survival were 33% (95% CI: 17-49%) and 60%, respectively.
The NRM was similar in patients who received single agent thiotepa/ fludarabine only in comparison to those who received

thiotepa/fludarabine plus other drugs such as busulfan, cyclophosphamide, or melphalan (15 vs 22%, *P*=0.5), but the cumulative incidence of relapse at 2 years was higher in the thiotepa/fludarabine only group (55% vs 13%, *P*= 0.004), resulting in an improved estimated 3-year PFS and OS for the thiotepa/fludarabine plus others regimens (61 vs 30%, *P*=0.02) and 74 vs 33%, *P*= 0.09) .
Discussion: We conclude thiotepa is rarely used as single agent or as part of the conditioning regimen in allogeneic stem cell transplantation for multiple myeloma, but this small analysis indicates that thiotepa deserves further investigations as conditioning regimen in MM.
Disclosure of Interest: None Declared.

PH-P532

"REAL WORLD DATA" ON CLINICAL FEATURES, PROGNOSIS AND POST AUTOLOGOUS HAEMOPOETIC STEM CELL TRASPLANTATION (AHCT) SURVIVAL IN MULTIPLE MYELOMA IN THE ERA OF NOVEL AGENTS: A REPORT OF THE HELLENIC SOCIETY OF HAEMATOLOGY IN 489 PATIENTS

E. Katodritou[1,], A. Pouli[2], E. Terpos[3], D. Mallouri[4], E. Eleutherakis-Papaiakovou[3], E. Giannopoulou[1], C. Apostolou[4], M. Kotsopoulou[5], S. Delimpasi[6], A. Symeonidis[7], I. Batsis[4], P. Kaloyiannidis[4], M. Vardaka[6], D. Christoulas[8], A. Papadopoulou[1], E. Verrou[1], P. Konstantinidou[1], A. Anagnostopoulos[4], M.-A. Dimopoulos[3], I. Sakellari[4]*
[1]Department of Hematology, Theagenion Cancer Center, Thessaloniki, [2]Department of Hematology, St. Savvas Oncology Hospital, Athens, [3]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, [4]Department of Hematology and Bone Marrow Transplantation Unit, G. Papanikolaou General Hospital, Thessaloniki, [5]Department of Hematology, Metaxa Cancer Hospital, Piraeus, [6]Department of Hematology and Bone Marrow Transplantation Unit, Evangelismos General Hospital, Athens, [7]Hematology Division, Department of Internal Medicine, University of Patras, Patras, [8]Department of Hematology, 251 General Air Force Hospital, Athens, Greece

Introduction: Autologous haemopoetic stem cell transplantation (AHCT) and novel agent-based (NAB) therapies have improved multiple myeloma (MM) outcome. However, prognostic factors for post-AHCT survival in the era of novel agents are not clearly defined and data regarding the role of post-AHCT consolidation/maintenance (C/M) therapy are inconclusive. The aim of the study was to record "real world data" on clinical features, prognosis and post-AHCT survival of a large cohort of consecutive MM patients treated with AHCT in Greece, in the era of novel agents. We also explored a possible role of post-AHCT C/M treatment, outside the setting of controlled trials.
Materials (or patients) and Methods: We reviewed the medical files of 489 consecutive MM patients (M/F: 278/211, median age: 56 years range: 31-79) treated with AHCT between 2000-2013 in 7 Greek Centers.
Results: At MM diagnosis 189 patients had ISS 1, 141 had ISS 2 and 90 had ISS 3 (no data: 69 patients). High risk cytogenetics i.e. del17p and/or t(4;14) were found in 24/104 with available data; 220 patients received conventional treatment and 263 NAB therapy. The median number of pre-AHCT cycles was 4 (range: 1-9); 470 patients underwent single and 19 tandem AHCT. Median pre-AHCT levels of Hb, albumin, β2 microglobulin and creatinine were 12.4 g/dL (8.8-17.0 g/dL), 4.1 g/dL (2.6-4.9 g/dL), 1.17 mg/L (1.0-2.2mg/L) and 0.87 mg/dL (0.59-2.52mg/dL), respectively; the median % of bone marrow plasma cells before AHCT was 3% (range: 0-40%); 378 patients were mobilized with cyclophosphamide + GCSF, 79 patients with GCSF and 32 patients with multi-agent chemotherapy. The median number of CD34+ cells infused was 5.9 $x10^6$/kg (range: 2.6-7.43 $x10^6$/kg). Median days for neutrophils >500/μL were 11.5 (range: 8-18); median days for PLT> 50.000/μL were 16 (range: 12-27); 176 patients had ≥very good partial response (vgPR) pre-AHCT and 203 had ≥vgPR post-AHCT; 266 patients received post-AHCT C/M treatment (consolidation: 80, maintenance: 186) and 223 did not; C/M treatment included interferon A or dexamethasone (36 patients), thalidomide (99 patients), bortezomib ± Dexamethasone (26 patients), Lenalidomide (78 patients) and bortezomib + Immunomodulator + Dexamethasone (27 patients). At the last follow-up, 337 patients were alive (68.9%) and 149 patients (30.5%) died (progression: 88%, AHCT-related: 2%, secondary malignancy: 2%). With a median post-AHCT follow-up of 56 months (95% CI: 51.2-60.7) the median post-AHCT time to progression and post-AHCT survival were 46 months (95% CI: 38-54) and 101 months (95% CI: 79-122), respectively. In the multivariate analysis, pre-AHCT NAB treatment and post-AHCT C/M therapy predicted positively, whereas the presence of del17p and/or t(4;14) predicted negatively for post-AHCT survival (*P*<0.05 for all parameters); ISS, type of post-AHCT treatment and disease status after AHCT did not predict for post-AHCT survival.
Discussion: In the era of novel agents, patients treated with AHCT enjoy prolonged survival outcomes. Pre-AHCT NAB treatment and C/M therapy independent of the type of agents used predict positively for post-AHCT survival, indicating the importance of continuous treatment in MM. Despite the use of novel strategies, high risk features is a strong negative predictor for post AHCT survival.
Disclosure of Interest: None Declared.

PH-P533

SINGLE CENTRE EXPERIENCE OF AUTOLOGOUS STEM CELL TRANSPLANT FOR MULTIPLE MYELOMA AND AL AMYLOIDOSIS IN DIALYSIS DEPENDENT PATIENTS

S. Alimam[1,], S. Gibbs[1], M. Waller[1], J. Woodward[1], F. Dignan[1], E. Tholouli[1]*
[1]Haematology, Central Manchester Foundation Trust, Manchester, United Kingdom

Introduction: Renal impairment is frequently encountered in Multiple Myeloma and AL Amyloidosis patients, with 20% of patients presenting in renal failure and 5% requiring dialysis. This is often perceived as a contra indication to effective high dose therapy which may have the potential of not only getting the patient into a deeper remission but also reversing their renal dysfunction. Autologous stem cell transplants can be performed safely in end stage renal impairment in Multiple Myeloma and AL Amyloidosis patients. We present our single centre experience.
Materials (or patients) and Methods: Between the periods of 2008 and 2012, there have been seven successful autologous stem cell transplantations in patients with dialysis dependent renal failure. Five patients had Multiple Myeloma and two AL Amyloidosis. There were five males and two females with a median age of 60 years (52–65). All patients underwent an autologous stem cell transplant in first remission. Six patients were on haemodialysis and one patient was on peritoneal dialysis. Only one patient was admitted to intensive care during their inpatient stay at time of stem cell transplant.
All patients were transplanted at Manchester Royal Infirmary, Manchester, UK, using Melphalan 140mg/m^2. They all received inpatient dialysis under the care of a renal physician (two to three times a week according to individual pre transplant schedule). One patient was on peritoneal dialysis which was performed successfully with no infections during the neutropenic phase. There was no increase in dialysis requirements during the inpatient stay.
Results: The median follow up was 3.6 years (1-5.3). Four of the seven patients became dialysis independent, two of whom received renal transplants. One patient resumed dialysis following decline in renal function after a 2 year period. However, during the period off dialysis he reported enhanced quality of life. One patient died following neutropenic sepsis and multi-organ failure within 100 days of transplantation. A second patient died 15 months post transplant from severe mitral regurgitation (a complication seen in long-term dialysis patients) having relapsed with myeloma at 12 months post stem cell transplant. All five patients alive at this time point continue to be in complete remission (two AL Amyloidosis, three Multiple Myeloma).
Discussion: In the correct setting, autologous stem cell transplantation is feasible in patients with dialysis dependent renal failure using Melphalan (140mg/m^2) conditioning in conjunction with dialysis. As demonstrated in our cohort, transplant related

complications do not differ from that of the non-dialysis patients. There is potential for cessation of dialysis, although in some cases this may only be temporary. Furthermore, autologous stem cell transplantation can induce complete remissions which subsequently may enable renal transplantation.
Disclosure of Interest: None Declared.

PH-P534
LONG-TERM FOLLOW-UP OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA PATIENTS

O. Lopez-Godino[1,], M. Cabrero[1], J. M. Bastida[1], M. Lopez-Parra[1], J. Labrador[2], E. Perez-Lopez[2], T. Caballero[3], F. Sanchez-Guijo[1], L. Vazquez[1], R. Garcia-Sanz[1], E. M. Ocio[1], N. Gutierrez[2], J. A. Perez-Simon[4], M. V. Mateos[1], C. del Cañizo[1], D. Caballero[1], L. Lopez-Corral[1]*
[1]Hematology Department, [2]Hospital Universitario de Salamanca-IBSAL, Salamanca, [3]Hospital Universitario Virgen del Rocio, Sevilla, Spain, [4]Hematology Department, Hospital Universitario Virgen del Rocio, Sevilla, Spain

Introduction: Allogeneic hematopoietic stem cell transplantation (HCT) is a potentially curative option in MM patients. However, the introduction of new drugs together with the transplant-related mortality/morbidity and the high relapse risk of this procedure make the use of HCT controversial.
Materials (or patients) and Methods: A total of 48 consecutive MM patients who underwent HCT in our center between 2000 and 2013 were retrospectively evaluated. Feasibility and efficacy of HCT was analyzed in terms of transplant-related mortality (TRM), progression free survival (PFS) and overall survival (OS).
Results: Median age was 54 years (30-65). 92% had received previous auto-transplant. Disease status at transplant was complete remission (CR) in 8 (16%), partial response or very good partial response (PR/VGPR) in 27 (56%), stable disease (SD) in 7 (10%) and progressive disease (PD) or non-responding disease (NRD) in 8 (17%). Twenty patients had previous extramedullary disease, 8 of them (17%) at transplant. Median time to HCT was 30 months (8–256) from diagnosis and 21 (3–111) from auto-transplant. Twelve patients (27%) had high-risk cytogenetic abnormalities.
All the patients but one received reduced intensity regimen (fludarabine-melphalan, plus bortezomib in 11 patients); Donor was unrelated in 13 patients (27%), 90% HLA 10/10, and median CD34-positive cells was 5,2 (2,2 – 13) x10^6/kg (from peripheral blood in 96%). Graft-versus-host disease (GVHD) prophylaxis consisted of calcineurin inhibitor-regimens plus MMF or metotrexate in 39 (81%) and tacrolimus and rapamicine in 8 (17%).
All patients engrafted. Cumulative incidence of acute GVHD (aGVHD) and grades 3-4 aGVHD was 54% and 18% respectively, (median onset:31 days, 6-172). Cumulative incidence of chronic GVHD (cGVHD) and severe cGVHD was 37% and 14% respectively (median onset: 197 days, 105-778) posttransplant.
Regarding response to transplant at day +100, 21/38 (55%) patients improved pre-HCT response: 5/8 of the patients who were in PD/NRD, 2/5 of the patients with SD, 12/22 of the patients with PR and 2/3 of the patients with VGPR. Among the 6 patients who were in CR, 5 patients maintained this response. Four patients were not evaluable. 38 patients subsequently relapsed or progressed, 20 of them with extramedullary involvement. Median time to relapse or progression was 11.8 months (2-150).
With a median follow up of 30 months (2-138) among patients alive, median EFS was 315 days. In multivariate analysis to develop cGVHD (mild (HR 0.31; 95% CI 0.1-0.9; *P*=0.04) moderate (HR=0.1(0.03-0.6) *P*=0.01) and severe (HR=0.1(0.05-0.6) *P*=0.01)) favourably influenced EFS, while time from auto-transplant >1 year to HCT adversely influenced outcome (HR 2.2(1.3-3.6) *P*=0.02).
The estimated 5 and 10 years OS was 41% and 20%. Overall TRM was 14% (6% at day +100). The main cause of death was progression disease (*n*=24). In multivariate analysis to develop grade 3-4 aGVHD adversely affected OS (HR 4.2(1.3-13.5) *P*=0.02), while chronic GVHD favourable influenced outcome (mild (HR 0.2(0.05-0.83) *P*=0.02) moderate (HR=0.2(0.04-0.88) *P*=0.03) and severe (HR=0.1(0.02-0.6) *P*=0.01).
Discussion: According to our results, HCT is an alternative therapy in high risk MM patients with low TRM, however risk of relapse is very high. Efforts should be focused on new strategies to control the minimal residual disease as well as the severe GVHD after HCT, maintaining the GVM effect.
Disclosure of Interest: None Declared.

PH-P535
A SECOND VACCINATION AGAINST INFLUENZA VIRUS IMPROVES IMMUNE RESPONSES IN PATIENTS WITH MULTIPLE MYELOMA

M. Hahn[1], P. Schnitzler[1], B. Schweiger[2], H. Goldschmidt[1], A. D. Ho[1], S. Michael[1,]*
[1]University Clinic Heidelberg, Heidelberg, [2]Robert Koch Institute, Berlin, Germany

Introduction: Influenza infection in multiple myeloma (MM) patients is often characterized by severe complications. Influenza vaccination is therefore generally recommended, however, immune response to vaccination is frequently insufficient. The aim of our diagnostic study was to determine the immune response after one and two doses of a novel trivalent influenza vaccine (Optaflu®) in MM patients.
Materials (or patients) and Methods: During the season 2012/13, vaccination with a trivalent (A(H1N1)pdm09, A(H3N2), B/Yamagata) influenza vaccine (Optaflu®, Novartis) was administered to 49 patients with confirmed diagnosis of MM at our outpatient clinic. Fourty-eight patients received one dose and 24 of these patients received a second dose of vaccine. Blood samples were taken prior to immunization and four weeks after each vaccination for evaluation of humoral response employing hemagglutination inhibition assays.
Results: Fourty-eight MM patients were vaccinated, none had protective immunity at baseline against all three viruses. After the first vaccination, seroprotection against all three antigens was achieved in 14% (7/49) of the patients. Of 42 patients without protective immunity, 24 received a second dose resulting in 33.3% (8/24) seroprotection. Patients who failed to seroconvert were more likely to receive an immunosuppressive therapy.
Discussion: We demonstrated in this pilot study that a single dose of influenza vaccine resulted in 14% seroprotection of MM vaccines. The frequency of protective titers could be more than doubled to 33.3% by a vaccine boost. In patients with MM the rate of seroconversion after a first dose of a trivalent influenza vaccine was poor, but increased after a second dose. In conclusion, two-dose vaccination with Optaflu® vaccine resulted in a significant, yet limited serological response. We therefore recommend an influenza vaccine boost for myeloma patients in general. A prospective, randomized study would be highly appreciated to confirm this recommendation.
Disclosure of Interest: None Declared.

PH-P536
MRD NEGATIVITY PROLONGS PFS BUT NOT OS AFTER UPFRONT AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

K. Remes[1], M. Hirviniemi[1], M. Putkonen[2,], V. Kairisto[3], V. Juvonen[3], M. Kauppila[2], U. Salmenniemi[2], T. Salmi[2], M. Itälä-Remes[2]*
[1]Clinical Haematology, Turku University Hospital and Turku University, [2]Dept of Clinical Haematology, Turku University Hospital, [3]Lab. of Molecular Genetics, Turku University Hospital, TYKSLAB, Turku, Finland

Introduction: Achievement of complete response (CR) has been considered a new goal of therapy for multiple myeloma (MM). The depth of the response may also be important. We have used a sensitive real-time quantitative polymerase chain reaction (qASO-PCR) to assess the level of minimal residual disease (MRD) in bone marrow of myeloma patients who had achieved CR or near-to CR (nCR) after upfront autologous stem cell transplantation (ASCT). Low or negative MRD was earlier shown to predict the ASCT

outcome[1]. We have now updated our data with a larger patient cohort.
Materials (or patients) and Methods: The aim was to evaluate prospectively the impact of MRD, assessed with qASO-PCR, on progression free (PFS) and overall survival (OS) in patients with MM who had attained CR/nCR (paraprotein not visible in electrophoresis but immunofixation positive) within six months after upfront ASCT performed in the time period from Oct. 1997 to Sept. 2010. From a total of 43 patients who were randomly selected for molecular analysis, 25 had reached low/negative MRD status, and 18 had high MRD. The groups were otherwise well balanced but in the MRDhigh group the proportion of nCR patients was higher, 8 out of 18, while the respective figure in the MRDlow/neg group was two out of 25. MRD was measured with a patient specific qASO-PCR, the method of which has been described earlier1. Allele-specific primers could successfully be designed for 90 % of patients. The median sensitivity of the PCR assay for those by whome the PCR target was not detectable was < 0.002 %. Kaplan-Mayer curves for PFS and OS for the MRDlow/negative and MRD high groups were produced.
Results: The median PFS for the MRDlow/neg and MRDhigh groups were 38 and 26 months (*P*=0.013), respectively. However, no significant difference was seen in OS (53 vs 52 months).
Discussion: Negative or low MRD is associated with a prolonged PFS but seems not to lead to a statistically significant benefit in OS when compared to high MRD in patients with a good response (CR or nCR) after upfront ASCT for MM. The most apparent cause for non-benefit in OS is probably the use of novel drugs, *viz*. bortezomib and lenalidomide, and late transplantations in the salvage protocols; they level off the early PFS benefit from upfront ASCT. Prospective studies are needed to work out how to maintain low or negative MRD status and translate this into a prolonged survival in MM.
[1]Putkonen M, Kairisto V, Juvonen V, *et al*. Depth of response assessed by quantitative ASO-PCR predicts the outcome after stem cell transplantation in multiple myeloma. *Eur J Haematol* 2010;85:416-23.
Disclosure of Interest: K. Remes Conflict with: advisory board and congress travel costs from Celgene, Janssen-Cilag and Mundipharma, M. Hirviniemi: None Declared, M. Putkonen Conflict with: Congress travel expenses from Celgene, Janssen-Cilag and Mundipharma, V. Kairisto: None Declared, V. Juvonen: None Declared, M. Kauppila Conflict with: Congress travel expenses from Mundipharma, U. Salmenniemi: None Declared, T. Salmi Conflict with: Congress travel expenses from Celgene, Janssen-Cilag and Mundipharma, M. Itälä-Remes: None Declared.

PH-P537
ALLOREACTIVE NK CELL THERAPY IN THE RAG2-/-C-/- MULTIPLE MYELOMA MODEL
G. Bos[1,], S. Sarkar[2], L. Wieten[2], A. Martens[3], W. Noort[3], R. Groen[3]*
[1]Internal Medicine, Hematology, [2]Maastricht University Medical Centre, Maastricht, [3]University Utrecht, Utrecht, Netherlands

Introduction: Multiple Myeloma (MM) is an incurable plasma cell malignancy residing in the bone marrow (BM). Cell based immunotherapy with allogeneic natural killer (NK) cells could be a new treatment option for MM because NK cells are known to mediate potent anti-tumor immunity. Here, we studied graft versus myeloma (GvM) responses and the development of graft versus host disease (GvHD) after immunotherapy with human allogeneic, KIR-HLA mismatched NK cells in *in vitro* and *in vivo* models.
Materials (or patients) and Methods: Freshly isolated NK cells killed KIR-HLA mismatched human MM cell lines in a flow cytometry based cytotoxicity assay *in vitro*. Killing efficiency varied between the cell lines and could partially be explained by differences in HLA expression. One of the MM cell lines (U266), was injected in immunodeficient $RAG2^{-/-}gc^{-/-}$ mice. At day 21, 23 and 25 after tumor injection, allogeneic PBMC or purified NK cells were intravenously injected in MM bearing mice and MM outgrowth was monitored real time by bioluminescent imaging.
Results: Infusion of unactivated NK cells decreased MM severity in one out of four treated mice. Analysis of the BM revealed that the infused NK cells had migrated to BM. Infused PBMCs efficiently eradicated established MM in all treated mice, and in this group T cells were found in the BM. However, in PBMC receiving mice, GvM effects coincided with the development of GvHD which will severely hamper clinical application of PBMC infusion. On the other hand, NK cells could be infused at high numbers without causing GvHD, emphasizing the therapeutic opportunities of NK cell therapy. We additionally show that the GvM response could be enhanced by activation of the NK cells with IL-2 and by conditioning of the mice with low dose chemo/radiotherapy (50 mg/kg cyclophosphamide and 2x2gy TBI).
Discussion: Our study illustrates the safety and therapeutic potential of NK cell therapy in MM. Thereby, our study can be a first step towards cell based immunotherapy for MM patients without the induction of GvHD.
Disclosure of Interest: None Declared.

Paediatric Issues

PH-P538
TCR-ALPHA/BETA-DEPLETED HSCT IN PATIENTS WITH PRIMARY IMMUNODEFICIENCY DISEASES
D. Balashov[1,], M. Maschan[1], Y. Skvortsova[1], L. Shelikhova[1], E. Gutovskaya[1], D. Shasheleva[1], I. Shipitsina[1], M. Ilushina[1], Z. Shekhovtsova[1], V. Tzetlina[1], R. Khismatullina[1], O. Tatarinova[1], E. Kurnikova[1], E. Boyakova[1], A. Levadniy[1], J. Muzalevsky[1], G. Novichkova[1], A. Maschan[1]*
[1]Dmitriy Rogachev Federal Research Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation

Introduction: Current experience with TCRαβ-depleted HSCT is limited to a few studies mainly in patients with malignant diseases transplanted from haploidentical donors. The key objects for this approach are reduction of GVHD risk, fast immune reconstitution and preservation of GVL-effect. However, infections, GVHD and GVHD-associated complications still remain significant problems in patients with non-malignant diseases transplanted from haploidentical or match unrelated donors (MUD).
Aim of our study was to examine the effect TCRαβ-graft depletion for HSCT from MUD or haploidentical donors in patients with primary immunodeficiency diseases (PID).
Materials (or patients) and Methods: In 2012-2013 twelve patients (male:female = 9:3; median age 1,8 years [0,2-6,2 years]) with different PIDs were transplanted with TCRαβ/CD19-depletion of the grafts. Indications for HSCT were CGD (*n*=1), Hyper-IgM (*n*=1), WAS (*n*=4), HLH (*n*=1), SCID (*n*=3) and unidentified PID (*n*=2). Stem cell source was peripheral blood from 10/10 HLA-MUD (*n*=8) or haploidentical donors (*n*=4).
All patient received Treosulfan-based reduced intensity condition regimens with top-up of Campath-1H or ATG (horse) as serotherapy. The median number of TCRαβ+ cells was 7 x 10^3/kg (0.8 x 10^3/kg – 3.9 x 10^4/kg) and median number of CD34+ cells was 15 x 10^6/kg (10.3 x 10^6/kg – 61.0 x 10^6/kg). GVHD prophylaxis included Tacrolimus (*n*=6) or Tacrolimus with short course of MTX (*n*=6).
Results: Engraftment occurred in all patients; median time to neutrophil recovery was +15 day (Day +13 - +21), median time to platelet engraftment was +12 day (Day +9 - +20). GVHD grade II occurred in 30% of patients and we had no patients with GVHD grade III-IV. There were two late graft rejections (patients with CGD, [*n*=1] and Hyper-IgM, [*n*=1]) 3 and 6 months after primary HSCT; second HSCT were performed in both cases with good preliminary results. Other serious complications after HSCT were CMV-retinitis (*n* = 1) and secondary hemophagocytic syndrome associated with BCGitis (*n* = 1).

Figure 1. Recovery of CD3+ cells

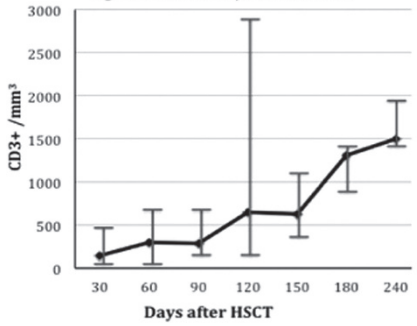
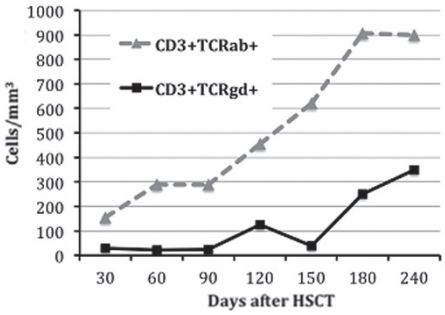


Figure 2. Recovery of TCRαβ/TCRγδ T-cells



With median follow up of 10 months (range, 4-15 months) all patients are alive and rate of immune reconstitution is encouraging in all cases (Fig. 1,2).
Discussion: TCR αβ/CD19 depletion – promising alternative technology for clinical application in haploidentical or matched unrelated transplantation. Low incidence of GVHD and rate of immune reconstitution are sufficiently factors for decreasing of TRM and increasing the chance to long-term survival.
Disclosure of Interest: D. Balashov: None Declared, M. Maschan Conflict with: received lecture's fee from Maltenyi Biotec, Y. Skvortsova: None Declared, L. Shelikhova: None Declared, E. Gutovskaya: None Declared, D. Shasheleva: None Declared, I. Shipitsina: None Declared, M. Ilushina: None Declared, Z. Shekhovtsova: None Declared, V. Tzetlina: None Declared, R. Khismatullina: None Declared, O. Tatarinova: None Declared, E. Kurnikova: None Declared, E. Boyakova: None Declared, A. Levadniy: None Declared, J. Muzalevsky: None Declared, G. Novichkova: None Declared, A. Maschan: None Declared.

PH-P539
SUBSTANTIAL RATE OF MIXED CHIMERISM AFTER TREOSULFAN-BASED CONDITIONING IN CHILDREN WITH NON-MALIGNANT DISEASES

H. Fouz[1,], F. Schuster[1], M. Siepermann[1], F. Babor[1], V. Friemann[1], E. Schmidt[1], A. Borkhardt[1], R. Meisel[1]*
[1]Department of Paediatric Oncology, Haematology and Immunology, Duesseldorf University Hospital, Duesseldorf, Germany

Introduction: Treosulfan-based conditioning for haematopoietic stem cell transplantation (SCT) shows limited extramedullar toxicity and thus appears favorable especially in patients with non-malignant diseases.
Materials (or patients) and Methods: Retrospective analysis of clinically significant mixed chimerism (MC; defined as >10% recipient cells) in 13 patients (median age 2 years) with non-malignant diseases after treosulfan-based conditioning from 2009 to 2013. Underlying diseases were Wiskott-Aldrich syndrome (WAS) (*n*=2), mucopolysaccharidosis type I (MPS I) (*n*=1), immunodeficiency syndrome (ID) (*n*=5), hemophagocytic lymphohistiocytosis (HLH) (*n*=1) and beta-thalassemia major (TM) (*n*=4). Donors were 10/10 HLA-matched unrelated (*n*=5), sibling (*n*=7) and one haploidentical donor. All patients received treosulfan and fludarabine, while thiotepa was added in seven cases. Pre-transplantat serotherapy (*n*=12) consisted of anti-thymocyte globulin (*n*=9) or alemtuzumab (*n*=3). Graft-versus-host disease (GvHD) prophylaxis was mainly based on cyclosporine (CSA) (*n*=12) with addition of methotrexate and/or mycophenolate mofetil in several cases. Chimerism was analyzed by short tandem repeats or XY-FISH.
Results: Conditioning was well tolerated, however, two patients with ID died due to pre-existing severe infections prior to engraftment. One patient suffered graft rejection after haploidentical transplantation which was successfully rescued after second haploidentical transplantation. Ten patients achieved primary engraftment with median neutrophil engraftment on day (d) 19.5 (range d 15-36).
Mixed chimerism occurred in four of the engrafted patients (40%) (MPS I, WAS, TM, ID). In one patient (ID) MC spontaneously dropped from 24.3% to 2.1% during first month and turned into full donor chimerism after day 100 (table1). MC persisted in three cases for a median follow up of 484 days (range 364-629 d) and rose to a median of 80% (range 60-90%). MC was detectable in T- and B-cell, myeloid and monocyte compartments in two patients (WAS, TM) and in T-cell and myeloid compartments in one patient (MPS I). CSA was thus tapered and two patients additionally received donor lymphocyte infusions (DLI). While no GvHD occurred, MC increased further despite these interventions (table1).
Notably however, only one patient (WAS) presented with recurrence of disease symptoms, i.e. low platelets requiring occasional platelet transfusions. Due to stable MC in the T-cell compartment (40-60%) retransplantation could be avoided and platelet count stabilized after splenectomy.
Discussion: Children with non-malignant diseases transplanted with treosulfan-based low toxicity conditioning appear to be at significant risk to develop MC. Interventions to reverse MC (i.e. tapering of CSA and DLI) seem of limited value. Since MC is not uniformly associated with symptoms of disease recurrence any intervention requires careful risk-benefit assessment. Long-term outcome with MC after treosulfan-based conditioning remains unclear and urgently warrants further analyses in larger prospective studies.
Disclosure of Interest: None Declared.

Table 1: Characteristics and course of patients with MC

First detection of MC (day: MC)		**Early CSA tapering** (day: MC)		DLI (day: MC)		**Last analysis** (day: MC)	
MPS I	+123: 10-20%	**MPS I**	+126: 10-20%	**MPS I**	+230/294: 30-40%	**MPS I**	+484: 40-60%
WAS	+12: 22%	**WAS**	+65: 10-20%	**WAS**	+117: 40-60%	**WAS**	+629: 60-80%
TM	+167: 10-20%	**TM**	+212: 20-30%	**TM**	None	**TM**	+364: 60-80%
ID	+19: 24.3%	**ID**	None	**ID**	None	**ID**	+300: 0%

PH-P540

UK EXPERIENCE OF UNRELATED CORD BLOOD TRANSPLANTATION IN PAEDIATRIC PATIENTS

P. Veys[1,], R. Danby[2,3,4], A. Vora[5], M. Slatter[6], R. Wynn[7], S. Lawson[8], C. Steward[9], B. Gibson[10], M. Potter[11], J. de la Fuente[12], G. Shenton[13], J. Cornish[9], A. Gennery[6], J. Snowden[5], D. Bonney[7], M. Velangi[8], A. Ruggeri[14], E. Gluckman[4], R. Hough[15], V. Rocha[2,3,4] and on behalf of Eurocord and BSBMT*

[1]Great Ormond Street Hospital for Children, London, [2]Oxford University Hospitals NHS Trust, [3]NHS Blood and Transplant Oxford Centre, Oxford, United Kingdom, [4]Eurocord, Hôpital Saint Louis APHP, Paris, France, [5]Sheffield Children's Hospital, Sheffield, [6]Royal Victoria Infirmary, Newcastle upon Tyne, [7]Royal Manchester Children's Hospital, Manchester, [8]Birmingham Children's Hospital, Birmingham, [9]Bristol Royal Hospital for Children, Bristol, [10]Royal Hospital for Sick Children Glasgow, Glasgow, [11]Royal Marsden Hospital, Sutton, [12]St Mary's Hospital and Imperial College, London, [13]Leeds General Infirmary, Leeds, [14]Eurocord, Hôpital Saint Louis APHP, Paris, [15]University College Hospital, London, United Kingdom

Introduction: The use of cord blood (CB) as an alternative stem cell source for Haematopoietic Stem Cell Transplantation (SCT) has been widely adopted in North America. The use of CB in the UK has been less favoured with preference given to the use of matched and mismatched adult unrelated donors in combination with *in-vivo* T-cell depletion with Alemtuzumab.

Objective: To assess the demographics and outcome of patients < 18 years of age undergoing unrelated CB transplantation (CBT) within the UK.

Materials (or patients) and Methods: All patients < 18 yrs undergoing CBT in the UK between 1998-2012 and reported to the BSBMT/Eurocord Registries were analysed. Patients included in prospective cord blood trials (*n*=1) were excluded.

Results: 330 patients aged between 0.1 and 17.9 years underwent CBT in the UK in a twelve year period. This reflects 16% of the total number of unrelated transplants performed during that time. 165 CBTs were performed for malignant (M) disease (ALL[73], AML[57], MDS/MPD[19], lymphoproliferative[13]) and 165 for non-malignant (NM) diseases (immunodeficiency [77], metabolic [45], bone marrow failure [24], HLH[18]). The median age and weight was less for NM patients 0.9 vs 6.7 years and 8.0 vs 23.6 kg respectively. Approximately half of all patients underwent CBT after 2009. Within the M group 74% received myeloablative conditioning, 73% received serotherapy, 82% a single cord, with a median TNC dose infused of 5.0 (1.1-34.4) x10^7/kg and CD34 dose infused of 1.97 (0.06-23.5) x 10^5/kg; 76% were 0/1 HLA antigen mismatches. Within the NM group 35% had myeloablative conditioning, 89% received serotherapy, 93% a single cord, with a median TNC infused of 11.0 (0.98-43.8) x 10^7/kg, and CD34 infused 3.98 (0.3-27.3) x 10^5/kg; 93% were 0/1 HLA mismatched.

Outcome: The overall survival (OS) for NM diseases at 2 years was 76%, with HLH patients doing the worst (49%). The median day to neutrophil (>0.5) and platelet (>20) recovery was 19 and 32 days respectively. The incidence of aGvHD (II-IV) and cGvHD was 20% and 8% respectively. The OS for the M group was 52% at 3 years, with a significantly worse outcome for lymphoproliferative disease (25%, *P*=0.01) and trends to better outcome with a TNC dose infused of >5 x 10^7/kg (63 v 46%; *P*=0.06), and 6/6 matched cords (69% (6/6) v 50% (5/6) v 41% (4/6); *P*=0.08). Median days to neutrophil (>0.5) and platelet (>20) recovery were 24 and 42 days respectively, and the incidence of aGvHD (II-IV) and cGvHD was 26% and 9% respectively. TRM was 15% at 100 days and 21% at 1 year and relapse 19% and 27% at 1 and 3 years respectively. There was no influence of year of CBT before/after 2009, the use of reduced intensity conditioning or the use of serotherapy. Focusing on children with acute leukaemia (*n*=130), the 3 year OS for AML was 58% and for ALL 48%; there was a trend to reduced survival with advancing disease status in ALL: CR1 64%, ≥ CR2 38%, and advanced disease 22% (*P*=0.08); surprisingly there was no influence of disease status in AML: CR1 68%, ≥ CR2 51%, and advanced disease 62% (*P*=0.74), although the numbers are fairly small.

Discussion: Despite the relative reluctance to use CBT in the UK the outcomes are good and very comparable to adult unrelated donor transplantation. HLH patients appear to do less well following CBT. The surprisingly good outcome (>50%) in advanced AML suggests the potential for a strong graft-versus-leukaemia effect but requires confirmation in a larger study.

Disclosure of Interest: None Declared.

PH-P541

CIDOFOVIR FOR HUMAN ADENOVIRUS INFECTIONS AFTER PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: LIMITED EFFECT ON VIRAL LOAD DESPITE CONSIDERABLE NEPHROTOXICITY

G. Lugthart[1,], M. A. Oomen[1], C. M. Jol-van der Zijde[1], L. M. Ball[1], D. Bresters[1], W. J. W. Kollen[1], F. J. Smiers[1], C. L. Vermont[1], R. G. M. Bredius[1], R. N. Sukhai[1], M. W. Schilham[1], M. J. D. Van Tol[1], A. C. Lankester[1]*

[1]Department of Pediatrics, Leiden University Medical Center, Leiden, Netherlands

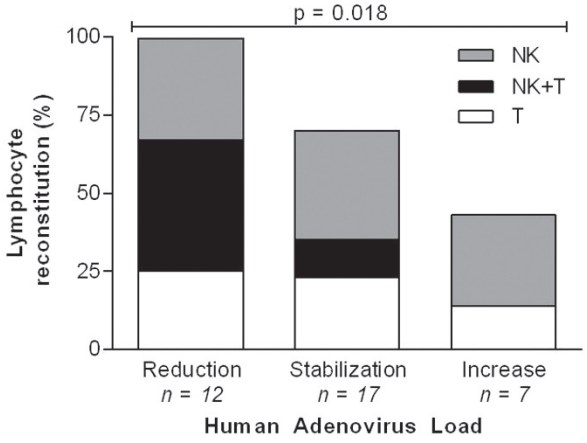
Introduction: Human Adenovirus (HAdV) viremia is a frequent and potentially fatal complication after pediatric allogeneic hematopoietic stem cell transplantation (HSCT). HAdV infections are generally treated with cidofovir, but studies on the effectiveness of cidofovir treatment are inconsistent and often lack data on the immunological status of cidofovir treated patients.

Materials (or patients) and Methods: In 36 children treated with cidofovir for HAdV viremia after HSCT, the change of plasma HAdV DNA load was measured 14 days after treatment initiation and related to concomitant lymphocyte reconstitution. Acute and chronic nephrotoxicity of cidofovir were evaluated by monitoring of glomerular and tubular kidney function.

Results: During cidofovir treatment, the viral load increased ≥1 log in 7 cases (19%), viral load stabilization occurred in 17 cases (57%) and ≥1 log viral load reduction was measured in 12 cases (33%). Viral load reduction was always accompanied by lymphocyte reconstitution and in only 5 cases (14%), viral load stabilization was reached in the absence of lymphocyte reconstitution. Eventually, HAdV viremia was cleared in 27/36 cases (75%). Glomerular and tubular nephrotoxicity occurred in 23% and 31% of cases, respectively. At latest follow up, 2/21 long-term survivors (10%) had cidofovir related chronic kidney disease.

Discussion: HAdV viremia progressed despite of cidofovir treatment in a substantial number of cases. A subgroup of patients possibly benefited from cidofovir through stabilization of the viral load but treatment was associated with considerable nephrotoxicity. Lymphocyte reconstitution was essential for reduction of the viral load during cidofovir treatment.

Disclosure of Interest: None Declared.



PH-P542

OUTCOMES AFTER A SEQUENTIAL CLOFARABINE AND CYTARABINE-BASED CHEMOTHERAPY FOLLOWED BY REDUCED-INTENSITY CONDITIONING AND ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN WITH PRIMARY REFRACTORY OR RELAPSED ACUTE MYELOID LEUKEMIA: A PILOT STUDY

A. Grain[1,], A. Sirvent[2], S. Marion[1], F. Méchinaud[3], M. Mohty[4], T. Guillaume[5], P. Chevallier[5], F. Rialland[6]*

[1]Hématologie pédiatrique, CHU de Nantes, NANTES, [2]Hématologie pédiatrique, CHU de Nice, Nice, France, [3]Pediatric Oncology Unit, University Hospital, Melbourne, Melbourne, Australia, [4]Service d'Hématologie clinique et de thérapie cellulaire, Hôpital Saint-Antoine, AP-HP, Paris, [5]Hématologie clinique, [6]Hématologie pédiatrique, CHU Nantes, NANTES, France

Introduction: The FLAMSA sequential treatment with chemotherapy followed by reduced-intensity conditioning (RIC) for allogeneic stem cell transplantation (allo-SCT) has been introduced few years ago for adult refractory acute myeloid leukemia (AML) showing high activity and relatively good survivals in this particular setting (Schmid, Blood, 2006). There is no study at our knowledge reporting the results of the sequential approach in pediatric refractory AML patients. Here we report our own experience in 6 children using a debulking chemotherapy combining clofarabine and Ara-C followed by RIC before allo-SCT.

Materials (or patients) and Methods: These preliminary results included 3 males and 3 females with a median age of 8,5 years (range: 3-19). All cases had received a sequential regimen before allo-SCT at the CHU of Nantes (*n*=5) or at the CHU of Montpellier (*n*=1) for primary refractory AML (*n*=1), refractory relapsed AML (*n*=3), slow responder relapsed AML (*n*=1) and blastic JMML (*n*=1). Sequential regimen consisted of 1) clofarabine 30 mg/m^2/d days-13 to -9, Ara-C 1g/m^2/d days-13 to-9 followed by RIC combining cyclophosphamide 60 mg/kg/d day-5, iv Busulfan 3.2 mg/Kg/d days -4 to -3 and ATG 2.5 mg/Kg/d days -3 to -2 in 5 patients or 2) clofarabine 30 mg/m^2/d days-13 to -9, Ara-C 1g/m^2/d days-13 to-9 followed by RIC total body irradiation 4 grays day-5, cyclophosphamide 40 mg/Kg/d days -4 to -3, and ATG 2.5 mg/Kg/d days -3 to -2 in 1 patient. One patient received a graft from a sibling donor while the five other patients received a graft from an unrelated donor (10/10 *n*=3; 9/10 *n*=2). GvHD prophylaxis consist in Ciclosporin and Mycophenolate Mofetil (MMF) (*n*=5), or Ciclosporin and Methotrexate (*n*=1).

Results: Engraftment was observed in 4 patients (67%) who achieved complete remission (CR) after transplant. The 2 patients which had an autologous reconstitution, relapsed and died rapidly. Considering the 4 patients achieving full engraftment and CR, two relapsed at day+60 and at 13 months post-allograft. The first one died. The two others patients are alive in CR at +35 and +51 months post-transplant.

Discussion: Patients with primary refractory and relapsed AML are rarely cured without undergoing allo-SCT. The treatment's management is currently debated. Using re-induction chemotherapy with the aim of obtaining a second CR before allo-SCT after myeloablative conditioning may leads to added organ severe toxicity, and low poor CR rate. Newer strategies using "sequential allo-SCT" have been developed and increased OS and LFS in patient with high risk diseases. Our pilot study shows encouraging results in survival, consistent with adults series. In our experience, cytoreductive chemotherapy and conditioning were well tolerated, as in the adult's study. To our knowledge, this is the first report of a sequential allo-SCT approach for refractory pediatric AML patients. Although the number of patients is limited in our cohort, the results showed here are very encouraging as 2 out of the 6 patients are alive in CR with full engraftment. These results have to be confirmed prospectively.

Disclosure of Interest: None Declared.

PH-P543

A PRELIMINARY POPULATION PHARMACOKINETIC MODEL FOR DOSE SELECTION OF TREOSULFAN USED IN CONDITIONING TREATMENT PRIOR TO HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN CHILDREN

P. J Van Den Berg[1,], M. Ruppert[1], K.-W. Sykora[2], R. Beier[2], D. W Beelen[3], R. A Hilger[3], M. E Scheulen[4], J. Wachowiak[5], E. R Nemecek[6], E. Spaans[1], M. Lioznov[7], U. Pichlmeier[7], B. Wulff[7], J. Baumgart[7]*

[1]Kinesis Pharma B.V., Breda, Netherlands, [2]Department of Pediatric Hematology/Oncology, Hannover Medical School, Hannover, [3]Clinic for Bone Marrow Transplantation, University Hospital Essen, [4]Department of Medical Oncology, West German Cancer Center, University Hospital Essen, Essen, Germany, [5]Department of Paediatric Oncology, Hematology and Transplantology, Poznan University of Medical Sciences, Poznań, Poland, [6]OHSU-Doernbecher Children's Hospital, Portland, United States, [7]medac GmbH, Wedel, Germany

Introduction: Treosulfan, an alkylating agent, is currently being developed by medac as a component of a conditioning regimen prior to HSCT in adults and children. Within a paediatric investigational plan (PIP) agreed by the Paediatric Committee of the European Medicines Agency, a population pharmacokinetic (PopPK) model had to be developed to select dosages for the agreed studies in paediatric patients from 1 month to 18 years of age.

Materials (or patients) and Methods: All available treosulfan pharmacokinetic data from 7 clinical studies consisting of data from 93 adults and 23 children (0.4 – 17 years) were used to develop a PopPK model for treosulfan using NONMEM version 7.1 and to investigate the potential influence of 6 covariates on treosulfan exposure (i.e. body surface area [BSA], age, body weight, height, renal function and use of diuretics). Paediatric dose selection was aimed to target a similar exposure in children as obtained in adults after intravenous administration of the clinically effective dose of 14 g/m^2/day treosulfan for 3 consecutive days for conditioning prior HSCT.

Results: When doses were calculated in g/m^2, the exposure to treosulfan (area under the curve, AUC) was significantly increased in children, compared to adults. The PopPK model for treosulfan consisted of two compartments with a first order distribution and elimination processes. A covariate analysis revealed that BSA was the only relevant covariate for clearance and volumes of distribution. The PopPK model provided an adequate fit to the data and model diagnostics revealed no significant bias.

Based on the estimated PopPK model, the functional relationship between clearance and BSA could be derived (see picture). For feasibility purposes, a simplified scheme of treosulfan dosing was deduced thereof to be applied in the planned paediatric studies:

BSA	Daily treosulfan Dose
≤ 0.5 m^2	10 g/m^2
> 0.5 – 1 m^2	12 g/m^2
> 1.0 m^2	14 g/m^2

$$DOSE_{model}(g) = \frac{Target\ AUC(\mu g * h/mL)}{1000} * 17.8 * \left(\frac{BSA}{1.75}\right)^{1.29}$$

Discussion: The PopPK model for treosulfan is robust and accurately predicted exposure in children older than 1 year of age. For infants a best estimate could be given only as the dataset merely included limited data of children below 1 year of age. The planned paediatric transplant trials will start with the deflected dosing table and will update the PopPK model accordingly to further investigate its validity in children with malignant or non-malignant diseases qualifying for HSCT.

Disclosure of Interest: P. Van Den Berg Conflict with: medac for paid consultancy services, M. Ruppert Conflict with: medac

for paid consultancy services, K.-W. Sykora: None Declared, R. Beier: None Declared, D. Beelen: None Declared, R. Hilger: None Declared, M. Scheulen: None Declared, J. Wachowiak: None Declared, E. Nemecek: None Declared, E. Spaans Conflict with: medac for paid consultancy services, M. Lioznov: None Declared, U. Pichlmeier: None Declared, B. Wullf: None Declared, J. Baumgart: None Declared.

PH-P544

IMPACT OF A CHANGE IN PROTECTED ENVIRONMENT ON THE OCCURRENCE OF SEVERE BACTERIAL AND FUNGAL INFECTIONS IN CHILDREN UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

C. Libbrecht[1,], C. Domenech[1], J. Bacchetta[2], C. Ploton[3], A. L. Bienvenu[4], V. Mialou[5], C. Galumbrun[6], Y. Bertrand[1]*

[1]Institute of Pediatric Haematology and Oncology, [2]Nephrology, [3]Bacteriology unit, Women-Mother and Child Hospital, [4]Mycology and Parasitology Unit, Croix Rousse Hospital, [5]Tissue and Cell Bank, Edouard Herriot Hospital, Lyon, [6]Heamatology Unit, La Timone Hospital, Marseille, France

Introduction: Infections are still a leading cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (aHSCT), particularly during the initial hospital stay. Many measures are taken to prevent those life threatening conditions, including strict isolation, but few guidelines exist. Protected environment can have important consequences, especially on children's quality of life. We compared here the incidence of severe bacteraemia and invasive fungal infections (IFI) in a large pediatric cohort undergoing aHSCT in two units, including major changes in term of human-to-human interaction and room isolation.
Materials (or patients) and Methods: We analysed the occurrence of severe infections from the beginning of the conditioning regimen to the end of the initial hospital stay in 286 patients undergoing aHSCT from 2002 to 2012 in our 2 centers. From 2002 to 2007, 144 procedures were performed in center 1 in laminar air flow rooms, wearing sterile gowns and gloves, hat and mask, eating sterile food. From 2008 to 2012, 142 procedures were performed in center 2 in positive air pressure rooms with HEPA filters, wearing clean gowns and mask, eating protected food. Furthermore, there was the possibility for one parent to sleep in his child's room in center 2. Children received the same oral decontamination and antibiotics/antifungals used for febrile neutropenia remained unchanged during the two periods. We recorded the occurrence of bacteraemia, defined by the association of fever with a positive blood culture (except for Coagulase negative staphylococci (CoNS) bacteraemia, where two distinct positive blood cultures were necessary), and proven IFI (according to the EORTC criteria).
Results: We reported 85 episodes of severe infections (29.7%): 44 episodes (30.6%) occurred in center 1 while 41 (28.9%) occurred in center 2 (*p: 0.53*). Infections were mostly bacteraemia with 80 episodes (94.1%) including 6 polymicrobial events: 41 episodes (28.5%) occurred in center 1 and 39 episodes (27.5%) in center 2 (*p: 0.71*). Gram Positive bacteria (*n*=71, 81.6%) were more prevalent than Gram Negative bacteria (GN) (*n*=16, 18,4%) with a large predominance of *CoNS* (*n*=57, 65.5%) in both centers. We observed a trend towards more GN bacteria in center 2 (*n*=12, 26.1%) compared with center 1 (*n*=4, 9.8%), not significant. Only the use of an unrelated donor was significantly associated with bacteraemia (*p: 0.003*). We recorded 5 IFI episodes: 3 candidemia and 2 mold infections. Three occurred in center 1 and 2 in center 2 (*p: 0.66*). Four patients died of infection (1.4%): 3 died from an IFI and 1 from bacteraemia.
Discussion: This is the first study comparing the occurrence of infection between 2 centers with different protected environments, but same protocols concerning antibiotics and prophylaxis. We showed similar infection rate in both centers. We noticed small changes: an increase in the proportion of GN as well as a later bacteraemia onset, but they never reached statistical significance. Graft source was independently associated with more bacteraemia, as described in the literature. These results support our decision to make life better for children undergoing HSCT, while keeping them safe in a protected environment.
Disclosure of Interest: None Declared.

PH-P545

BLINATUMOMAB IN PEDIATRIC PATIENTS WITH RELAPSED/REFRACTORY (R/R) B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKAEMIA (BCP-ALL): A PHASE I/II STUDY

F. Locatelli[1,2,], L. Gore[3], G. Zugmaier[4], R. Handgretinger[5], C. Rizzari[6], T. M. Trippett[7], A. Borkhardt[8], S. R. Rheingold[9], P. Bader[10], D. Bhojwani[11], T. M. Cooper[12], S. G. DuBois[13], M. M. O'Brien[14], C. M. Zwaan[15], C. Holland[16], N. Mergen[4], A. Fischer[4], M. Zhu[17], Y. Hijazi[4], J. Whitlock[18], A. von Stackelberg[19]*

[1]Department of Oncohematology, Ospedale Pediatrico Bambino Gesu, Rome, [2]University of Pavia, Pavia, Italy, [3]Children's Hospital Colorado, Aurora, United States, [4]Amgen Research (Munich) GmbH, Munich, [5]Department of Pediatric Oncology, University of Tübingen, Tübingen, Germany, [6]Department of Pediatrics, San Gerardo Hospital, University of Milano-Bicocca, Monza, Italy, [7]Memorial Sloan-Kettering Cancer Center, New York, United States, [8]Department of Pediatric Oncology, University of Düsseldorf, Düsseldorf, Germany, [9]Children's Hospital of Philadelphia, Philadelphia, United States, [10]Division for Stem Cell Transplantation and Immunology, Hospital for Children and Adolescents III, University of Frankfurt, Frankfurt, Germany, [11]Department of Oncology, St Jude Children's Research Hospital, Memphis, [12]Children's Healthcare of Atlanta, Atlanta, [13]Department of Pediatrics, University of California San Francisco School of Medicine, San Francisco, [14]Cincinnati Children's Hospital Medical Center, Cincinnati, United States, [15]Pediatric Oncology, Erasmus Medical Center, Sophia Children's Hospital, Rotterdam, Netherlands, [16]Amgen Inc., Rockville, [17]Amgen Inc., Thousand Oaks, United States, [18]Hospital for Sick Children, Toronto, Canada, [19]Department of Pediatric Oncology/Hematology, Charité Campus Virchow, Berlin, Germany

Introduction: Blinatumomab is an investigational bispecific T-cell engager (BiTE®) antibody that has been shown to produce complete remissions (CR) in CD19-positive BCP-ALL, thus providing a bridge to hematopoietic stem cell transplantation (HSCT), in an exploratory study in adults with r/r ALL. Cytokine-release syndrome (CRS) and CNS toxicities are medically important adverse events (AEs) associated with blinatumomab in adult patients. We report results from the phase I portion of an ongoing phase I/II multicenter study that evaluates the optimal dose, toxicities, and preliminary response rates of blinatumomab administered to paediatric patients with BCP-ALL.
Materials (or patients) and Methods: Eligible patients were <18 years old with BCP-ALL that was refractory, in second or later bone marrow relapse, or in any marrow relapse after allogeneic HSCT. Blinatumomab was given by continuous IV infusion (28 days on/14 days off; up to 5 cycles). Five dosing regimens were explored: 5, 15, and 30 μg/m^2/day and stepwise dosing of 5–15 or 15–30 μg/m^2/day (7 days at the lower dose, followed by the higher dose for the remainder of the cycle). Maximum tolerated dose (MTD) was the primary endpoint in the phase I portion of the study (Rolling 6 design). Secondary endpoints included toxicity, CR rate, and pharmacodynamic markers.
Results: Forty-one patients have received a total of 73 cycles. Nine (22%) patients had refractory disease and 7 (17%) had experienced ≥2 bone marrow relapses. Twenty-five (61%) patients had relapsed after allogeneic HSCT; none had prior autologous HSCT. CRS was dose-limiting. MTD was established at 15 μg/m^2/day based on dose-limiting toxicity assessment and data safety monitoring board recommendation. To reduce the risk of CRS, a stepwise dose of 5–15 μg/m^2/day was recommended for the phase II portion of the study. The phase II recommended dose was further evaluated in 18 patients across two age groups (2–6 and 7–17 years) in the phase I expansion part with PK analysis. One (6%) of those 18 patients developed CRS (grade 3). Among all 41 patients, the most common AEs (regardless of causality) were pyrexia (70% of patients), headache (37%), hypertension (32%), and anemia (29%). Forty-six percent of all patients developed ≥1

CNS event (all but one were grade 1 or 2), most commonly tremor (10%), anxiety (7%), and confusional state (7%). Across all dose levels, 15 (37%) patients attained CR (12 [30%] were MRD-negative), and 3 (7%) had partial remissions within the first two treatment cycles. Of the 15 patients attaining CR, 8 (53%) were able to proceed to allogeneic HSCT after completing blinatumumab therapy. Using body surface–area dosing, blinatumomab showed linear pharmacokinetics in paediatric patients. Transient elevations of serum cytokines (particularly interleukin-6, interferon-gamma, and interleukin-10) were observed in most patients, primarily within the first 2 days initiation of infusion.
Discussion: In the phase I portion of this study in paediatric patients with r/r BCP-ALL, 15 μg/m^2/day was established as MTD. CRS was dose-limiting, but administration of a stepwise dose, 5–15 μg/m^2/day, has been successful in ameliorating CRS. Thirty-seven percent of patients achieved CR, and more than half of those responders were able to receive allogeneic HSCT, thus confirming a potential bridge-to-transplantation role of blinatumomab.
Disclosure of Interest: F. Locatelli: None Declared, L. Gore Conflict with: Amgen Inc., G. Zugmaier Conflict with: Amgen Research (Munich) GmbH, Conflict with: Amgen Inc., R. Handgretinger: None Declared, C. Rizzari: None Declared, T. Trippett: None Declared, A. Borkhardt: None Declared, S. Rheingold Conflict with: Novartis, P. Bader: None Declared, D. Bhojwani: None Declared, T. M. Cooper: None Declared, S. DuBois: None Declared, M. O'Brien: None Declared, C. Zwaan: None Declared, C. Holland Conflict with: Amgen Inc., Conflict with: Amgen Inc., N. Mergen Conflict with: Amgen Research (Munich) GmbH, Conflict with: Amgen Inc., A. Fischer Conflict with: Amgen Research (Munich) GmbH, Conflict with: Amgen Inc., M. Zhu Conflict with: Amgen Inc., Conflict with: Amgen Inc., Y. Hijazi Conflict with: Amgen Research (Munich) GmbH, Conflict with: Amgen Inc., J. Whitlock: None Declared, A. von Stackelberg Conflict with: Amgen Inc.

PH-P546
MESENCHYMAL STEM CELL TREATMENT FOR STEROID RESISTANT GRAFT-VERSUS-HOST DISEASE IN CHILDREN

F. Erbey[1,], D. Atay[1], A. Akcay[1], E. Ovali[2], G. Ozturk[1]*
[1]Pediatric Hematology/Oncology & BMT Unit, Bahcelievler Medicalpark Hospital, [2]Hematology, Acibadem Labcell, Istanbul, Turkey

Introduction: Severe graft-versus-host disease is a life threatening complication after allogeneic transplantation with hematopoietic stem cells. Mesenchymal stem cells have immunomodulatory effects. The aim was to study the effect of mesenchymal stem cell infusion on graft-versus-host disease.
Materials (or patients) and Methods: Fifteen patients with steroid-resistant severe graft-versus-host disease were treated with mesenchymal stem cells, between June, 2011, and December, 2013. We recorded response, transplantation-related deaths, and other adverse events after infusion of mesenchymal stem cells.
Results: The median age of the patients was 7 years (min-max range: 2-18 years) with 9 males and 6 females. The median dose of mesenchymal stem cell was 1.33 x 10^6 (min-max range 1.00 - 2.00 x 10^6) cells per kg bodyweight. Twelve patients received one dose, two received two doses, and one received three doses. No patients had side-effects during or immediately after infusions of mesenchymal stem cells. Seven patients achieved complete response, two partial response after mesenchymal stem cells infusion. The total effective rate was 60% (9/15). Mean follow-up period was 289 (38–809) days post-transplantation, 8 patients survived and 7 died. Overall survival rate was 53,3%. The causes of death included graft-versus-host disease (n = 2), graft-versus-host disease with concomitant infection (n = 2), leukemia relapse (n = 2) and infection (n = 1), respectively.
Discussion: Mesenchymal stem cells derived from the bone marrow are effective therapy for childrens with steroid-resistant graft-versus-host disease.
Disclosure of Interest: None Declared.

PH-P547
PREVALENCE AND SEVERITY OF LATE EFFECTS AFTER ALLOGENEIC HSCT FOR HAEMATOLOGICAL MALIGNANCIES IN CHILDREN TRANSPLANTED UNDER THE AGE OF THREE YEARS

D. Bresters[1,], A. Lawitschka[2], R. Duarte[3], A. Rovo[4], C. Peters[2] and Complications & Quality of Life and Paediatric Diseases Working Parties*
[1]Pediatrics, LUMC, Leiden, Netherlands, [2]Stem Cell Transplantation Unit, St. Anna Kinderspital, Vienna, Austria, [3]Hospital Duran i Reynals, Catalan Institute of Oncology, Barcelona, Spain, [4]Hematology, University Hospitals, Basel, Switzerland

Introduction: Several studies have been published on late effects after haematopoietic stem cell transplantation (HSCT) in childhood, but few have focused on children transplanted at a very young age. The aim of this study is to assess the prevalence of and risk factors for late effects in these young children and compare with older children undergoing HSCT. Also, risk factors for late effects in these young children will be analysed.
Materials (or patients) and Methods: This is a multicentre cross-sectional study. Patients transplanted for a haematological malignancy under the age of three years and alive at least 5 years after HSCT were identified from the EBMT database. Paediatric HSCT centres in Europe were sent a questionnaire for each eligible patient regarding the presence and severity of selected late effects: endocrine function (growth, thyroid and gonadal function), CNS problems and secondary malignancy. After return of the completed questionnaires, data were entered in a database and supplemented with patient data in the EBMT database for analysis. The following risk factors for late effects will be analysed: sex, age, conditioning regimen, donor type and stem cell source, acute GVHD, and chronic GVHD.
Results: From the EBMT database 650 potentially eligible patients were identified. 295 (45%) questionnaires were returned and valid for data collection. 171 of 295 (58%) patients are male. Median age of the study group at HSCT is 1.8 years (range 0-3 yr). The majority of patients received a myeloablative conditioning regimen (197 of 209 (94%) patients in whom conditioning was registered). In 66 of 295 (22%) patients this included total body irradiation with a dose of at least 6 Gy) and 2 patients received total lymphoid irradiation <6 Gy. Median follow-up of the study group is 10.5 years (range: 4.8 to 31.6 yrs). The frequency of late effects is as follows: 26% (n=77) of patients had only one of the selected late effects, 22% (n=65) of patients had 2 or 3 late effects and 2% (n=6) had 4 or 5 late effects. Severe or disabling late effects were present in 8 of 295 (3%) patients. The most prevalent late effect was thyroid dysfunction in 30% (88/289 with thyroid function known, hypothyroidism in 79). Growth disturbance was present in 27% (n=78) and gonadal dysfunction was present in 27 of 153 (18%) patients aged 12 years or more at time of study. CNS problems were present in 14% (39/288), cognitive problems (n=39, 14%) and concentration impairment (n=32, 12%) being the most prevalent CNS late effects. Nine patients developed a secondary malignancy at a median of 11.1 years (range: 0.94 to 22.75yrs) post HSCT. Risk factors for these late effects will be analysed and presented.
Discussion: The preliminary results of this study indicate that 50% of the patients had at least one endocrine or CNS late effect and/or a secondary malignancy at a median follow-up of 10.5 years after HSCT performed before age 3 years. The analysed late effects have been described as the most common after HSCT in any paediatric age group and the prevalence of these problems does not appear to be increased in young children. Interestingly, at this young age 23% received a conditioning regimen including irradiation, a known risk factor for late effects. The avoidance of irradiation in children should be a major goal for the upcoming years.
Disclosure of Interest: None Declared.

PH-P548

VIRAL INFECTIONS IN CHILDREN AND ADOLESCENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: SINGLE CENTER 10 YEAR EXPERIENCE

H. David Escobar Pirela[1,], J. María Fernández Navarro[1], C. Fuentes Socorro[1], M. D. M. Andrés Moreno[1], A. Verdeguer[1]*
[1]Paediatric Oncology, Hospital Universitari i Politecnic La Fe, Valencia, Spain

Introduction: Viral infections are a common complication in children after hematopoietic stem cell transplantation (HSCT) which has been related to high rates of morbidity and mortality.
Materials (or patients) and Methods: To describe our center's experience in management of viral infections in children after HSCT and their prevalence, we conducted a cross sectional study, reviewing medical records of children who underwent HSCT in our clinic unit between January 2002 and December 2011. All HSCT recipients were included in the study regardless the stem cells' source, type of transplant, underlying diagnosis and disease status at the moment of transplantation. Patients had PCR pre-emptive screening test weekly for CMV, EBV and ADV which were either quantitative or qualitative. Other viruses were screened only in response to suggestive signs and symptoms of disease. Viral infection was defined as two positive consecutive PCR results in the absence of clinical signs/ symptoms or one positive associated with a compatible clinical picture. Statistical association between viral infections and risk factors was achieved through binary logistic regression analysis
Results: 224 patients underwent HSCT in the study period (137 autologous, 58 allogeneic 29 CBT). Mean age 6.94 years (± 4.4). Most prevalent underlying diseases were: Acute Lymphoblastic Leukemia (LLA) 67%, Acute Myeloid Leukemia (ALM) 20% and Neuroblastoma 3.63%. Seventy three viral infection episodes were diagnosed in 55 patients (prevalence: 24.55%): CMV (16 cases, 21.91%), HHV6 (16 cases, 21.91%), ADV (12 cases, 16.43%) and BKV (10 cases, 13.69%). Infections were more prevalent in cord blood recipients (9%). Seventy percent of the viral infections received specific treatment. Eight patients died due to viral infections. ALL OR 4.1 (95% CI 1.48-11.37) and allogeneic stem cell transplantation (AHSCT) OR 3.75 (95% CI 1.68-8.36) were associated with a higher risk of viral infection (Table1).

Table 1. Statistical analysis results

Variable	p	OR	CI
Allogeneic	0.001	3.75	1.68-8.365
Autologous	ns	ns	ns
Age	ns	0.95	0.88-1.03
TBI	ns	1.14	0.38-3.39
Busulphan	ns	0.93	0.39-2.25
ALL	0.006	4.11	1.49-11.37
ALM	ns	2.09	0.68-6.41

Discussion: In our center, viral infection prevalence is higher than previously reported, although mortality is lower. A probable explanation could be our pre-emptive screening test policy. CMV and HHV6 were the most common etiologies. Viral infection prevalence was higher in allogeneic HSCT, especially when cord blood was the stem cell´s source. ALL and AHSCT were the only factors associated with a higher risk of viral infections in our patients.
Disclosure of Interest: None Declared.

PH-P549

REDUCED INTENSITY CONDITIONING REGIMEN AND ALLOGENEIC STEM CELL TRANSPLANTATION FROM RELATED OR UNRELATED HLA IDENTICAL DONOR IN ADVANCED EWING SARCOMA

A. Prete[1,], R. Rondelli[1], R. Luksch[2], A. Pession[1], F. Locatelli[3], F. Fagioli[4]*
[1]Pediatric Oncology and Hematology Department, University of Bologna, Bologna, [2]Pediatric Oncology and Hematology Department, IRCCS Istituto Tumori di Milano, Milano, [3]Pediatric Oncology and Hematology Department, IRCCS Bambin Gesù, Roma, [4]Pediatric Oncology and Hematology Department, Ospedale Regina Margherita, Torino, Italy

Introduction: Allogeneic hematopoietic stem cell transplantation (allo-SCT) may provide donor cytotoxic T cell/NK cell-mediated disease control in patients with Ewing Sarcoma (ES). However, little is known about the prevalence of graft-versus-ES effects and only a few case experiences have been reported. We evaluate in a prospective national wide protocol the feasibility and efficacy of a reduced intensity conditioning regimen (RIC) followed by allo-SCT from related (RD) or unrelated HLA donor (UD) in advanced Ewing Sarcoma.
Materials (or patients) and Methods: From 2009 and 2012, 14 pts, aged 5-22 years, affected by resistant or relapsed ES, were enrolled and submitted to an allo-SCT after a RIC consisting of Thiotepa 15 mg/kg and Melphalan 140 mg/sqm. The donor was a RD in 9 cases and an UD in 5. At time of transplant 5 pts were in CR, 1 in VGPR, 5 in PR and 3 with active disease. Seven pts received allo-SCT as first line graft. Graft versus host disease (GVHD) prophylaxis consisting of Cyclosporin A in related and Cyclosporin A + Anti-lymphocytic serum and short term methotrexate in UD setting. SC sources were bone marrow.
Results: The reconstitution of bone marrow function was obtained in all the pts. Acute GVHD of grade II occurred in 5 pts and a complete marrow donor chimerism was observed after a median time of 40 and 60 days in sibling and UD setting respectively. After a median follow-up of 14 (5-42) months, 7 pts relapsed, 5 dead 9 are alive and well. The 2 years probability of OS (SE) and EFS (SE) were respectively of 0.51 (15.4) and 0.43 (12.2) respectively. The 100 days probability of TRM was 0.0.
Discussion: The use of allo-SCT in patients with advanced ES is currently experimental but in a subset of patients it may constitute a valuable approach for consolidating CR. Related Donor, CR and first-line allo-SCT assure the best outcome and may be purposed in prospective trials.
Disclosure of Interest: None Declared.

PH-P550

INFUSION-RELATED FEBRILE REACTION:RISK FACTOR FOR ENGRAFTMENT SYNDROME FOLLOWING PAEDIATRIC HAPLO-IDENTICAL ALLOGENEIC HEMATOPOIETIC STME CELL TRANSPLANTATION

Y. Chen[1,], X. jun Huang[1], L. ping Xu[1], F. rong Wang[1], Y. Wang[1]*
[1]Peking University People's Hospital, Beijing, China

Introduction: Engraftment syndrome(ES) has been recognized as an early immune reaction during neutrophil recovery after hematopoietic stem cell transplantation (HSCT) characterized by noninfectious fever and skin rash. Few data on ES after paediatric haplo-identical allogeneic hematopoietic stem cell transplantation was reported. Our previous study showed the association between infusion-related febrile reaction (IRFR) and subsequent immune reactions in adults patients following haplo-identical transplant. To determine risk factors for ES and to explore the association between IRFR and ES in a paediatric transplant cohort, we analyzed 82 consecutive recipients of myeloablative haplo-identical allo-HSCT in our institute.
Materials (or patients) and Methods: Clinical data of 82 recipients received haploidentical Allo-HSCT from Jan, 2010 to Dec, 2012 was investigated. Major clinical events included infusion-related febrile reaction (IRFR), engraftment syndrome (ES) and graft-versus-host disease (GVHD). IRFR was defined as unexplained fever >38.3°C

presented with no evidence of infection within 24 hours following the infusion of allogeneic peripheral harvest according to previous reports from our institute. Engraftment syndrome was defined as febrile reaction almost with skin rash and/or other immune reactions within 96 hours of engraftment according to the diagnostic criteria described by Spitzer and Maiolino. Possible risk factors included patient and donor age,patient and donor gender,HLA disparity,risk stratification of primary disease(standard risk/high risk), MNC dose,CD34+ cell dose,CD3+ cell dose and the occurrence of IRFR.
Results: Paediatric patients (median age, 12 years; range, 2-18 years) received busulfan-based myeloablative conditioning regimen. Primary diseases included acute myeloid leukemia (*n*=29), acute lymphoblastic leukemia (*n*=36), chronic myeloid leukemia (*n*=6) and other diseases (*n*=11). The median times to neutrophil recovery and platelet recovery were 12(9-21) days and 16(5-86) days. 37(45.1%) out of all the patients had engraftment syndrome at a median of 9(7-16) days. Patients presented with fever (97.3%) skin rash (75.7%), diarrhea (37.8%) hepatic disfunction (8.1%) and hypoxemia (5.4%). Multivariate analysis identified IRFR (*P*=0.005; HR=2.701; 95% CI=1.355-5.387) as only high risk factor for ES after haplo-identical allo-HSCT. Overall survival did not significantly differ between patients with and without ES.
Discussion: IRFR,as a unique clinical reaction seen in haplo-identical allogeneic stem cell transplant,was associated with the occurrence of ES in paediatric patients.Our results suggested the potential role of IRFR as predictor of subsequent immune reactions in haplo-identical transplant setting.
Disclosure of Interest: None Declared.

PH-P551
HOME-CARE DURING NEUTROPENIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN AND ADOLESCENTS IS SAFE AND MAY BE ADVANTAGEOUS TO ISOLATION IN THE HOSPITAL
O. Ringdén[1,], M. Remberger[1], J. Karlsson Törlen[2], M. Engström[2], G. Fjaertoft[2], J. Mattsson[1], B.-M. Svahn[2]*
[1]Laboratory Medicine, Therapeutic Immunology, KAROLINSKA INSTITUTET, [2]Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital Huddinge, Stockholm, Sweden

Introduction: After allogeneic stem cell transplantation (ASCT), children are isolated in hospital to prevent neutropenic infections. Fifteen years ago, we challenged this routine by allowing patients to be treated at home during the neutropenic phase. Initially, mainly adult patients were included in home-care. A matched-pair analysis showed that patients treated at home had several advantages compared to isolation in the hospital, such as early discharge to the outpatient clinic, fewer days of total parenteral nutrition, fewer episodes of acute graft-versus-host disease (GVHD), lower transplant-related mortality (TRM), and improved survival (Svahn *et al*, *Blood* 2002). Subsequently home-care in this setting was used on a routine basis. Children have a lower probability of and a lower severity of acute GVHD than adults. Thus, we do not know whether home-care would reduce the risk of acute GVHD in children in the same way as found in adults. The main reason for the present study was to determine whether it is safe to treat children at home.
Materials (or patients) and Methods: Patients living within two hours' drive from the hospital were given the option of treatment at home after ASCT. Daily visits by an experienced nurse and phone calls from a physician from the unit were included in the protocol. We compared 29 children and adolescents treated at home with 58 matched hospital controls.
Results: The children spent a median time of 13 days at home (range 2–24 days). Before discharge to the outpatient clinic after ASCT, they spent a median of 6 (0–5) days in hospital. Time to absolute neutrophil count $>0.5\times10^9$/L and to reach platelets $>30\times10^9$/L was not significantly different in the home-care and hospital-care children. Need for platelet transfusion and erythrocyte transfusion was similar in the two groups. The cumulative incidence of acute graft-versus-host disease (GVHD) grades II–IV was 21% in the home-care children and 39% in the controls (*P*=0.1). Chronic GVHD and probability of relapse were similar in the two groups. Transplant-related mortality at five years was 11% in the home-care patients and 18% in the controls. Overall survival at three years was 77% and 62%, respectively (*P*=0.33). None of the patients died at home and no adverse events occurred. Median costs were 38,748 Euro in the home-care patients and 49,282 Euro in those treated in the hospital (*P*=0.2).
Discussion: Initially, our aim was to improve the quality of life of our patients by treating them at home instead of isolating them in the hospital. Children in particular appreciate much more being at home than being locked up in an isolation room for most of the day. The result must be treated with caution because of the limited numbers. However, there tended to be less acute GVHD, lower TRM and a better survival in children treated at home, to the same extent as we have seen in many more adult patients (Svahn *et al*, Blood 2002). Inclusion of many more children in the home-care program will be necessary to establish whether or not these observations have true statistical significance. Our overall experience of home-care in adults involves more than 200 patients, and to date, none of them have died at home.
We conclude that it is safe for children and adolescents to be treated at home during the pancytopenic phase after ASCT.
Disclosure of Interest: None Declared.

PH-P552
THE PHARMACOKINETIC PROFILE OF TREOSULFAN IN PEDIATRIC PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
M. Ten Brink[1,], R. Bredius[2], J. Zwaveling[1], F. Smiers[2], J. den Hartigh[1], H.-J. Guchelaar[1], A. Lankester[1] and Pediatric Diseases and Inborn Errors*
[1]Clinical Pharmacy and Toxicology, [2]Pediatrics, Leiden University Medical Center, Leiden, Netherlands

Introduction: Treosulfan is an alkylating agent applied in regimens prior to allo-HSCT in children. It has strong myeloablative and immunosuppressive activity and a mild toxicity profile in comparison to busulfan. In contrast to busulfan, pharmacokinetic (PK) data to optimize treosulfan dosing are still scarce in pediatric patients. We developed a population pharmacokinetic model and limited sampling strategy (LSS). Furthermore, we describe the PK profile of 22 pediatric patients and an interim analysis on the correlation with clinical outcome.
Materials (or patients) and Methods: Patients received treosulfan based conditioning prior to their HSCT for various malignant and non-malignant indications. Intravenous treosulfan was combined with fludarabine and thiotepa; the treosulfan dose was 42 g/m^2 (or 30 g/m^2 if BSA<0.5 m^2 (*n*=1)), divided over 3 days. Multiple (*n*=6) sampling scheme was used in the first 20 patients to develop a PK model and LSS which was applied in 2 patients. Samples were collected after the first dose of treosulfan and measured with a reversed phase high pressure liquid chromatography method using UV detection. The PK model en LSS were developed with non-linear mixed-effects modelling. This model and LSS were applied to determine the PK profile of each individual patient.
Results: A total of 22 patients were included into the study with a median age of 4.4 years (0.1-16.8 years) and a median follow-up of 1.1 year (0.3-2.4 years). The cohort included patients with hemoglobinopathy (*n*=14), hematologic malignancy (*n*=6) or immune deficiency (*n*=6). A one-compartment model was used and clearance and volume of distribution were allometrically scaled using body weight. The scaling exponent for clearance was fixed at 0.75 and for volume of distribution at 1.0. The population estimates for clearance and volume of distribution were 6.9 L/h and 13.2 L for a patient of 20kg, respectively. Treosulfan AUC could be adequately determined based upon two serum samples, at 4 and 7 hours. The average AUC was 1625±251 mg*hr/L and interpatient variability was 15%. The toxicity profile was in general mild; elevated liver enzymes, mucositis and skin toxicity were the most common toxicities. Neutrophil engraftment was 83% in patients receiving a first SCT and 75% patients receiving a second SCT. In this limited cohort we did not find a relation between treosulfan exposure and SCT outcome parameters e.g. engraftment, chimerism, toxicity and survival.

Discussion: In this study a bio-analytical method, PK model and LSS for treosulfan were developed and validated. Furthermore, PK parameters of 22 pediatric patients were analysed. This interim analysis did not demonstrate a relation between treosulfan exposure and SCT outcome. However, there is need for further analysis in a larger group of patients and in disease specific subgroups.
Disclosure of Interest: None Declared.

PH-P553
MONONUCLEAR CELL COLLECTION USING THE OPTIA FOR CHILDREN WEIGHING LESS THAN 12.5 KG

N. Comey[1], O. Smith[2], A. O'Marcaigh[2,]*
[1]Haematology/Oncology, Our Lady's Children's Hospital Crumlin, [2]Haematology, Our Lady's Children's Hospital, Dublin, Ireland

Introduction: The new Optia has recently replaced the older COBE Spectra previously used for mononuclear cell (MNC) collections at this institution. There are limitations to the Optia system for smaller patients as a minimum inlet flow rate (IFR) of 10ml/min is required compared to 2-3ml/min for Spectra. The following strategy was devised to overcome this limitation by using a citrate/heparin combination and increasing the inlet:anticoagulant (AC) ratio.
Materials (or patients) and Methods: For patients with a total blood volume (TBV) of < 700ml, heparin is continuously administered during the procedure. This is achieved by adding heparin (1:1000 iu/ml preservative free) directly to the anti-coagulant (ACD-A). 5000 iu heparin is added to 500ml ACD-A giving a concentration of 10iu heparin / ml ACD-A. The procedure may then be run at Inlet:AC of 30:1. For every 30ml whole blood processed patient will receive 1 ml of heparin/citrate combination. Our default AC infusion rate is set at 0.9mls/min but this may safely be increased to 1.2mls/min if patient has high platelet count or evidence of clumping in the circuit. For patients with a TBV of 700 to 1000ml, the required IFR can be achieved by altering the inlet: AC ratio only.
Discussion: The above strategy has been devised to overcome the limitations imposed by the minimum flow rate required by the Optia system. It allows collection to be performed without exceeding recommended maximum AC infusion rates.
Disclosure of Interest: None Declared.

PH-P554
FAISABILITY OF OVARIAN TISSUE CRYOPRESERVATION IN PAEDIATRICS BEFORE TRANSPLANTATION: A GAMBLE ON THE FUTURE

F. Chambon[1], A. David[2], F. Brugnon[3], J. L. Pouly[3], L. Janny[3], A. S. Gremeau[3], E. Merlin[3], E. Rochette[3], E. Doré[3], F. Isfan[3], F. Deméocq[3], J. Kanold[3,]*
[1]CRCTCP, INSERM CIC 501, CHU Clermont Ferrand, Clermont Ferrand, [2]CHU St Etienne, St Etienne, [3]CHU Clermont Ferrand, Clermont Ferrand, France

Introduction: Because of a significant improvement in the survival of children and adolescents with cancer, fertility preservation has to be a major concern for paediatric oncologists.
The aim of our study was to report all our ovarian tissue cryopreservation's (OTC) cases before stem cell transplantation (SCT) in order to specify the interest and indications of this method and to study the clinical and hormonal outcome in girls.
Materials (or patients) and Methods: From September 2000 to September 2013, 32 girls had an OTC before SCT. Eight patients had no malignant disease and 24, a malignant disease. After informed consent, the surgical ovarian collection consisted in the biopsy of a third of each ovary by laparoscopy which was frozen by a slow cooling protocol. A histological analysis and a follicular account were performed.
Results: Among our 32 patients, OTC's indications were 13 auto-SCT and 19 allo-SCT. Ovarian tissue harvest was performed by intraumbilical laparoscopy using a 3 to 7-mm laparoscop. Two 3 to 10-mm trocars were used. No major postoperative complications occurred excepted for one patient with sickle cell disease

[PH-P553]

Pt TBV mls	400	500	600	700	800	900	1000
Initial IFR with default inlet:AC	4.2	5.3	6.3	7.4	8.4	9.5	10.5
Increased inlet:AC ratio	30:1	30:1	30:1 or 15:1	15:1	14;1	14:1	14:1
IFR	10.5	13.2	15.8/10.5	10.2	10.5	11.1	12.3
AC infusion rate	0.9	0.9	0.9/1.2	1.0	0.9	0.9	0.9
Add Heparin preservative free 5000iu to 500mls ACD-A for Inlet:AC ratio -30:1	Heparin to ACD-A.	Heparin to ACD-A	Heparin to ACD-a for inlet:ac 30:1 no heparin if ac 15:1	If platelets high / evidence of clumping consider increasing AC infusion rate and lowering inlet: AC ratio.			

[PH-P554]

Primordial follicles at harvesting and future hormonal evaluation of 32 girls who had OTC before HSCT in our center (median, range)

At cryopreservation			At hormonal evaluation						
Nb of Girls.	Age (yrs)	Primordial follicles / mm²	Nb of Girls	Age (yrs)	FSH (IU/L)	LH (IU/L)	Oestradiol (pmol/L)	AMH (pmol/L)	Inhibine B (pg/mL)
≤ 10 yrs 14	8 [2 – 10]	14 [0-83]	9 [5 – 20]	12	71,4 [0,3-137,2]	15,4 [0,2-51,1]	40 [40-340]	1,8 [0,7-2,5]	< 15
≤ 16 yrs 11	15 [13 – 16]	8 [5-47]	9 [14-19]	17	75 [0,2-167]	32,4 [4-92]	36 [18-69]	2,5 [0,7-3,5]	< 15
> 16 yrs 7	18 [17 - 19]	3 [1-8]	5 [17-26]	20	72,9 [6,9-79,8]	32,2 [8,3-36,6]	51 [18-201]	3 [1,2-5,6]	< 15
Total 32	14 [2 – 19]	10 [0 – 83]	23 [5 – 26]	17	72,9 [0,2 – 167]	28,7 [0,2 – 92]	40 [18 - 340]	2,3 [0,7 – 5,6]	< 15

and protein S deficiency who had a severe haemorrhage of one ovary. The following chemotherapy regimens were not delayed and started at a median range of 10 days [1-81] after OTC. The anatomopathologic analysis showed 10 primordial follicles/mm^2 [0-83] and no malignant cells in any ovarian tissues. The median follow-up after harvest was 29 months [0-111], 21 girls were alive in complete remission, 1 was still on treatment and 10 died. Hormonal results were evaluable for 23 patients with a median age at 17 yrs [5-26] and 14 were in premature ovarian failure.
Discussion: Feasibility of OTC with sample of a third of each ovary seems to be an appropriate method before transplantation with no consequences on therapeutic program for children to preserve potentially fertility.
Disclosure of Interest: None Declared.

PH-P555
RESULTS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PRIMARY IMMUNODEFICIENCIES IN CHILDREN IN MEXICO: A MULTICENTRIC REPORT

R. Navarro-Martin del Campo[1,], M. Perez-Garcia[2], O. González-Ramella[1], A. Olaya-Vargas[2], D. Aguilar-Escobedo[3], T. D. L. Flores-Cacho[3], A. L. Orozco-Alvarado[1], O. Gonzalez-Llano[4], C. Mancias-Guerra[5], M. D. L. A. del Campo-Martinez[6], I. Montero-Ponce[6], F. Gaytan - Morales[7], M. T. Pompa-Garza[8], T. Muñoz-Ronquillo[8], R. Amador-Sanchez[9], M. Ortiz-Sandoval[1] and Asociación Mexicana de trasplante de células Hematopoyéticas y Terapia Celular Pediátrica*
[1]Unidad de Trasplante de Progenitores Hematopoyeticos, Hospital Civil de Guadalajara, Guadalajara, [2]Unidad de Trasplante de Progenitores Hematopoyeticos, [3]Banco de Sangre, Instituto Nacional de Pediatria, Distrito Federal, [4]Unidad de Trasplante de Progenitores Hematopoyeticos, [5]Banco de Sangre de Cordon Umbilical, Hospital Universitario de Monterrey, Monterrey, [6]Unidad de Trasplante de Progenitores Hematopoyeticos, Centro Medico Nacional la Raza, [7]Unidad de Trasplante de Progenitores Hematopoyeticos, Hospital Infantil de Mexico, Distrito Federal, [8]Unidad de Trasplante de Progenitores Hematopoyeticos, Unidad Medica de Alta Especialidad 25 IMSS, [9]Unidad de Trasplante de Progenitores Hematopoyeticos, Centro Médico Nacional Noreste IMSS, Monterrey, Mexico

Introduction: Hematopoietic stem cell transplantation (HSCT) is the definitive treatment for some primary immunedeficiencies (PID). In 2010 all the pediatric HCT Centers in Mexico formed the "Mexican Association of Pediatric Stem Cell Transplantation and Cellular Therapy". This represents the first report of the association describing the clinical outcome in HSCT for PID.
Materials (or patients) and Methods: Between January 1998 to March 2013 all PID patients in whom a HSCT was performed in 7 pediatric HCT centers in Mexico, were retrospectively analyzed.
Results: A total of 57 patients were included. Average age was 29 months (range from 2 to 158). Time from diagnosis to transplantation was 20 months (range from 1 to 110). Diagnosis included severe combined immunodeficiency in 30% (*n*=17), Wiskott-Aldrich Syndrome in 24% (*n*=14), Griscelli-syndrome in 10% (*n*=6), Shwachman-Diamonn 2% (*n*=1), Chédiak-Higashi syndrome in 2% (*n* =1), others PIDs (in 32% *n*=18). Graft source included: related bone marrow (RBM) in 7% (*n*=4), unrelated cord blood in 72% (*n*=41), and mobilized peripheral blood (PMB) in 21% (*n*=12). Median cell dose by settings was as follows: in RBM median mononuclear cell dose was 2.34x10^8/kg and median CD34 cell dose was 2.48x10^6/kg. In PMB median nucleated cell dose was 8.6 x10^8/kg and median CD34 cell dose was 6.3x10^6/kg, whereas in UCB Median Nucleated cell dose was 2.9x10^8/kg and median CD34 cell dose was 2.1x10^6/kg. In all but 1 case, GVHD prophylaxis was given. Conditioning regimens included myeloablative in 63% of patients (*N*=36) and non-myeloablative in 37% (*n*=21). Engraftment occurred in 63% of patients (*n*=36). The average time for neutrophil engraftment was 20 days (range from 8 to 54). Fourteen patients (24%) developed an II-IV grades aGVHD, whereas cGvHD was presented only in 7 cases (12%). Transplant related mortality was 26% considering deaths before day 100. The main causes of death were infection and graft failure. Overall survival of all cases was 61%.
Discussion: This is the first report of HSCT for PID children in Mexico. The mean source of Hematopoietic stem cells used was UCB (71%) with good results in our population. Early diagnosis and prompt performance of SCT with an optimal donor and conditioning regimen contributed to the favorable outcomes.
Disclosure of Interest: None Declared.

PH-P556
THE RECENT SINGLE CENTRE EXPERIENCE WITH PEDIATRIC SOLID TUMORS OF A NEW STEM CELL TRANSPLANTATION AND CELL THERAPY UNIT

J. L. Fuster[1,], V. Cabañas[1], M. Blanquer[1], A. Sánchez-Salinas[1], J. Monserrat-Coll[1], J. Francisco Pascual-Gázquez[1], A. Galera[1], E. Llinares[1], M. Bermúdez-Cortés[1], J. María Moraleda[1]*
[1]Stem cell transplantation and cell therapy unit, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain

Introduction: Increased treatment intensity may improve disease-free survival (DFS) for certain pediatric solid tumors and autologous stem cell rescue allows dose escalation. This strategy is referred to as high-dose chemotherapy with autologous stem cell rescue or autologous hematopoietic stem cell transplantation (ASCT). The role of allogeneic transplantation (alloSCT) for the treatment of refractory/relapsed pediatric solid tumor has recently gained much attention; particularly haploidentical transplantation (haplo) is considered a tool to explore the inherent alloreactivity of donor natural killer cells.
Materials (or patients) and Methods: Here we analyze our single centre experience with 28 transplant procedures (21 ASCT) in 23 pediatric patients with solid tumors, including 5 haplo and 2 allo-SCT from a matched sibling donor (MSD).
Results: Haplo was carried out in 2 patients with Ewing's sarcoma in second complete remission (CR) after metastatic relapse, in 2 patients with advanced (relapsed) neuroblastoma, and in 1 patient with advanced (metastatic relapsed) osteosarcoma; 2 of these 5 patients required subsequent allo-SCT from a MSD after rejection of the primary graft. The remaining 21, correspond to ASCT procedures in 20 patients diagnosed with high risk neuroblastoma in partial remission (8), Ewing's sarcoma (3), intracranial germ cell tumor (GCT) (2), abdominal theratoid/rhabdoyd tumor (2), relapsed refractory Wilms' tumor (1), Hodgkin lymphoma in second CR (1), relapsed osteosarcoma (1), retroperitoneal primitive neuroectodermal tumor (PNET) with tumor spillage (1), and supratentorial PNET (1). Two patients (1 supratentorial PNET in second partial response, 1 refractory intracranial GCT) underwent double (tandem) ASCT. Regarding ASCT, the median age at transplantation was 5 years (3.5-8.5). One patient dead of septic shock after a second conditioning regimen (tandem transplant) for supratentorial PNET. One patient affected of intracranial GCT, and heavily pretreated with platinum compounds developed tubulopathy and severe ototoxicity after transplantation. No other major complications were registered. With a median follow-up post ASCT of 36 months (14.5-53.7) the median progression free survival (PFS) and median overall survival (OS) were 11 and 33 months respectively. The overall estimated 3-year PFS and OS were 43% and 47%, respectively. The median PFS was significantly shorter in patients who did not achieve a CR before ASCT (4.5 months) as compared with those who had a CR (not reached) with a hazard ratio of 4.5 (95% confidence interval, 1.3 to 15.4; *P*=0.03). Of note, 2 patients affected of metastatic relapse of Ewing's sarcoma undergoing haplo in second CR are alive in remission, one of them after 25 months from haplo. Four of 8 high-risk neuroblastoma patients are alive in remission after an average follow-up time of 31 months (range 15-55). Eight patients dead, 7 from disease progression (4 high-risk neuroblastoma, 2 Ewing's sarcoma, 1 osteosarcoma) and 1 from septic shock.
Discussion: ASCT is an effective and safe treatment for high-risk chemosensitive pediatric solid tumors, both as first line or as salvage therapy after relapse. CR before ASCT improves PFS. Haplo may be a therapeutic option to control the disease for very high risk patients such as relapsed Ewing's sarcoma in second remission.
Disclosure of Interest: None Declared.

PH-P557

MAJOR ABO INCOMPATIBLE BONE MARROW TRANSPLANTATION IN CHILDREN: DETERMINING WHAT RESIDUAL VOLUME OF DONOR RED CELLS CAN SAFELY BE INFUSED FOLLOWING RED CELL DEPLETION

K. Patrick[1,], W. Lau[1], J. Doyle[2], A. Gassas[1], E. McDougall[1], M. Ali[1], S. Courtney[1], M. Egeler[1], T. Schechter Finkelstein[1]*

[1]Division of Haematology/Oncology, The Hospital for Sick Children, Toronto, [2]Division of Pediatric Hematology/Oncology, Cancer Care Manitoba, Manitoba, Canada

Introduction: Red cell depletion of major ABO incompatible bone marrow (BM) reduces the risk of acute haemolysis during heamatopoietic stem cell transplantation (HSCT). However, residual red cells remain and in children, this volume can be significant relative to their body weight. We sought to determine the volume of incompatible red cells (iRBCs) that can safely be given to children, in the context of allogeneic HSCT from a major ABO mismatched donor.

Materials (or patients) and Methods: All patients undergoing HSCT using fresh BM from a donor with a major ABO blood group mismatch between 1st January 2000 and 1st August 2013 at The Hospital for Sick Children, were identified. A retrospective chart review was conducted.

Results: 78 patients with a median age of 9.2 years (range 0.16 to 18 years) were identified. BM was red cell depleted using pentaspan sedimentation in 74 of the cases. Antihistamines, acetaminophen and hyperhydration (125ml/m^2/hr for 2 hours before and at least 4 hours after BM infusion) were given to all patients. The duration of the BM infusion varied, depending on the volume of BM, the weight of the child and the volume of iRBCs. The median duration was 4.5 hours (0.3ml of iRBCs/kg/hr).

The median volume of iRBCs transfused was 1.6ml/kg (range 0.1-10.6ml/kg).

Patients were observed for clinical and biochemical signs of haemolysis, including hypotension, haemoglobinuria, significant increases in creatinine, defined as a greater than 50% increase from baseline and significant increases in unconjugated bilirubin, defined as greater than the upper limit of normal (ULN). 43 patients had no signs of haemolysis. 24 patients had a significant rise in unconjugated bilirubin. 12 patients had clinically apparent haemoglobinuria. 2 patients had a significant rise in creatinine but neither required dialysis. 2 patients became hypotensive, one required a single fluid bolus and one required a brief period of inotropic support which was felt most likely to be related to infection, due to the absence of other signs of haemolysis.

The only serious reaction attributable to the infusion of incompatible BM, rather than intercurrent infection, occurred in a 13 year old boy who received 3.9ml/kg of iRBCs. The BM was given at a rate of 135ml/hr (1.75ml of iRBCs/kg/hr). He developed hypoxia, an unconjugated bilirubin 4 times the ULN and a doubling in creatinine. Symptoms improved with prolonged hyperhydration, oxygen and steroids.

With a median follow up of 8.5 years, 51 patients were still alive. Of the 27 deaths, 18 were due to non-relapse mortality, although none were attributable to the infusion of ABO incompatible BM.

Discussion: We describe a large cohort of children who received HSCT from major ABO incompatible donors and demonstrate that with careful hydration, close attention to urine output and monitoring of biochemical markers of renal function and haemolysis, at least 3ml/kg of iRBCs can safely be given to children. It should be expected that a degree of haemolysis will occur and this should be monitored for. We recommend that if more than 3ml/kg of iRBCs have to be given, BM is divided into aliquots and given at 4 to 8 hour intervals to allow monitoring of renal function between each infusion.

This information answers a highly relevant clinical question, for which no clear evidence based recommendation could previously be made.

Disclosure of Interest: None Declared.

PH-P558

COMMON GAMMA CHAIN- AND JAK3-DEFICIENT SCID, CONDITIONED VERSUS UNCONDITIONED TRANSPLANT: A SINGLE CENTRE EXPERIENCE

G. Kharya[1,], Z. Nademi[1], T. Leahy[1], K. Brigham[2], D. Barge[3], S. Hambleton[1,4], M. Abinun[1,4], T. Flood[1,4], A. Cant[1,4], M. Slatter[1,4], A. Gennery[1,4]*

[1]Department of Pediatric Immunology, [2]Hematopoietic Stem Cell Laboratory, [3]Regional Immunology Laboratory, Newcastle upon Tyne Hospital NHS Foundation Trust, [4]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction: Haematopoietic stem cell transplant (HSCT) is curative for SCID. The role of conditioning chemotherapy in determining post-HSCT immunoreconstitution, particularly of B cells, continues to be debated. Common gamma chain (CgC)- and JAK3-deficient SCID are permissive for donor T cell engraftment following infusion of stem cells – B cell engraftment is less frequent. We analysed immunoreconstitution and function in CgC and JAK3 SCID patients receiving conditioning chemotherapy versus unconditioned infusion.

Materials (or patients) and Methods: Retrospective data collection for all CgC/JAK3 SCID HSCT between October 1989 - September 2013.

Results: 38 patients (27 CgC, 11 JAK3) were analysed. Median age at diagnosis 5 (range 1-11) months, 14 (37%) diagnosed at birth. Median age at HSCT 6 (range 1-12) months. In 5, donor was matched sibling, 8 match related family, 1 matched unrelated, 4 unrelated cord blood and 20 TCD haplo-identical parents. 27/38 (71%) received conditioning chemotherapy (CC) (Bu8/Cy:15, Bu16/Cy:2, Treosulfan base:8, Others:2), 11/38 (29%) were unconditioned infusions (UCC). 24/38 (63.2%) received immunosuppression with CSA alone or in combination with methylprenisolone or mycophenolate mofetil. Mean CD34+ cell dose in CC was 7.66x10^6/kg (0.48x10^6/kg for cord blood transplant) and 8.25x10^6/kg for UCC. 28/38 (74%) patients are alive and well. 10/38 (26%) patients died (8 in immediate post transplant period, 2 died 2.5 years post transplant), 8/27 (30%) CC patients died (2 due to direct regimen related toxicity, 6 due to flaring of pre-existing infection) and 2/11 (18%) UCC patients died. 13 patients developed grade I-II GVHD mostly skin, 1 grade IV liver GVHD requiring liver transplant. Immune reconstitution and function was analysed in 30/38 patients (28 alive till date, 2 late deaths at 2.5 years). All have 100% donor T cell chimerism, 26/30 (87%) have naive T cells >200. B cell chimerism mirrored myeloid chimerism and was 100%:9; 50-99%:3; 10-49%:4; <10%: 14 patients. Class switch memory B cell (CSM) percentage was analysed as a marker of B cell function in 27/30 patients (for whom data was available). 12/27 (44%) patients have CSM > 1% (9/12 (75%) in CC, 3/12 (25%) in UCC). 15/27 (56%) patients have CSM <1% (8/15 (53%) in CC, 7/15 (47%) in UCC). 16/30 (53%) patients were on immunoglobulin (Ig) replacement (8/20 CC, 8/10 in UCC) till last follow-up, whereas 14/30 (47%) were IVIG independent with good vaccine response (12/20 CC, 2/10 UCC). Of 9 patients who received Bu8Cy conditioning and TCD marrow, 3 developed myeloid chimerism, all of whom had Campath 1-M TCD, compared to 6 who had cell-selected TCD, none of whom had myeloid chimerism.

Discussion: Although small numbers, these data suggest that CgC/JAK3 SCID have good T cell immunoreconstitution and function irrespective of whether they receive conditioning chemotherapy. B cell immune reconstitution and function are more likely when patient receives conditioning chemotherapy. However, for those receiving TCD marrow, more chemotherapy may be required to ensure myeloid engraftment, which may increase the mortality risk.

Disclosure of Interest: None Declared.

PH-P559

HAEMATOPOIETIC STEM CELL TRANSPLANTATION FOR RAG1/2 SEVERE COMBINED IMMUNODEFICIENCY OR OMENN SYNDROME: A SINGLE CENTRE EXPERIENCE

R. Elfeky [1,], Z. Nademi [1], K. Brigham[2], D. Barge[3], S. Hambleton[1,4], M. Abinun[1,4], T. Flood[1,4], A. Cant[1,4], M. Slatter[1,4], A. Gennery[1,4]*

[1]Department of Pediatric Immunology, [2]Hematopoietic Stem Cell Laboratory, [3]Regional Immunology Laboratory, Newcastle upon Tyne Hospital NHS Foundation Trust, [4]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

Introduction: Mutations in Recombinase activating genes (RAG)1/2 represent 20% of severe combined immunodeficiency (SCID). Patients have an extended phenotypic spectrum including SCID and Omenn syndrome (OS). We report the detailed haematopoietic stem cell transplant (HSCT) outcome of a series of SCID/OS RAG-deficient patients.

Materials (or patients) and Methods: From 1997-2012, 18 patients (9 male) with confirmed *RAG1/2* mutations received HSCT at our centre. Median age at diagnosis was 1.7 months (range 0-7), median age at transplant 3.5 months (range 0.5-9), median follow-up 41.5 months (range 2-180). 10 patients had OS, 8 had SCID. Data on clinical presentation, type of transplant (HLA match), conditioning regimen, immunoreconstitution (IR) and outcome were assessed with a comparison between OS/SCID together with a comparison of outcome as regards T-cell depleted (TCD) vs T-cell replete (TCR) transplant and busulphan (Bu) vs treosulphan (Treo) -based regimens.

Results: 18 patients received 26 transplants; 9 TCD (of which 5 had OS). Main conditioning protocols were either Treo-based (*n*=8, 2 TCD); or Bu-based (*n*=5, all TCD). 2 were unconditioned (UC); one alive and well with few naive T cells, no B cells 96m post-transplant. The other patient had graft failure and died within 2 months. There were no differences among OS/SCID; TCD/TCR and Treo-/Bu-based regimens as regards age at transplant, CD34+ dose, duration to engraftment. 17 patients (94%) engrafted with naïve T cells in 11/14 cases for whom data were available, and immunoglobulin (Ig) independency in 9/14. Latest myeloid chimerism was 100% donor in 5 patients, partial donor in 9. Four patients had donor myeloid chimerism from 1-10%; 3 of them have good immune reconstitution and are immunoglobulin independent. In these, early chimerism showed initial donor myeloid >50% in all 4 patients. Absent donor myeloid chimerism was present in 3 patients; all had poor T cell immunoreconstitution and are immunoglobulin dependent. Seven patients (38%) died post-transplant, 5 during the first 6 months. 6/7 had engrafted (1 suffered graft loss); donor myeloid chimerism was 100% in 3 of these, partial in 1 and 0 in 2 patients. Mortality was higher among OS than SCID, TCD than TCR, and Bu-based than Treo-based patients. Main cause of death among OS was disseminated viral infection (4 patients), of whom 3 had grade 2/3 aGVHD and were immunosuppressed. SCID deaths were engraftment pneumonitis (*n*=1) and aGVHD grade 2 with EBV-driven cerebral lymphoproliferative disease (*n*=1).

Discussion: SCID had better IR and survival than OS. Main cause of death among OS was infections. TCR transplant carry a better outcome than TCD. 100% donor myeloid chimerism seemed associated with poor outcome. The conditioning data suggested that some conditioning is required to get early myeloid donor chimerism, which enables B cell function and thymopoiesis with naive T cells.

Disclosure of Interest: None Declared.

[PH-P559]

	OS (n=10)	SCID (n=8)	**TCD (n=9)**	**TCR (n=9)**	Treo-based (n=8)	Bu-based (n=5)	UC/ Other (n=5)
Naïve T cells median (range)	94.5(0-636)	161 (0-860)	**106.5 (0-860)**	**144 (0-636)**	172 (0-860)	132 (0-161)	22(0-322)
Naïve T cells	5/8(62%)	6/6(100%)	**4/6(66%)**	**7/8(87%)**	7/7(100%)	2/3(66%)	2/4(50%)
Ig dependent	4/8(50%)	1/6(16%)	**3/6(50%)**	**2/8(25%)**	1/7(14%)	1/3(33%)	3/4(75%)
Mortality	5(50%)	2(25%)	**5(55%)**	**2(22%)**	1(12%)	3(60%)	3(60%)

PH-P560

ANTI-THYMOCYTE GLOBULIN (ATG) EXPOSURE IS RELATED TO OVERALL SURVIVAL IN CHILDREN RECEIVING ALLOGENEIC-HEMATOPOIETIC CELL TRANSPLANTATION (HCT): TOWARDS INDIVIDUALIZED DOSING TO IMPROVE SURVIVAL

R. Admiraal[1,2,3,4,], C. Jol-van der Zijde[3], C. van Kesteren[1,2,4], M. van Tol[3], C. Knibbe[4], R. Bredius[3], J. J. Boelens[1,2]*

[1]Pediatric stem cell transplantation unit, [2]U-Dance, Labarotory Applied Immunology, University Medical Center, Utrecht, [3]Department of Pediatrics, Leiden University Medical Centre, [4]Department of Pharmacology, Leiden Academic Centre for Drug Research, Leiden, Netherlands

Introduction: To prevent graft versus host disease (GvHD) and rejection in unrelated hematopoietic cell transplantation (HCT), children receive anti-thymocyte globulin (ATG) in the conditioning regimen. The therapeutic window is critical as over-exposure results in delayed immune reconstitution (IR) and under-exposure GvHD: both causing significant morbidity and mortality. As the optimal exposure is unknown we studied the association between active ATG (Thymoglobulin) exposure and outcomes as a step towards an evidence based dosing regimen of Thymoglobulin.

Materials (or patients) and Methods: All pediatric patients transplanted between 2004 - 2012 receiving Thymoglobulin in the two study centers were included. Only first HCTs were included, patients mounting IgG anti-ATG antibodies were excluded. Different exposure related variables such as AUC (area under the curve) were calculated using a validated PK model and were tested for their association with the endpoints: event-free survival (EFS), overall survival (OS), acute GvHD and T-cell reconstitution. T-cell reconstitution was defined as a CD3+ T-cell count > 100 x10^6/L in two consecutive occasions within 100 days after HCT. aGvHD was classified using the Glucksberg criteria. Cox proportional hazard and regression models were used.

Results: Pharmacokinetic and -dynamic data were available from 196 pediatric HCT's; 28 were excluded due to 2nd/3rd HCT (13) or antibodies (15). OS was 67% with a median follow-up of 130 weeks (1-440). Post-HCT exposures correlate best with the endpoints. Survival was higher in patients with a low (<20 AU*days/L) post-HCT AUC (80.4% vs 60.7%, *P*=0.026). Multivariate predictors for lower OS were high (>20 AU*days/L) post-HCT AUC (*P*=0.024), mismatch donor (*P*=0.01) and malignancy (*P*=0.007). In addition, low post-HCT AUC of Thymoglobulin was associated successful T-cell reconstitution (*P*<0.001) while incidence of aGvHD was comparable irrespective of post-HCT AUC (*P*=0.48) (fig 1).

Discussion: High (>20 AU*days/L) post-HCT exposure of Thymoglobulin is associated with a lower OS and a lower probability of successful IR. These results may contribute to individualized dosing guidelines of Thymoglobulin in children, aiming to improved survival.

Disclosure of Interest: None Declared.

[PH-P560]

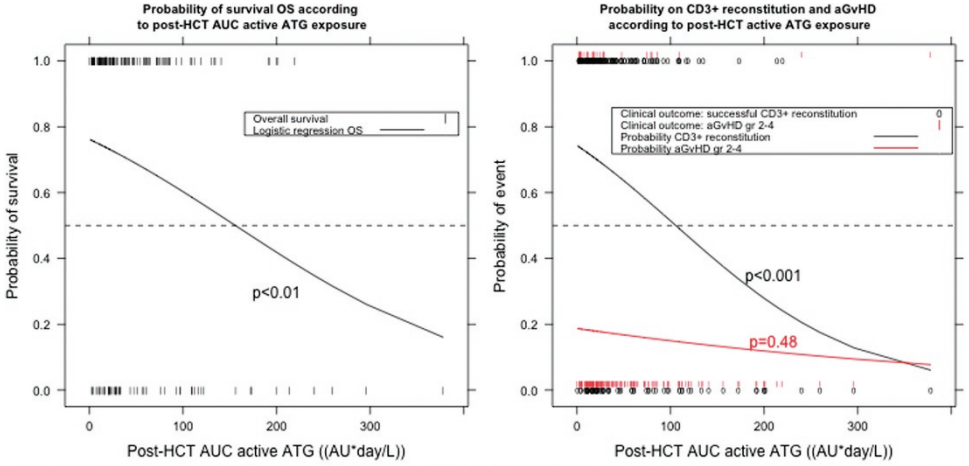


Figure 1. Left panel: Post-HCT AUC of active Thymoglobulin in (non)survivors (black l) with a logistic regression depicting the chance of survival versus post-HCT AUC of active Thymoglobulin (black line). Dashed line: 50% chance of event.
Right panel: Post-HCT AUC of active Thymoglobulin of patients with an (un)successful T-cell reconstitution (black 0) and patients with GvHD grade 2-4 (red l) with a logistic regression depicting the chance of successful reconstitution versus post-HCT AUC of active Thymoglobulin (black line) and the chance of developing GvHD grade 2-4 versus post-HCT AUC of active Thymoglobulin (red line). Dashed line: 50% chance of event

PH-P561
INTERIM ANALYSIS OF THE RANDOMIZED PROSPECTIVE EXERCISE THERAPY STUDY IN THE PEDIATRIC STEM CELL TRANSPLANTATION (BISON)

A. Senn-Malashonak[1,], S. Arndt[1], K. Siegler[1], A. Rosenhagen[2], T. Klingebiel[3], W. Banzer[2], P. Bader[1]*
[1]Department for Stem Cell Transplantation and Immunology, University Hospital Frankfurt Goethe University, [2]Department of sports medicine, Johann Wolfgang Goethe Universität Frankfurt am Main, [3]Center of children and adolescents, University Hospital Frankfurt Goethe University, Frankfurt am Main, Germany

Introduction: Hematopoietic stem cell transplantation (SCT) is associated with a rapid decline of physical and psychosocial resources. Besides treatment-related side effects and complications, particularly children suffer of immobility during and after therapy. Even several years later young survivors present post-transplant impairments in physical fitness and quality of life. For adults evidence suggests a beneficial effect of a supportive sport therapy on i.a. cardio-respiratory function, corticosteroid-induced myopathy, psychological stress, fatigue and somatic side effects. The purpose of this RCT is to evaluate the physical and psychological impact of an exercise intervention program during pediatric PBSCT.
Materials (or patients) and Methods: After informed consent all participants (n=50) were randomized into a sport (IG) or control group (CG). While the IG involved a standardized 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. Primary outcome measures were aerobic capacity and isometric muscle strength. Additionally body composition, medical parameters and psychosocial questionnaires were assessed at hospital admission and discharge. For statistical analysis we used descriptive statistics, Wilcoxon-signed-rank and Mann-Whitney U-test via SPSS.
Results: In three years 39 patients (11.1±3.4 yrs; IG n=19; CG n=20) completed the study successfully. Transplanted children and adolescents were hospitalized for 40.5±11.8 days. First results indicate that the IG was discharged 2.6 days earlier than the CG. For muscle strength the CG declines (highly) significant about -15% (hand grip: p≤ 0.004; knee extension: p≤ 0.011), while the IG-values were stable (hand grip: -3.0%) or even increased (knee extension: 12.0%). Furthermore the 6-Minute-Walking-Test represents highly significant reduction in the walking distance of the CG (P= 0.003, mean: -14.2 ±16.7%). Likewise post inter-group comparison was even highly significant (P= 0.002). In spiroergometry (n=27) decreases of VO_2 max/kg were observed in both groups (P=.000; IG: 19%, KG: 24%). The analyses of the health-related quality of life questionnaires show significant deterioration only for subscales of the parent report (IG: Total-Quality of Life P= 0.000; KG: Physical Wellbeing P= 0.030).
Discussion: The results indicate that sports therapy can improve the loss of physical performance during PBSCT and may influence medical parameters. Current studies with children undergoing cancer therapy support these findings. Following researches consider sustainability of a structured sports therapy. Therefore the BISON study supervises children and adolescents until day +200 after transplantation in outpatient setting. The recruitment will continue until December 2014.
Disclosure of Interest: None Declared.

PH-P562
NON-TBI CONDITIONING FOR CHILDREN WITH ALL BEFORE FIRST ALLOGENEIC HSCT

N. Zubarovskaya[1], C. Diaz de Heredia[2], F. Locatelli[3], J.-H. Dalle[4], P. Bader[5], J. Wachowiak[6], A. Yesilipek[7], C. Peters[1,] on behalf of PDWP*
[1]Stem Cell Transplantation, St. Anna Children's Hospital, Vienna, Austria, [2]Stem Cell Transplantation, Hospital Vall d'Hebron, Barcelona, Spain, [3]Department of Pediatric Hematology and Oncology, IRCCS Ospedale Pediatrico Bambino Gesù, Rome-Vatican, Italy, [4]Stem Cell Transplantation, Hôpital Robert Debré, Paris, France, [5]Klinik für Kinder- und Jugendheilkunde III, Onkologie und Hämatologie, Johann Wolfgang Goethe Universität, Frankfurt, Germany, [6]Department of Pediatric Oncology, Hematology and Transplantology, Poznan University of Medical Sciences, Poznan, Poland, [7]Department of Pediatric Hematology, Akdeniz University Medical School, Antalya, Turkey

Introduction: Total body irradiation (TBI) in combination with chemotherapeutic medicines is the gold standard for conditioning regimen in children with high risk acute lymphoblastic leukemia (ALL). Some situations preclude the application of TBI, e.g. young patient's age, pre-existing morbidities or centre's decision. As only few studies focus on the frequency and outcome of

chemo-conditioning in children with ALL, the EBMT PD WP initiated a retrospective study on this topic.
Materials (or patients) and Methods: We identified 1728 children and adolescents with ALL who received a first HSCT between 2000 and 2012 and who were registered in the EBMT data base.
Results: The majority of patients were transplanted in first or subsequent remission, 11% were not in complete remission at time of HSCT. The most common donor type were unrelated donors (52,9%), followed by matched siblings (32.8%), other relatives (13,9%) and some others like twins. The stem cell source was bone marrow in 43.6% and peripheral blood stem cells in 37.8%. 18.1% received cord blood and the remaining different combinations of grafts. For 90.8% of patients the centres intended a myeloablative conditioning, and for 9.8% a reduced toxicity or a reduced intensity conditioning regimen was chosen. The preferred non-TBI conditioning regimen was a combination of busulfan and cyclophosphamide (48%), followed by a triple-drug regimen consisting of busulfan, cyclophosphamide and etoposide; the remaining patients received different combinations like fludarabine/thiotepa/melphalan or treosulphan. Acute GVHD occurred in 52% (overall grade III/IV: 14,2%), chronic GVHD was reported for 15.2% (7.1% limited, 7.1% extensive).
At time of analysis, 51.4% of patients were alive and 45,9% had died. Causes of death were relapse (49.8%) or transplant related complications (42.7%). There was a significant improvement over time, as patients transplanted after 2008 had an overall survival of 60,9% with comparable relapse incidence but lower incidence of non-relapse mortality. Compared to the whole cohort, children who were transplanted below the age of 4 years had a lower relapse incidence (28.6%) and an overall survival of 56.3%. 336 children were under 2 years of age at time of HSCT and the overall survival was 56.3 % despite the high relapse risk of predominantly infant leukaemia phenotypes.
Discussion: The preliminary results of this study indicate that more than 50% of children with ALL who received no TBI as conditioning regimen for allogeneic HSCT from different donors in different remission status are alive. This observation justifies and requests a prospective evaluation whether TBI is still superior compared to conditioning regimen with chemotherapy only in comparable cohorts of patients. As most paediatric ALL-patients are cured with contemporary chemotherapy protocols, only a small proportion requires an allogeneic HSCT. International cooperation is essential to answer the question of the most appropriate conditioning regimen for these patients.
Disclosure of Interest: None Declared.

PH-P563

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CONGENITAL DISORDERS. A SINGLE CENTRE EXPERIENCE

M. Faraci[1,], G. Morreale[2], S. Giardino[2], F. Bagnasco[3], P. Terranova[2], D. Di Martino[4], M. Di Rocco[5], E. Lanino[2]*
[1]SCT Unit-Haematology-Oncology Department, G. Gaslini Children's Research Institute, [2]SCT Unit-Haematology-Oncology Department, [3]Epidemiology, Biostatistics and Committees Unit, [4]Haematology-Oncology Department, [5]3Unit of Rare Diseases, Department of Paediatrics, G. Gaslini Children's Research Institute, Genova, Italy

Introduction: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative option for a variety of congenital disorders. We retrospectively analyzed results in children who underwent HSCT for congenital disorders other than bone marrow failure syndromes at G. Gaslini Children Research Institute over the last 25 years.
Materials (or patients) and Methods: Overall, 51 pts (75% males) underwent 57 transplant procedures, 6 children having received a second HSCT for graft failure (2), graft rejection (3), and severe chronic GvHD (1). Diagnosis were primary immunodeficiency (PI) in 32 cases (56%), inborn error of metabolism (IEM) in 16 (28%) and osteopetrosis in (OP) 9 (16%). Median age at HSCT was 2.1 yrs (0.1-14.5). Donors were matched related (MRD) in 13 cases and alternative (AD) in 44 (unrelated volunteer, 22; unrelated cord blood: 22). Stem cell source were bone marrow (BM, 31),cord blood (CB, 22) and peripheral blood stem cells (PBSC, 4). Preparative regimen was myeloablative in 49 cases, either Busulphan (34) or Treosulfan (15) based; 8 transplant were prepared by a reduced-intensity regimen. Fludarabine was part of conditioning in 23 cases.
Results: Acute GVHD occurred after 32/49 evaluable HSCTs (grade 1-2: 20; grade 3: 12); chronic GvHD occurred in 25/46 children at risk (limited:14; extensive:11).Chimerism measurement by study of short tandem repeat DNA sequence showed graft failure in 5 pts (3 PI, 2 IEM), primary rejection in 3 (1 PI, 1 IEM, 1 OP), mixed chimerism in 4 (2 IEM, 1 PI, 1 OP), and full donor in 41 HSCTs (72%). 11 (21.6%) pts died: 7 of infective complications, 2 of organ toxicity (1 MOF and 1 of VOD), 2 of hemorrhagic events (Table 1). At 3 yrs since HSCT, the overall survival (OS) was 77.4% (I.C. 62.9-86.8) with a median follow-up of 4.6 yrs (0-20.7) and there was not statistical difference between AD and MRD.A trend in favour of better OS was observed in recipients of CB *vs* BM transplant.
Discussion: In our experience the probability of long-term survival after HSCT for children with congenital disorders is high; the mortality was higher in the group of PI (25%) *vs* IEM (13%) and *vs* OS (11%) The superior survival we observed in CB transplant recipients confirms this source as the 1st choice for small children with non-malignant diseases. We regularly follow these patients in a life-long surveillance program to prevent and treat late complications.
Disclosure of Interest: None Declared.

PH-P564

CLOFARABIN/FLUDARABIN + EXPOSURE-TARGETED BUSULFAN IN MYELOID AND LYMPHOBLASTIC LEUKEMIAS IN CHILDREN: FIRST RESULTS OF A DUTCH NATIONAL COHORT

M. Bierings[1,], J. Boelens[1], B. Versluys[1], W. Kollen[2], R. Bredius[2], A. Lankester[2]*
[1]Pediatric HCT team, Utrecht University Children's Hospital, Utrecht, [2]Pediatric HCT team, Leiden University Medical Centre, Leiden, Netherlands

Introduction: Busulfan (Bu) as myeloablative agent is used in conditioning regimens prior to hematopoietic stem cell transplantation (HCT). In the recent past we reported on the relation between Bu exposure and outcome and defined an optimal exposure to minimize rejection, toxicity and relapse. From there we replaced classic cyclophsphamide by fludarabine (flu) to further reduce toxicity. This has also recently been published. In vitro a synergistic antileukemic effect of clofarabin (clo) to the Bu/Flu combination has been shown. We thus reasoned that the Clo Flu Bu combination might be optimal as an antileukemic conditioning regimen. Furthermore, recent interest to replace total body irradiation (TBI) by a chemotherapeutic approach to reduce late effects (endocrine disorders, second malignancies) accelareted our interest in chemo-based conditioning regimens. Within the framework of the Dutch Childhood Oncology Group (DCOG) it was decided to explore the Clo Flu Bu regimen for safety and efficacy for lymphoid and myeloid malignancies.
Materials (or patients) and Methods: Our conditioning regimen consisted of clofarabine 30 mg/m^2 over 1 hour, followed by fludarabin 10 mg/m^2 in 1 hour, followed by a 3-hour infusion of once-daily busulfan (weight-based dosing) for 4 consecutive days. The cumulative target area under the curve (AUC) for Bu was 90 mg*h/l. Serotherapy in unrelated donors (expect AML patients receiving cord blood) and greaft-versus-host-disease (GvHD) prophylaxis were according to standard protocols. Primary endpoint: Leukemia-free-survival (LFS). Other endpoints: acute and chronic GvHD, veno-occlusive disease (VOD), non-infectious lunginjury, neutrophile (@day 60) and thrombocyte engraftment (@day 180). A risk factor analysis was performed using univariable and multivariable COX regression.
Results: 38 patients were included (18 AML CR2, 11 ALL CR1 (of which 2 infants), 6 ALL CR2/3, 3 MDS (RAEB-t). 6/12 ALL patients with available MRD status prior to HCT were positive: $2>10^{-3}$ and $4 <10^{-3}$. Donors used: 14 unrelated cord blood, 9 (matched) family donors (1 haplo id), and 14 matched unrelated marrow donors.

Median follow-up 226 days (range 18-895). The estimated 1-year LFS was 68 +/- 10 % (AML 70%, ALL 67%. MDS 67%), with an estimated NRM@1 year 13 +/- 6% and relapse@1 yr 18 +/- 6 %. Other endpoints: neutrophil engraftment 100% @d60, trombocyte engraftment 76+/- 6% @d180, aGvHD 2-4@d180 was 18 +/- 6% (gr 3-4: 6 +/- 4%), extensive cGvHD@1 year 8 +/- 6% and no VOD was noted. One graft failure was noted in a MDS patients grafted with a MUD. He was succesfully regrafted with a cord blood graft.
Discussion: This cohort of children with ALL, AML and MDS was conditioned with a chemotherapeutic regimen that showed acceptable short term safety and efficacy. Longer follow up in a larger cohort is necessary to confirm efficacy as well as a potential reduction of late effects.
Disclosure of Interest: None Declared.

PH-P565

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CONGENITAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: 17-YEARS SINGLE PEDIATRIC CENTRE EXPERIENCE

S. Giardino[1,], M. Faraci[1], G. Morreale[1], F. Bagnasco[2], C. Micalizzi[3], S. Marcenaro[4], E. Lanino[1]*
[1]Hematopoietic Stem Cell Transplantation Unit, Dept. of Pediatric Hematology and Oncology, [2]Epidemiology and Statistics, [3]Hematology Unit, Dept. of Pediatric Hematology and Oncology, [4]Laboratory of Clinical and Experimental Immunology, Gaslini Children Research Institute, Genoa, Italy

Introduction: Reduced intensity conditioning (RIC) regimens have improved survival of patients with congenital hemophagocytic lymphohistiocytosis (HLH) undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). We report the experience of HSCT-Unit of Gaslini Children Hospital (GCH) in allo-HSCT in congenital HLH patients (pts).
Materials (or patients) and Methods: From 1995 until 2012, 15 children (12 male), affected by congenital HLH (5 familiar hemophagocytic lymphohistiocytosis 2 [FHL2], 4 FHL3, 2 FHL5, 1 XLP-1, 1 Chediak-Higashi Syndrome, 2 FHL-Unknown Gene) were diagnosed in GCH and treated with chemotherapy according to international protocols (HLH-94 in 9, HLH-2004 in 12) followed by allo-HSCT; one FHL 3 patient received a 2nd HSCT for primary graft failure. Ten of them (67%) had central nervous system (CNS) involvement either at diagnosis or later during first line therapy (Table 1). Median age at HSCT was 2,6 years (0,25 – 14,5). Alternative Donor (AD) was selected in all cases but in two twins transplanted with a partially matched related donor (mother). Stem cells source was bone marrow in 10 cases, cord blood in 5 and peripheral blood in one. A Busulfan (Bus)-based Myeloablative Conditioning (MAC) regimen was used in 7 cases (43,75%), all but one performed before 2005, while RIC Bus-free was used in 9 (56,25%), all but one performed after 2005. In patients transplanted with AD (87,5%), GvHD prophylaxis included ATG in 11 (68,75%) or anti-CD52 in 3 (18,75%).
Results: Engraftment was achieved in all pts but one, who received a second HSCT because of primary graft failure. Acute-GvHD was observed in 10 cases (62,5 %) (50% grade 1-2, 12,5% grade 3); chronic-GvHD in 7 (46,6%) (13,3% limited, 33,3% extensive). A mixed chimerism was observed in one patient, without disease relapse. CNS toxicity (posterior reversible encephalopathy syndrome [PRES]) was observed in 2 pts (13,3%), who had CNS involvement at diagnosis. Neurological evaluation at last follow up showed CNS residual damage in 6 pts, represented by temporomesial sclerosis with epilepsy in 2, brain atrophy with psychomotor delay in 3 and ataxic-spastic syndrome in 1. With a median follow up after HSCT of 3.71 years (1 month - 17.17 years), 12 (80%) patients are alive and disease-free and 3 died (20%) because of transplant related events (1 VOD, 1 sepsis, 1 hemorrhagic complication). Cumulative DFS probability at 3-years was 77,1% (CI 43,2 – 92,2), with trends in favour of better survival for patients conditioned with RIC (MAC 71,4% [CI 25,8 – 91,9] vs RIC 87,5% [CI 38,7 – 98,1]) and transplanted in more recent years (≤ 2005: 62,5% [CI 22,9 – 86,1] vs > 2005: 100%) (Figure 1). Of note, all three patients who received anti-CD52 are alive and disease free, compared to 9/12 who received ATG.
Discussion: Our data shows that patients with congenital HLH transplanted from AD in more recent years, using RIC regimens, experience an excellent outcome, as also reported by other groups. Larger studies maybe necessary to confirm these observations.
Disclosure of Interest: None Declared.

PH-P566

SINGLE CENTRE RETROSPECTIVE ANALYSIS OF 34 PATIENTS WITH X-SCID TRANSPLANTED WITH PARENTAL HLA- HAPLOIDENTICAL GRAFTS

D. Kleila[1,], A. Schulz[1], C. Schuetz[1], I. Furlan[1], M. Wiesneth[2], K.-M. Debatin[1], W. Friedrich[1], M. Hoenig[1]*
[1]Department of Pediatrics, University Medical Center Ulm, [2]Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Transfusion Service Baden Württemberg-Hessen and Institute of Transfusion Medicine, University of Ulm, Ulm, Germany

Introduction: X-linked Severe Combined Immunodeficiency (X-SCID) is caused by genetic defects in the common gamma chain (IL2RG) of the receptors for IL-2,-4,-7,-9,-15 and IL-21. IL2RG deficiency is the most common SCID entity.
Materials (or patients) and Methods: We retrospectively analysed data from 34 patients with IL2RG deficiency subsequently transplanted with T-cell depleted HLA-haploidentical parental grafts in our institution between 1982 and 2005.
Results: : Overall survival (OS) was 82% (28/34) with a median follow up of 10.8 years (1.4 - 24.6). Two methods for T-cell depletion of the grafts were used: sheep erythrocyte/ soy bean lectin sedimentation between 1982 and 1995 for 20 pts, CD34 positive selection/ *in vitro* T-cell depletion between 1996 and 2005 for 14 pts. In the first group OS was 80% (16/20) with a median follow up of 16.9 years (1.4 - 24.6), in the second group 86% (12/14) with a median follow up of 8.5 years (1.9 - 12.7). 20 pts were transplanted with and 14 pts without conditioning. OS was 85% and 79% respectively. Two pts died due to infectious complications (BCG, EBV), in four pts with respiratory failure no infectious agent was identified; no pt died due to GvHD. T-cell engraftment was found in all pts except one who failed to engraft paternal cells without conditioning but was successfully retransplanted with a maternal graft. Successful retransplantations with conditioning were performed in two pts 6 and 12 years after the first attempt without conditioning because of recurrent infections (in spite of Ig-substitution) and autoimmune hepatitis respectively. Stable B-cell function without the need for Ig-substitution was noted in 14/17 long term survivors (82%) after conditioning and in merely 3/11 pts (27%) treated without conditioning.
Discussion: For pts with X-SCID without an available HLA-compatible donor, haploidentical transplantation with and without conditioning is a therapeutic option with excellent survival. The probability of posttransplant B-cell function can be increased by conditioning.
Disclosure of Interest: None Declared.

PH-P567

IMMUNE RECONSTITUTION AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION WITH TCR ALFA/BETA AND CD19 DEPLETION IN CHILDREN WITH NON MALIGNANT DISEASES. A SINGLE CENTRE EXPERIENCE.

M. P. Boaro[1,], M. Tumino[1], C. Mainardi[1], M. Pillon[1], E. Calore[1], M. V. Gazzola[1], A. Strano[1], R. Destro[1], S. Varotto[1], M. C. Putti[1], L. Sainati[1], G. Basso[1], C. Messina[1]*
[1]Pediatrics, Pediatric Hematology Oncology Padova, Padova, Italy

Introduction: HSCT is currently used for the treatment of some non malignant diseases. In the last years, in order to prevent graft versus host disease (GVHD) and post-transplant lymphoproliferative disease (PTLD), techniques of *in vitro* graft manipulation are improving. In this setting TCR αβ/CD19 depletion represent a

recent promising strategy, due the maintenance of large numbers of immune cells in the graft.
Materials (or patients) and Methods: Between 2011 and 2013, in the Haemathology and Oncology Pediatric Department of Padova, 9 patients (7/9 non Caucasian) were treated with HSCT with TCR αβ and CD19 depletion (median age at transplant: 46 months, median follow up: 16 months), for non malignant diseases: mucopolisaccaridosis (1), severe aplastic anemia (1), primary immunodeficiencies (1 Wiscott-Aldrich syndrome, 1 IL10R defect, 1 SCID RAG1 defect, 1 CD25 defect, 1 Omenn Syndrome), Fanconi anemia (FA, 1), sickle cell disease (1). Three patients (Lansky Performance Scale, LPS, <10) were transplanted in a safe setting (Pediatric Intensive Care Unit) for ongoing severe infection (2 Pseudomonas Aeruginosa, 1 Pneumocystis Jirovecii, 1 Aspergillosis). Two patients received a matched allograft, the other 7 children received a related haploidentical SCT. G-CSF mobilized peripheral blood stem cells were infused previous *in vitro* TCR αβ and CD19 depletion (CliniMACS, Miltenyi). All patients were conditioned by myeloablative regimen. GVHD prophylaxis consisted of rabbitATG and cyclosporine (CsA, 8) or mycophenolate (MFM, 1). Immunosuppressive therapy was continued when TCR αβ infused were >25000/Kg (CsA, 6. MFM, 1), with progressive tapering and withdrawal at 6 months post HSCT. Analysis on immune reconstitution was performed at the engraftment and monthly for 6 months.
Results: All patients engrafted, with a median time of 15 days. Two patients rejected 40 days after engraftment from haploHSCT (1 SCID, 1 FA). 8 patients are alive, 1 died for TRM after the second transplant (FA). Patients with ongoing infection at the moment of stem cell infusion, recovered without complications. No GVHD grade III-IV was registered. None of the patients developed severe infection. A number of 6/9 patients suffered from CMV-reactivation without clinical nor radiological involvement, with prompt response to specific antiviral therapy. Any patient developed PTLD. Chimerism was 100% of the donor in 6/8 patients; two children (sickle cell disease and SCID for RAG1 defect) developed a stable mixed chimerism, without signs of underling disease. About immunoreconstitution, our data at +30 from engraftment showed: a median of 259 (range 6-1215) CD3 T cell/ul, 50 (range 0-180) CD4 T cell/ul, 111 (4-535) CD8 T cell/ul, 244 (32-650) NK cells/ul, 268 (0-638) CD19 cells/ul. In particular, CD4 (absolute count >200/ul) and CD19 normal range (13-27%) were reached from +90 and +60 day respectively.
Discussion: In our experience HSCT with TCR αβ/CD19 depletion represents a valid strategy to treat pediatric non malignant disease in terms of fast engraftment, low TRM, no severe GvHD development, and rapid immunereconstitution without evidence of severe infections.
Disclosure of Interest: None Declared.

PH-P568
BUSULFAN-BASED MYELOABLATIVE PREPARATIVE REGIMENS FOR PEDIATRIC AML RESULT IN EXCELLENT OUTCOMES AT DAY 100
D. A Jacobsohn[1,], B. Logan[2], M. Fei[2], M. A Pulsipher[3], W. Navarro[4] and on behalf of the 09-MRD study team*
[1]Blood and Marrow Transplantation, Children's National Health System, Washington, [2]CIBMTR, Medical College of Wisconsin, Milwaukee, [3]Division of Hematology / Blood and Marrow Transplant, University of Utah School of Medicine, Salt Lake City, [4]Transplant Medical Services, National Marrow Donor Program, Minneapolis, United States

Introduction: Historical data suggest similar overall survival for pediatric patients undergoing allogeneic hematopoietic cell transplant (HCT) for acute myeloid leukemia (AML) using busulfan (BU)-based myeloablative (MA) regimens as compared to total body irradiation (TBI)-based MA regimens. There has been a shift to increased use of BU MA regimens as compared to TBI MA regimens in pediatric AML patients. Therefore, understanding the short-term toxicities and early non-relapse mortality from BU MA regimens in these patients is crucial.
Materials (or patients) and Methods: We used data from an ongoing, prospective multi-institutional study (PBMTC ONC1001/CIBMTR 09-MRD) to assess the incidence of toxicity and day 100 mortality by MA regimens. A total of 43 subjects enrolled in 09-MRD (2011-2013) had BU MA conditioning. All patients were in morphologic remission, had not had prior HCT, and had organ function suitable for MA transplant. Median age was 7 years (1-22), with 49% males and 51% females. Eighty-eight percent had Karnofsky/Lansky ≥90%, with 74% of patients having a comorbidity index of 0 or 1. Disease status was CR1 in 72% and CR2 in 28% and 91% were in cytogenetic remission. Donor types were: HLA-identical siblings in 19%, other relatives 5%, 7-8/8 unrelated donor 42%, 4-6/6 single cord blood in 28%, and 5-6/6 double cord bloods in 7%. Only 5% of patients overall received PBSC.
Results: The following were the cumulative incidences (CI) of toxicities at day 100, based on CIBMTR data form 2100: infection 75% (95% CI 61-87%), idiopathic pneumonitis 5% (0-13%), liver toxicity 21% (10-34%), other organ toxicity 24% (12-37%). In terms of graft-versus-host disease (GVHD), incidence of grade 2-4 was 21% (10-35%) and grade 3-4 0%. The CI of relapse was 19% (9-31%) and transplant-related mortality (TRM) 0%, for a disease-free-survival (DFS) of 81% (69-91%) at day 100 and an overall survival (OS) of 95% (87-100%).
Discussion: While this is not a prospective treatment study, these data suggest that significant day 100 toxicities in BU-based MA HCT are acceptable and the incidence of GVHD is low, despite 81% of patients receiving an alternative-donor graft. Moreover, a day 100 TRM of 0 suggests BU-based MA regimens are very well-tolerated in children with AML. These excellent results may be due to pharmacokinetic-based dosing which are standard now, although these data were not collected.
Disclosure of Interest: None Declared.

PH-P569
IS HEPCIDIN A BETTER MARKER OF IRON OVERLOAD IN CHILDREN TREATED WITH HEMATOPOIETIC STEM CELL TRANSPLANTATION?
A. Zaucha-Prażmo[1,], J. Gożdzik[2], K. Drabko[1], M. Woźniak[2], J. R. Kowalczyk[1]*
[1]Department of Pediatric Hematology, Oncology and Transplantology, Medical University, Lublin, [2]Department of Pediatric Transplantation, Children University Hospital, Krakow, Poland

Introduction: Recent studies suggest the association of iron overload with complications of hematopoietic stem cell transplantation (HSCT).
The aim of the study was to estimate the significant marker of iron overload and to assess its correlation with transplant related complications in children treated with HSCT.
The study was approved by Ethical Committee of the Medical University of Lublin, Poland.
Materials (or patients) and Methods: 39 children,15 girls and 24 boys with median age 9,9 years (0.6-16.2 year) treated with HSCT were enrolled into the study. 25 patients with median age 8.8 years (range 0.6-17) were transplanted for malignant diseases, 14 with median age 9.9 years (range 0.2-15.7) - for non-malignant. 9 autologous and 30 allogeneic transplantations were performed, of which 20 transplants were from matched unrelated donors (MUD), 8 from matched sibling donors (MSD) and 2 from mismatched family donors (MMFD). Control group consisted of 10 children – 5 girls and 5 boys, with median age of 9.8 years (2.8-15.1) without anemia and infection. In all transplanted children ferritin, hepcidin -25, soluble transferrin receptor – sTfR and iron levels were assessed before conditioning. The same parameters were measured in the control group.
Organ toxicities (renal, hepatic, gastrointestinal, mucosal) were evaluated according to Bearman RRT scale.
Results: Significantly higher median hepcidin and ferritin plasma concentrations (28.9 ng/ml and 933 ug/l respectively) were found in transplanted patients, when compared to controls (median hepcidin value was 11.2 ng/ml, median ferritine 22 ug/l) [$P<0.005$].

Median hepcidin and ferritin levels did not differ between patients treated for malignant (36,3 ng/ml and 1024 ug/l respectively) and non-malignant diseases (25,3 ng/ml and 117 ug/lrespectively) .
Median values of sTfR, as well as median iron levels in children before HSCT (1,3 ug/ml and 13,2 umol/l respectively) did not differ compared to controls (1,0 ug/ml and 17,9 umol/l respectively).
In 19/39 (43,6%) patients organ complications > 2 grade were diagnosed, in 20/39 children no organ complications or complications of 1 and 2 grade occurred. No difference was found in median ferritin, hepcidin sTfR and and iron levels between these groups of patients.
In 17/39 patients (43,6%) high pre-transplant ferritin concentration (>1000 ug/l) was diagnosed (median 1902 ug/l). In this group of patients median hepcidin level was higher (62,8 ng/ml) than in low ferritin (< 1000 ug/l) group (median ferritin 117 ug/ml; median hepcidin 21 ng/ml) [P<0,05]. No difference was found in the incidence of transplant related organ toxicities and infections in low and high ferritin/hepcidin group.
Discussion: According to our analysis children undergoing stem cell transplantation are at risk of iron overload, which can be defined as well as high hepcidin-25 or ferritin serum concentration.
We have not found a significant association between the pretransplant serum hepcidin/ferritin levels and the cumulative incidence of organ toxicities and infections.
Further studies are necessary to determine the correlation between iron overload and the incidence of complications in HSCT patients.
Disclosure of Interest: None Declared.

PH-P570

ANTIFUNGAL PROPHYLAXIS WITH POSACONAZOLE VS. FLUCONAZOLE OR ITRACONAZOLE IN PEDIATRIC PATIENTS WITH NEUTROPENIA

M. Döring[1,], M. Eikemeier[1], A. Schulz[2], U. Hartmann[3], C. P. Schwarze[1], M. Ebinger[1], R. Handgretinger[1], I. Müller[4]*
[1]Department of Pediatric Hematology and Oncology, University Children`s Hospital Tübungen, Tübingen, [2]Department of Pediatric Hematology and Oncology, University Children`s Hospital Ulm, Ulm, [3]University Pharmacy, University Children`s Hospital Tübungen, Tübingen, [4]Clinic of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: Pediatric patients with hemato-oncological malignancies and neutropenia resulting from chemotherapy have a high risk of acquiring invasive fungal infections.
Oral antifungal prophylaxis with azoles, e. g. fluconazole or itraconazole, is preferentially used in pediatric patients after chemotherapy, even though only a few studies have been published. We also administered posaconazole based on data from studies in adult patients. Retrospectively, we compared the safety, feasibility and initial data on efficacy of posaconazole vs. fluconazole and itraconazole in pediatric patients and adolescents during neutropenia after chemotherapy.
Materials (or patients) and Methods: In this single center study, 93 pediatric patients with a median age of 12 years (range 2 - 17years) with neutropenia (absolute neutrophil count of 500 cells) from a minimum of 5 days after intravenously chemotherapy, who received fluconazole, itraconazole or posaconazole as antifungal prophylaxis were analyzed. 32 of the 93 pediatric patients received antifungal prophylaxis with fluconazole, while 31 were given antifungal prophylaxis with itraconazole. 30 other pediatric patients received antifungal prophylaxis with posaconazole. The observation period was defined as the day before start with oral antifungal prophylaxis with one of the three azoles, to the end of intensive chemotherapy and recovery from neutropenia or until occurrence of a proven or probable invasive fungal infection.
Results: The duration of the periods of prolonged neutropenia in the group of children treated with fluconazole had a mean of 8.9 ± 3.2 days. In the group of pediatric patients treated with itraconazole the mean was 12.6 ± 6.1 days, while the mean for the group of children treated with posaconazole was 17.4 ± 11.4. Proven invasive fungal infections were reported in 2 (6.3%) pediatric patients in the fluconazole or itraconazole group and in 1 (3.3%) pediatric patient in the posaconazole group. Possible fungal infections occurred in 3 (9,5%) pediatric patients in the fluconazole or itraconazole group and in none (0%) of the pediatric patients in the posconazole group. The percentage of patients with adverse events potentially related to clinical drugs were 15.6% in the itraconazole group, 12.9% in the fluconazole group and 10% in the posaconazole group. Posaconazole vs fluconazole or itraconazole are comparably effective in preventing invasive fungal infections in pediatric patients with posaconazole being slightly more effective in pediatric patients with neutropenia after chemotherapy.
Discussion: *Conclusion:* Posaconazole vs fluconazole or itraconazole are comparably effective in preventing invasive fungal infections in pediatric patients with posaconazole being slightly more effective in pediatric patients with neutropenia after chemotherapy.
Disclosure of Interest: None Declared.

PH-P571

VALIDATION OF A TEST DOSE STRATEGY FOR ADMINISTERING INTRAVENOUS BUSULFAN IN THE SETTING OF PEDIATRIC MYELOABLATIVE STEM CELL TRANSPLANTATION: CLINICAL AND PHARMACOKINETIC RESULTS

T. Nishikawa[1,], Y. Okamoto[1], T. Tanabe[1], K. Kurauchi[1], S. Nakagawa[1], T. Abematsu[1], Y. Kodama[1], Y. Shinkoda[1], Y. Kawano[1]*
[1]Pediatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Introduction: Intravenous busulfan (iv-Bu) is considered to have a much more predictable pharmacokinetic (PK) profile than oral-Bu. However there remains strong variation. For cases in which real-time therapeutic drug monitoring (TDM) is not applicable, new approaches using PK analyses of test doses have been proposed.
In the present study, we tested whether the administration of a test dose of iv-Bu before HSCT can be used to predict the PK of the first dose of iv-Bu in children and investigated the association between the clinical outcomes and PK results.
Materials (or patients) and Methods: iv-Bu was administered as a two-hour infusion every six hours for four days. Five dose levels (0.8-1.2 mg/kg) were evaluated according to body-weight strata. Following the administration of a single dose of iv-Bu as a test dose and the first dose of Bu during the conditioning regimen, blood samples were collected from each patient at seven points. Dose adjustment was not allowed between the administration of the test and treatment doses. The Bu concentrations in plasma were measured using HPLC. The area under the curve (AUC) for both the test dose and first dose was calculated using the linear trapezoidal rule.
Results: Twenty-three children from 8 months to 17 years of age were enrolled in this study. The underlying diseases included AML (n = 10), advanced solid tumors (n = 6), and others (n = 7). The conditioning regimens included Bu/LPAM (n = 13), Bu/CY (n = 6) and Bu/FLU/ATG (n = 4). The stem cell source was allogeneic BM in 12 patients, PB in one patient, CB in four patients and autologous PB in six patients. Two patients developed graft failure. Overall, 11 of the 23 patients (48%) relapsed after HSCT. Nine patients died due to their underlying disease, and four patients died due to TRM. The three-yr OS and EFS rates were 40% and 19%, respectively.
At the first dose, 11 (58%) of 19 patients achieved an AUC within the 900–1500 μM × min therapeutic window. Underexposure was observed in four (21%), and one patient with the lowest AUC (686 μM × min) developed graft failure. Although overexposure was observed in four, no patients developed VOD. Three patients who developed lethal Bu lung disease had an AUC of 881, 1004, and 2353 μM × min, respectively. The relapsed patients had lower AUC values than the patients who remained in remission (median: 1056 vs. 1275, P = 0.21).

The AUC for the first dose was compared with that for the test dose. A linear regression analysis showed a statistical correlation between these values ($r = 0.65$; $P < 0.05$).
Discussion: The body-weight strata dosing schedule used in the present study was associated with a low rate of achievement of the target AUC. The risk of graft failure among patients undergoing allogeneic HSCT should be minimized, keeping the AUC above 900 μM × min. When the maximum antitumor effect is required, then maintaining a high Bu exposure within the target AUC may be necessary.
Three patients developed lethal Bu lung disease, not correlated with the AUC.
Although a statistical correlation was observed between the first dose and test dose AUC values, it is very difficult to predict the first dose AUC precisely based on the test dose AUC. Developing a test dose strategy for assessing the efficacy of iv-BU treatment would be a useful means of excluding extremely poor or extensive metabolizers.
Disclosure of Interest: None Declared.

PH-P572
TANDEM HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL RESCUE IN HIGH RISK NEUROBLASTOMA PATIENTS: A SINGLE CENTER EXPERIENCE
S. J. Kim[1,], H. Kim[1], J. W. Lee[1], K. D. Park[1], H. Y. Shin[1], H. S. Ahn[1]*
[1]Seoul National University, Seoul, Republic Of Korea

Introduction: Treatment outcome of pediatric cancer has improved substantially during the past two decades, but limited progress has been achieved in improving the survival rate of high risk neuroblastoma. High-dose chemotherapy (HDCT) has been employed to improve survival, but many patients still suffer from relapse after HDCT. The intent of this research was to investigate the clinical effectiveness of tandem HDCT combined with autologous stem cell rescue therapy and establish a regimen that maintains a low TRM and relapse rate.
Materials (or patients) and Methods: We retrospectively analyzed 60 children with high risk neuroblastoma who received single or tandem HDCT during the period between October 1997 and December 2010. The event-free survival (EFS), overall survival (OS), and toxicities were assessed.
Results: Sixty patients diagnosed with high-risk neuroblastoma received single or tandem HDCT and autologous stem cell rescue. Thirty two patients received single HDCT and 28 patients received tandem HDCT. Patients ≥ 18 months consisted 78.1% of the single group and 82.1% of the tandem group. Patients in the single HDCT group received a conditioning regimen that consisted of melphalan, etoposide, and carboplatin (MEC) or topotecan, melphalan, carboplatin (CTM). The tandem HDCT group received topotecan, thiotepa, and carboplatin (TTC) with MEC. In June of 2007, a modification of carboplatin dose was implemented as patients were observed to experience renal toxicity.
The probability of 5-year event-free survival (EFS) and overall survival (OS) was higher in patients who received tandem HDCT compared to the patients who received single HDCT. The EFS of the single and tandem group was 48.8% and 63.9%, respectively (P=0.23). The OS of the single and tandem group was 77.6% and 85.7%, respectively (P=0.54). Treatment related mortality (TRM) was observed in 2 of the 32 patients that received single HDCT, and 2 of 28 patients that received tandem HDCT. A decrease in TRM attributed to renal toxicity was observed in patients who received lower doses of carboplatin.
Discussion: Tandem HDCT could improve survival in high-risk neuroblastoma patients. Conditioning regimen related to renal toxicity may play a factor in increasing TRM, and further investigation of an optimal conditioning regimen is warranted.
Disclosure of Interest: None Declared.

PH-P573
THYMOGLOBULINE IN EFFICIENT EXPANSION OF CYTOKINE-INDUCED KILLER (CIK) CELLS AND POSSIBILITY OF CIK CELL CLINICAL APPLICATION IN POSTTRANSPLANT SETTING.
M. Mielcarek[1,], B. Rybka[1], R. Ryczan[1], D. Noworolska-Sauren[1], M. Ussowicz[1], K. Kalwak[1]*
[1]Deparment of Bone Marrow Transplantation, Paediatric Oncology and Haematology, Wroclaw Medical University, Wroclaw, Poland

Introduction: Cytokine-induced killer (CIK) cells are expanded *in vitro* with IFN-γ, αCD3 monoclonal antibodies (mAb) and IL-2. In 2010 Bonanno presented a protocol with polyclonal thymoglobuline (TG) substituting mAb showing a successful differentiation of CIK cells. Due to high relapse rate adoptive CIK cell immunotherapy aims at controlling minimal-residual disease by the immune system, leading to effective tumour cell killing in post-transplant setting.
Materials (or patients) and Methods: Peripheral blood mononuclear cells from 5 healthy potentially haploidentical donors were activated with IFN-γ on day 0 and fed with 50 ng/mL of mAb (standard protocol) or 500 ng/mL of TG (modified protocol) with IL-2 on day 1. Culture medium exchange with further IL-2 stimulation was performed every 2-3 days for 21 days. Aliquots of CIK cells were harvested weekly for cellularity, viability, cytotoxicity (against NK-sensitive K562 cells) and immunophenotype (standard lymphocyte subpopulations, regulatory T cells, TCRαβ/γδ). In one case cytotoxicity against fresh isolated MDS/sAML patient blasts was tested. In this case after local ethical board acceptance and obtaining a signed informed consent the treatment with CIK cells stimulated with TG was applied in a paediatric patient in progression of sAML (status post 4 alloHSCT, incl.1 haplo from KIR-mismatched father and 3 DLIs with no features of GvHD). 1st TG-derived CIK cells were infused 4 weeks after the last DLI in the previously applied cell dose ($1x10^6$ CD3+cells/kg) with hydrocortisone, clemastine and metamizol prophylaxis without any cytoreductive chemotherapy. 2nd infusion was performed 4 weeks after with increased dose of $2.7x10^6$ CD3+cells/kg with clemastine and metamizol prophylaxis. 3rd infusion was done 3 weeks after with increased cell dose of $5x10^6$ CD3+cells/kg with clemastine. Two last doses were applied after chemocytoreduction of blasts. The patient was monitored for early and late immunological reactions.
Results: The expansion of CIK cells stimulated with TG was more vigorous than mAb-driven CIK cells. This culture showed more efficient K562 and fresh isolated blasts target cells lysis when compared with standard protocol. The frequency of Treg cells as well as TCRαβ cells was lower for the studied protocol in comparison with standard setting. Infusion of TG-derived CIK cells did not cause any side effects in the recipient. No features of GvHD was observed. After the 1st infusion circulating peripheral blasts were reduced from 66% to 19% within 24 hours as well as transient autologous signal drop was noted. The patient died of progression 2,5 months after the 3rd infusion and 4 months from the 1st one.
Discussion: TG allows sufficient clinical grade functional CIK cell expansion without concomitant generation of Treg cells (a subset implicated in the down-regulation of anti-tumor immunity) and with reduction of TCRαβ cells (a population correlated with GvHD). Infusion of TG-derived CIK cells obtained from previous haematopoietic stem cell donor seems to be safe in allogeneic stem cell recipients who failed DLI and progressed without any features of GvHD. Further studies on safety and efficacy of TG-derived CIK cell therapy are required. Supported by NCN grant NN407686940.
Disclosure of Interest: None Declared.

PH-P574

REDUCED INTENSITY CONDITIONING HAPLOIDENTICAL BONE MARROW TRANSPLANTATION WITH A POST-INFUSION CYCLOPHOSPHAMIDE APPROACH IS FEASIBLE AND ENABLES FULL DONOR ENGRAFTMENT FOR CHILDREN SUFFERING FROM SICKLE CELL DISEASE

A. Katsarou[1,], Y. Harrington[1], F. O'Boyle[1], N. Killeen[1], N. O'Brien[1], E. Warner[1], S. Chakravorty[1,2], L. Karnik[1], J. de la Fuente[1,2]*
[1]Department of Paediatrics, St. Mary's Hospital, [2]Centre for Haematology, Imperial College London, London, United Kingdom

Introduction: Sickle cell disease has a high risk of morbidity and mortality and bone marrow transplantation currently offers the only chance of cure. However, less than 25% of children have a matched familial donor free of the condition and the chances of finding an unrelated donor are low.
Materials (or patients) and Methods: Between July and September 2013 we performed three haplointetical BMT for patients with homozygous HbSS disease and severe cerebrovascular disease at St Mary's hospital. All children had suffered at least one cerebrovascular event and had evidence of disease progression despite adequate transfusion therapy. The source of stem cells was G-CSF primed maternal bone marrow. Two patients had the same blood group as the donor and one had a major ABO incompatibility. Two patients were CMV+/+ and one -/+.
Results: Endogenous haemopoiesis was suppressed for three months pre-transplantation with the use of hypertransfusions, hydroxycarbamide 30 mg/kg and azathioprine 3 mg/kg. The conditioning regimen comprised ATG (Thymoglobulin) 0.5 mg/kg day -9 and 2 mg/kg days -8 to -7 (total 4.5 mg/kg), thiotepa 10 mg/kg day -7, fludarabine 30 mg/m^2 days -6 to -2 (total 150 mg/m^2), cyclophosphamide 14.5 mg/kg days -5 and -4 (total 29 mg/kg) and TBI 2 Gy on day -1. GvHD prophylaxis was provided with cyclophosphamide 5 mg/kg day +3 and +4, MMF and sirolimus (target 10-15 ng/mL). MMF was weaned after day +35 over two weeks after molecular evidence of donor haemopoiesis. The cell dose was 5.13 x 10^8 TNC/kg and 1.123 x 10^6 CD34+/kg, 11.82 x 10^8 TNC/kg and 5.158 x 10^6 CD34+/kg, and 10.117 x 10^8 TNC/kg and 2.617 x 10^6CD34+/kg, respectively.
Neutrophil engraftment occurred on day +16, +17 and +21, respectively. All patients showed chimerism studies >95% donor in whole blood and T cell fraction on days +28, +60 and +90 and patient 1 is off all immunosuppression since day +119.
Patient 1 developed a possible IFI of the lungs and CMV pneumonitis, reactivated HSV type 1 and type 2 and had haemorrhagic cystitis. Patient 2 had a possible IFI of mastoid. Patient 3 has a possible IFI of the lung. All infectious complications had complete resolution following first line treatment and none of the patients had organ failure. Patient 1 developed acute GvHD of the skin stage 1 with complete response to topical steroids and patient 3 acute GvHD skin stage 3 with complete response to the use of MSC. None of the patients developed VOD.
Discussion: In summary, these constitute the first three haploidentical transplants reported for sickle cell disease with the use of a post-infusion cyclophosphamide and reduced intensity conditioning in the paediatric population. This approach has led to prompt engraftment enabling the cure of patients with no related or unrelated donors. Although infectious complications are common all patients responded promptly and had full resolution.
Disclosure of Interest: None Declared.

PH-P575

INTERNATIONAL STUDY ON OUTCOMES OF HAEMATOPOIETIC STEM CELL TRANSPLANT FOR DNA-DSB REPAIR DEFECTS.

J. Slack[1,], M. A Slatter[1], B. Gaspar[2], M. Albert[3], S. Ghosh[4], R. Meisel[4], T. Guengoer[5], G. Karasu[6], K. Drabko[7], A. Lange[8], K. Kalwak[9], S. Pasic[10], C. Heilmann[11], K. Imai[12], P. Shaw[13], K. Schultz[14], M. Eapen[15], E. Horwitz[16], A. R Gennery[1] and IEWP EBMT, CIBMTR*
[1]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, [2]Institute of Child Health, University College, London, United Kingdom, [3]Dr. von Haunersches Kinderspital der LMU, Munich, [4]Heinrich-Heine Universität, Düsseldorf, Germany, [5]Kinderspital, Zurich, Switzerland, [6]Goztepe Medicalpark Hospital, Istanbul, Turkey, [7]Medical University of Lublin, Lublin, [8]Polish Academy of Sciences, Wroclaw, [9]Polish Academy of Sciences, Wroclaw, Poland, [10]Mother and Child Health Institute, Belgrade, Serbia, [11]BørneUngeKlinikken, Copenhagen, Denmark, [12]Tokyo Medical and Dental University, Tokyo, Japan, [13]Paediatrics and Child Health, University of Sydney, Sydney, Australia, [14]BC Children's Hospital, Vancouver, Canada, [15]Medical College of Wisconsin, Milwaukee, [16]Children's Hospital of Philadelphia, Pennsylvania, United States

Introduction: Patients with DNA-dsb repair defects often present with recurrent infection, bone marrow failure and predisposition to lymphoid malignancy. The systemic nature of the repair defect may cause patients not to tolerate myeloablative conditioning. We report multi-centre outcomes of HSCT in patients with Nijmegen Breakage Syndrome (NBS), DNA ligase 4 deficiency (LIG4), Cernunnos deficiency (XLF), and Ataxia Telangiectasia (AT).
Materials (or patients) and Methods: Centres were contacted through EBMT IEWP and CIBMTR. Retrospective data were collected via a questionnaire, including conditioning regimen, outcome post-HSCT, incidence of GvHD. Conditioning regimens were classified as myeloablative (MAC) (busulfan or treosulphan) or reduced intensity (RIC) (typically a combination of fludarabine and melphalan or very low dose cyclophosphamide, including combinations of targeted antibodies and alemtuzumab).
Results: 23 patients identified, from 17 transplant centres in 11 countries, including NBS (8), LIG4 (7), XLF (4), AT (4). Median age at HSCT was 52 months (range 22-240), median follow-up 13.5 month (range 2-102). 8 received MFD, 11 MUD, 4 mis-matched donors, 3 UCBT, 12 BM, 8 PBSC. 16/23 (70%) received RIC, of whom 14 (88%) are alive, 7 underwent MAC, of whom 2 (29%) are alive. Acute GvHD (grade 1-3) was common occurring in 14/23 (61%) cases, 12 ≥ grade 2 skin+/- gut/liver, 5 died. Acute GvHD occurred in 4/4 XLF, 4/8 NBS, 2/7 LIG4 and 3/4 AT patients. 5 developed chronic GvHD, 2 died.
Discussion: HSCT can be successful for patients with DNA-dsb repair defects. A good short-term outcome can be achieved, particularly if RIC is used. Irradiation is not necessary as part of conditioning, and should be avoided. GvHD is common, and not well tolerated. Long-term follow up will be required to determine whether these patients are at greater risk of developing secondary malignancies.
Disclosure of Interest: None Declared.

PH-P576

VORICONAZOLE AS ANTIFUNGAL PROPHYLAXIS DURING NEUTROPENIC PHASE IN CHILDREN UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

J. R. Molina[1,], D. Buenasmañanas[1], E. García-Torres[1], A. Rodríguez-Villa[1], R. Rojas[1], C. Martín[1], J. Serrano[1], P. Gómez[1]*
[1]Hematology, University Hospital Reina Sofia, Córdoba, Spain

Introduction: Children undergoing allogeneic stem cell transplantation (Allo-SCT) are at high risk of acquiring invasive fungal disease (IFD). However, in the last years the use of antifungal prophylaxis with anti-mold agents (especially azoles) has significantly reduced the incidence of IFD in this kind of pediatric patients. In this study we have prospectively assessed the safety and efficacy of voriconazole as primary antifungal prophylaxis during neutropenic phase in children undergoing Allo-SCT.

Materials (or patients) and Methods: From Oct-2004 to Nov-2013 a total of 77 Allo-SCT performed in 72 children and adolescents (<18 years) were included in this prospective study; median age was 9 years (range: 1-17). Acute lymphoblastic leukemia was the most frequent underlying disease (*n*=46). In all except 6 patients myeloablative conditioning regimen was administered while 39 children received antithymoglobulin. Stem cell source was bone marrow in 40 (51.9%) patients, peripheral blood in 15 (19.5%) patients and cord blood in the other 22 (28.6%). All pediatric patients received oral or intravenous voriconazole at a dose of 5 mg/kg/12 hours (*n*=23) or 7 mg/kg/12 hours (*n*=54) until a top dose of 200 mg/12 hours from day -1 to day +75 or later in patients with acute graft versus host disease (aGVHD). In this study we have just analyzed neutropenic phase, from stem cell infusion day to one week after myeloid engraftment (>500/mm^3 neutrophils). IFD (proven or probable) was diagnosed according to EORTC/MSG criteria. Voriconazole plasma levels were not monitored. AGA was measured twice a week from jun-2006 in 63 children.
Results: Median engraftment was 16 days (r: 10-67) and no child died during neutropenic phase. Only two (2.6%) patients developed IFD (probable aspergillosis) within prophylaxis with voriconazole during neutropenic phase but none of them died due to IFD and both are alive today (28 and 22 months after Allo-SCT, respectively). In this large series, voriconazole prophylaxis failed in 11 (14.3%) children while a total of 66 (85.7%) patients successfully completed the prophylaxis without adverse events, no empirical or preemptive antifungal therapy neither IFD. Six (7.8%) receptors needed an empirical (*n*=4) or preemptive (*n*=2) antifungal therapy, voriconazole was stopped and replaced with amphotericin B in these six patients. In 3 (3.9%) children were detected grade III/IV adverse events due to voriconazole prophylaxis and needed a definitive withdrawal. These 3 receptors presented persistent hepatic damage with cytolysis enzymes elevation but it was readily and quickly solved in all cases once stopped voriconazole; no other adverse event was detected in this high risk phase. In our study 6 children <2 years old were included, voriconazole was stopped in one of them and empirical treatment was started for persistent fever; the rest successfully completed voriconazole prophylaxis.
Discussion: According to the data of our series, voriconazole as primary antifungal prophylaxis during neutropenic phase in children undergoing Allo-SCT is effective in reducing the incidence of IFD (2.6%). Voriconazole is also well tolerated with a low percentage of adverse events easily solved once the drug was stopped without life-threatening complications, moreover only 7.8% of children needed empirical or preemptive antifungal treatment.
Disclosure of Interest: None Declared.

PH-P577

SUCCESSFUL TREATMENT FOR SECONDARY AML AND MDS FOLLOWING MYELOABLATIVE HEMATOPOETIC STEM CELL TRANSPLANTATION FOR CHILDREN, ADOLESCENTS AND YOUNG ADULTS

O. Maher[1,], L. Cooper[2], N. Tarek[1], L. L Worth[2], D. Petropoulis[2], D. A Lee[2], R. E Champlin[3], G. Rondon[3], P. Tewari[2]*
[1]Pediatrics, [2]Pediatric Cellular Therapy, [3]Stem Cell Transplantation and Cellular Therapy, UT MD Anderson Cancer Center, Houston, United States

Introduction: Hematopoietic stem cell transplantation (HSCT) provides potential curative treatment for secondary myelodysplastic syndrome (sMDS) and acute myeloblastic leukemia (sAML). There is only limited literature reporting outcomes of HSCT for children and young adults with sMDS/sAML. We report the outcomes of 33 pediatric and young adult patients with sMDS and sAML following myeloablative chemotherapy and HSCT.
Materials (or patients) and Methods: A retrospective analysis of patients age <26 with sMDS and sAML treated at UT MD Anderson Cancer Center with HSCT was conducted using descriptive measures.
Results: Thirty-three consecutive patients (median age 19, range 2-25) with sMDS (*n*=9) and sAML (*n*=24) received myeloablative HSCT at our center between 1990 and 2013. Twenty-five patients were male, primary diagnosis as follows: severe aplastic anemia (*n*=6), acute lymphoblastic leukemia (*n*=5), osteosarcoma (*n*=5), Hodgkin's Lymphoma (*n*=5), Germ-cell tumor (*n*=4), soft-tissue sarcoma (*n*=3), Non-Hodgkin lymphoma (*n*=3) and other (*n*=2). The median time between primary diagnosis and HSCT was 51 months (range 5-172). Twelve (36.4%) patients were found to be resistant to induction chemotherapy and were transplanted in relapse. Transplant regimens and GVHD Prophylaxis are described in the table below. Donor sources include Marrow (*n*=15), Apheresis product (*n*=10), and Cord blood (*n*=8). Twenty-nine (88%) patients tolerated transplant procedure well and achieved successful donor engraftment. Median time to engraftment was 12 days (range 8-31). Acute Graft versus host disease (aGVHD) occurred in 17 patients, of which only 3 had grades 3-4. At a median follow up of 10.7 months (range 0.3-236) 14 patients remain alive. Causes of death were as follows: disease recurrence (*n*=11), GVHD (*n*=2), Graft Failure (*n*=2), Infection (*n*=2), Multi-organ failure (*n*=1), Hemorrhage (*n*=1).
Discussion: This is the largest reported Pediatric and young adult cohort of patients that underwent HSCT for secondary malignancies or secondary AML. In this young age group, despite exposure to prior therapies, myeloablative regimens are well tolerated with low rates of Grades 3 and 4 GVHD. Highest cause of mortality remains disease relapse/recurrence, with 11 (33%) of patient mortality attributable to this. While disease relapse and treatment-related mortality remain major challenges, many patients benefit following Myeloablative chemotherapy and allogeneic HSCT across regimens and donor sources.
Disclosure of Interest: None Declared.

[PH-P577]

Prep Regimen Used	N		GVHD Prophylaxis Used	N	
Fludarabine/Busulfan	8		Tacrolimus/Methotrexate	16	
Thiotepa/Bulsufan/Cytoxan	5		Tacrolimus/Mycophenolate	4	
Melphalan/Thiotepa/Fludarabine	5		Tacrolimus/Mycophenolate/Cytoxan	4	
Fludarabine/Melphalan	4		Cyclosporine/Methotrexate	3	
Fludarabine/Clofarabine/Bulsulfan/TBI	3		Cyclosporine/Steroids	2	
Bulsulfan/Cytoxan	3		Cyclosporine	1	
TBI/Cytoxan/Thiotepa	2		Tacrolimus	1	
TBI/Cytoxan/Etoposide	2		Unknown	2	
Fludarabine/Melphalan/TBI	1				

PH-P578

ANESTHESIA FOR BRONHOSCOPY IN PEDIATRIC PATIENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION AND HIGH DOSE CHEMOTHERAPY

M. Bochishcheva[1,], A. Lazarev[1], E. Goncharova[1], I. Zinnatullin[1], A. Volkova[1], A. Kozlov[1], M. Bogomolniy[1], L. Zubarovskaya[1], B. Afanasyev[1]*

[1]R.M. Gorbacheva Memorial Institute Of Children Oncology, Hematology And Transplantation. First State Medical Universtity Named after Pavlov I.P., St Petersburg, Russian Federation

Introduction: Bronchoscopy performed with the use of modern equipment and adequate anaesthesia can be safely used for pediatric patients suffering from oncological and hematological diseases.

Objectives of our study were the comparison of different anesthetics in children with oncological and hematological diseases during bronchoscopy, assessment of complications, and selection of preferable method of anaesthesia.

Materials (or patients) and Methods: We analyzed retrospectively 165 episodes of anesthesia provided to patients aged 8 months -13 years undergoing bronchoscopy in 112 (68%) patients after hematopoietic stem cell transplantation (HSCT) and 53 (32%) patients after high dose chemotherapy. All the procedures were performed in the operating room with constant monitoring of heart and breathing rate, pulsoximetria, ECG, blood pressure (every 3 min). All the patients had spontaneous ventilation. We used the following methods of anesthesia– halothane 19% (*n*=31), sevofluran 53% (*n*=87), propofol IV 28% (*n*=47), combined with local anaesthetic (Lidocain 2%) for high-resolution CT guided bronchoscopy with endoscopic video system Olympus with external diameter of the distal end ranging from 3.6 mm. to 4.9 mm. At the end of the procedure all the patients were transported to the post anesthetic room where they were monitored until full recovery from anesthesia. Patients from intensive care unit (ICU) were not included in this research.

Results: There were no serious complications and no one required further ventilation or transportation to the ICU. All complications after anesthesia were transient, required correction or resolved spontaneously. The complications associated with halothane included agitation 19.3% (*n*=3), laringospasm 6.45% (*n*=2), dysrhythmia 6.45% (*n*=2), hypotension 9.6% (*n*=3), transient hypoxia during emergence 9.6% (*n*=3) and other 2/2% (*n*=1). Propofol has some advantages such as quick induction and recovery providing better control, however the complications included hypotension 6.3% (*n*=3), dysrhythmia 8.5% (*n*=4), apnea 0.4%, (*n*=1). Only 2.3% complications were noted when sevofluran was given (2 patients had agitation during induction).

Discussion: At the moment of bronchoscopy sevofluran is optimal for anesthesia in indicated group of pediatric patients due to quick induction, considerably fast recovery, low rate of complications during anesthesia and emergence. It also provides extra comfort to bronchologist by suppressing cough, swallowing reflex and bronchodilator effect.

Disclosure of Interest: None Declared.

PH-P579

CURRENT RESULTS WITH TRANSPLANTATION OF TCRAB/CD19 DEPLETED STEM CELLS FROM HAPLOIDENTICAL DONORS IN CHILDREN

P. Lang[1,], T. Feuchtinger[1], H.-M. Teltschik[1], M. Schumm[1], P. Schlegel[1], M. Pfeiffer[1], M. Ebinger[1], C.-P. Schwarze[1], R. Handgretinger[1]*

[1]Hematology / Oncology, University Children's Hospital, Tuebingen, Germany

Introduction: T-cell depletion is an effective method to prevent Graft-versus-Host Disease (GvHD) in haploidentical stem cell transplantation.

Materials (or patients) and Methods: In order to increase T-cell depletion efficacy while maintaining anti-tumor and anti-infectious activity of the graft, we have evaluated a new method which removes αβ+ T-cells via a biotinylated anti-TcRαβ antibody followed by an anti-biotin antibody conjugated to magnetic microbeads while retaining γδ+ T-cells, Natural killer (NK) cells and others. CD19+ B-cells were concomitantly depleted to prevent EBV-LPD. The CliniMACS® system was used for manipulation of PBSCs from full haplotype mismatched family donors.

Results: The overall depletion of αβ+ T-cells was highly effective with 4.6 log. Patients received a median number of only 14 x 10^3/kg residual αβ+ T-cells. Recovery of CD34+ stem cells was 72%, and the median number of infused CD34+ stem cells was 12x10^6/kg. Additionally, the patients received potential antileukemic effector cells: 107x10^6/kg CD56+ NK cells and 11x10^6/kg γδ+ T-cells.

Diagnoses: ALL (*n*=20), AML/MDS/JMML (*n*=9), nonmalignant diseases (*n*=4), solid tumors (*n*=2); disease status: CR2-CR6 (*n*=17), active disease (*n*=11, 45%). 23 patients received a 2nd/3rd SCT (65%). The conditioning regimen comprised fludarabin 40mg/m^2 or clofarabin 50mg/m^2 (day -8 to d -5), thiotepa 10mg/kg (d -4), melphalan 70mg/m^2 (d -3 and d -2). OKT3 was used as rejection prophylaxis from day -8 to day -1 in the first 7 patients and was substituted since 2011 by a reduced ATG-F dose (15mg/kg) given at start of the regimen in order not to impair NK and γδ+ T-cells of the grafts (1 mg/kg d -12, 4 mg/kg d -11, 5 mg/kg d -10 and -9; *n*=28 patients). Short course MMF (until day +30) was given in 25 patients. Graft rejection occurred in 14% of the patients. However, after reconditioning and second stem cell donation, final engraftment was achieved in all patients. Median time to reach neutrophil and platelet recovery in patients with primary engraftment was 10 and 11 days respectively. All patients showed a rapid immune reconstitution with 250 (OKT3 conditioning) and 273 (ATG conditioning) CD3+ T-cells/µl, 30 (OKT3) and 47 (ATG) CD3+4+/µl and 300 (OKT3) and 382 (ATG) CD56+ NK-cells/µl at day +30 post-transplant. γδ+ T-cells started to expand faster than αβ+ T-cells in the early post-transplant period (156 vs 82 cells/µl at day +30) whereas at day +90, αβ+ T-cells were predominant (170 vs 134 cells/µl). Acute GvHD grade 0-I occurred in 25 patients (71%); 6 patients had GvHD II (17%), 3 patients had GvHD III (9%) and one patients experienced GvHD grade IV (3%). 3 patients experienced chronic GvHD (8%). Incidence of acute GvHD was not influenced by the number of residual T cells or by type of serotherapy. 2 year EFS was: 80% (nonmalignant diseases); 37% and 9% (acute leukemias, any CR and active disease). Patients with any CR and 1st SCT showed better results than patients with subsequent SCT (100% vs 30%).

Discussion: These data indicate that transplantation of TcR αβ+/CD19 depleted cells from a haploidentical donor results in fast immune reconstitution and low incidence of both acute and chronic GvHD. OKT3 could be substituted by ATG without negative effects. The anti-leukemic efficacy of this approach in comparison to other methods of T-cell depletion needs to be evaluated with a longer patient follow-up.

Disclosure of Interest: None Declared.

PH-P580

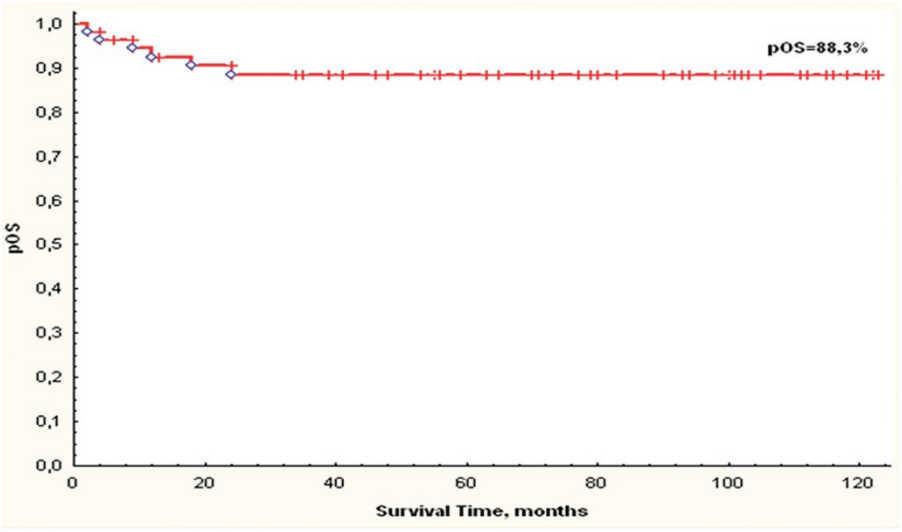
LOW DOSE CYCLOPHOSPHAMIDE AND FLUDARABINE-BASED CONDITIONING REGIMEN FOR PEDIATRIC MATCHED RELATED DONOR HEMATOPOIETIC STEM CELL TRANSPLANTATION IN SEVERE APLASTIC ANEMIA

E. Skorobogatova[1,], D. Balashov[2], P. Trakhtman[2], M. Maschan[2], K. Kirgizov[1,2], G. Novichkova[2], A. Roumyantsev[2], A. Maschan[2]*

[1]The Russian Children's Research Hospital, [2]Dmitriy Rogachev Centre of Paediatric Hematology, Oncology and Immunology, Moscow, Russian Federation

Introduction: Use of high dose cyclophosphamide (Cy) in preparative regimens is associated with endothelial damage, hemorrhagic cystitis and potential carcinogenicity. Fludarabine (Flu) is a potent immunosuppressant devoid of significant visceral toxicity. We hypothesized that introduction of Flu as part of preparative regimen will allow lowering of Cy dose without compromising overall efficacy of transplantation.

[PH-P580]



Materials (or patients) and Methods: Fifty-five children with acquired severe aplastic anemia (SAA), 30 males, 25 females, median age 12,5 (4-17) years, received HSCT from matched siblings (6 – syngeneic) between September 2003 and August 2013. Graft sources included BM (*n*=34), PBSC (*n*=18), PBSC+CB (*n*=2), PBSC+BM (*n*=1). Conditioning regimen included Cy 100 mg/kg (d-5,-4,-3,-2), Flu 100 mg/m^2 (d -5,-4,-3,-2), ATGAM 100 mg/kg (d -4,-3,-2,-1). GvHD prophylaxis included: CsA+MTX – 9% (n - 5) or CsA+MMF – 91% (n- 50).
Results: Overall 53(96%) of patients engrafted. Rejection rate was 7,5% (*n*=4) (on 1, 4, 6, 30 months after HSCT). Second HSCT was performed in 5 cases (4 – for patients with rejection, 1 – for primary graft failure). Three patients survived after the second HSCT with good engraftment, 2 patients died. Direct toxicity of conditioning regimen was minimal. No signs of acute GvHD were observed in 33 patients (62,2%), grade I-II aGVHD – in 17 patients (32,0%), grade III-IV aGVHD – in 2 patients (3,7%). Limited cGvHD was diagnosed in 3 patients, extensive cGvHD – in 2 patients. At median follow up of 84(4-123) months the estimated probability of 10 years OS was 88,3%, EFS – 82,2%. The causes of death were sepsis (*n*=2), CMV-infection (*n*=2, patients after second HSCT) and cGvHD (*n*=2).
Discussion: Our results suggest that low Cy/Flu conditioning regimen is well tolerated and ensures stable engraftment, minimal toxicity and low GVHD rate in the majority of pediatric SAA patients.
Disclosure of Interest: None Declared.

PH-P581

COMPARISON OF TWO DOSES OF ANTITHYMOCYTE GLOBULIN IN PEDIATRIC PATIENTS WITH APLASTIC ANEMIA WHO RECEIVED ALLOGENEIC BONE MARROW TRANSPLANTATION

H. Yabe[1,], N. Uchida[2], S. Takahashi[3], Y. Ohno[4], Y. Kouzai[5], H. Kanamori[6], S. Mori[7], T. Nagamura[8], K. Kato[9], M. Murata[10], R. Suzuki[11], Y. Atsuta[11], S. Kojima[12]*
[1]Department of Cell Transplantation and Regenerative Medicinep, Tokai University School of Medicine, Isehara, [2]Department of Hematology, Federation of National Public Service Personnel Mutual Aid Associations TORANOMON HOSPITAL, [3]Division of Molecular Therapy, The Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, [4]Department of Internal Medicine, Kitakyushu Municipal Medical Center, Fukuoka, [5]Department of Transfusion Medicine, TOKYO METROPOLITAN TAMA MEDICAL CENTER, Tokyo, [6]Kanagawa Cancer Center, Yokohama, [7]St. Luke's International Hospital, [8]The Institute of Medical Science, The University of Tokyo, Tokyo, [9]Japanese Red Cross Nagoya Daiichi Hospital, [10]Nagoya University, [11]Department of HSCT Data Management and Biostatistics, [12]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan

Introduction: Allogeneic bone marrow transplantation (BMT) has become the treatment of choice for severe aplastic anemia (SAA) over the past decades and the outcomes of BMT have improved even from unrelated donors. Inclusion of rabbit antithymocyte globulin (rATG; ThymoglobulinR) as a part of conditioning regimen is known to reduce the incidence and severity of acute and chronic graft-versus-host disease (GVHD). However, the optimal dose of rATG is not still established in BMT for childhood SAA. We retrospectively compared the results of two doses of rATG in allogeneic BMT.
Materials (or patients) and Methods: A total of 230 pediatric patients aged 1 to 15 years who received allogeneic BMT for SAA with the conditioning regimen including rATG at a dose of 5 mg/kg (ATG-5 group; 81 patients) or 10 mg/kg (ATG-10 group; 149 patients) were enrolled in this study.
Results: There were no statistical differences between two groups in age and the male to female ratio. Thirty-eight patients in ATG-5 group suffered from congenital AA and 43 patients acquired, while 23 patients in ATG-10 group suffered from congenital AA and 126 patients acquired (*P*=0.0001). Moreover, ATG-5 group received BMT more recently (2002 to 2011 vs 1994 to 2011), and received BMT from unrelated donor more than HLA-identical

sibling (*P*=0.0029) compared to ATG-10 group. Primary engraftment was observed in 78 of 81 patients in ATG-5 group and 143 of 149 patients in ATG-10 group (*n.s.*). Grade II to IV and Grade II to IV acute GVHD developed 17.5% and 6.3% of patients in ATG-5 group and 15.7% and 6.9% of patients in ATG-10 group (*n.s.*), respectively. Chronic GVHD was recognized 21.1% of patients in ATG-5 group and 17.5% of patients in ATG-10 group (*n.s.*). Positive CMV antigenemia was detected in 44.7% of patients in ATG-5 group and 38.5% of patients in ATG-10 group. EBV-reactivation treated by rituximab was observed in 6 (7%) of patients in ATG-5 group and 13 (9%) of patients in ATG-10 group. Cumulative incidence of primary and late graft failure at 2 years was 7.6% in ATG-5 group and 12.5% in ATG-10 group (*n.s.*). Ten patients died in ATG-5 group, and the causes of death were as follows; primary or late graft failure (2), organ failure (2), infection (1), acute GVHD (1), hepatic VOD (1), ARDS (1), secondary cancer (1). Nineteen patients in AATG-10 group died, and the causes of death were as follows; Infection (8), organ failure (4), primary or late graft failure (3), ARDS (1), TMA (1), chronic GVHD (1), secondary cancer (1). Overall survival rates at 5 years after BMT were 85.7% in ATG-5 group and 89.0% in ATG-10 group (*n.s.*). Event-free survival rates at 5 years after BMT were 86.0% in ATG-5 group and 83.5% in ATG-10 group (*n.s.*).
Discussion: There were no statistically differences between ATG-5 group and ATG-10 group in the outcome of allogeneic BMT in this study. However, 5 mg/kg of rATG is preferable because comparable outcomes were obtained in ATG-5 group that included large proportion of unrelated donor BMT.
Disclosure of Interest: None Declared.

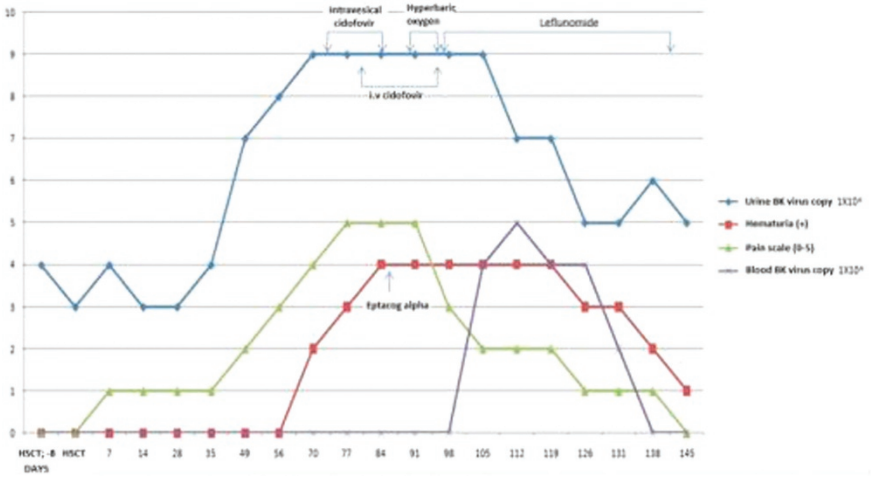
PH-P582

LEFLUNOMIDE TREATMENT FOR THE BK VIRUS-ASSOCIATED HEMORRHAGIC CYSTITIS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION RECIPIENTS: REPORT OF TWO PEDIATRIC CASES FROM A SINGLE-CENTRE

O. Gursel[1,], I. Eker[1], O. Babacan[1], H. Cem Irkilata[2], B. Karaman[3], O. Koc[4], A. Emin Kurekci[1]*
[1]Pediatric Hematology, [2]Urology, [3]Radiology, [4]Pediatrics, Gulhane Military Medical Faculty, Ankara, Turkey

Introduction: There isn't specific antiviral drug with proven efficacy against BK virus (BKV) replication and for BKV hemorrhagic cystitis (HC). Leflunomide is an immune suppressive drug with antiviral activity. To the best of our knowledge, these are the first reported cases of a pediatric BKV-HC patients, who received leflunomide therapy
Materials (or patients) and Methods: Case 1. A 14 years-old male patient with relapsed ALL, underwent haploidentical HSCT from his 49 years old mother. Conditioning consisted of total body irradiation (TBI), thiotepa, melphalan and rabbit ATG. 4.5 x 10^6/kg CD34+ stem cells were transplanted. There were no BKV HC, but he underwent 2th haploidentical HSCT from his 23 years old sister on the 50th day, because of primary graft failure. Cyclophosphamide, rabbit ATG and fludarabine given for conditioning. Full engraftment was achieved. Neutrophil, platelet and erythrocyte recovery were 21 days, 30 days and 35 days respectively after the 2th HSCT. On the 24th day of the 2th HSCT (74th day of the first HSCT), urine BKV loads increased to 9×10^9 copies/ml with HC. There were no improvement in BKV HC despite bladder irrigation, intravesical and i.v cidofovir, eptacog alpha, hyperbaric oxygen respectively; also despite nephrostomy and cystostomy because of pelviectasis. Oral leflunomide therapy was started with a dosage of 40 mg/day, on the 105th day of HSCT, after the increase of viral BKV load to 4×10^5 copies/ml in blood also. After three days of this dosage, it is reduced to 20 mg/day. On the 10th day of leflunomide therapy, his symptoms, especially pain and sypmtoms resolved, viral BKV was negative in blood and 10^5 copies/ml in urine (Figure).
Case 2. A 15 years-old male patient with CML underwent matched unrelated donor HSCT after his second remission. Conditioning consisted of TBI, rabbit ATG and cyclophosphamide. 5.8 x 10^6/kg CD34+ stem cells were transplanted. Full engraftment was achieved with neutrophil, platelet and erythrocyte recovery being 16 days, 17 days and 23 days after the HSCT respectively. On the 15th day of the transplantation, urine BKV loads increased to 9×10^9 copies/ml with HC. He received the same therapies of case 1, but there were no improvement. Oral leflunomide therapy was started with the same dosage on the 43th day of HSCT but discontinued after two weeks, because of no improvement in BKV HC. Bladder irrigation went on and he was discharged on the 94th of HSCT, after the resolution of his signs and symptoms. Viral BKV load was negative in blood throughout the HSCT and decreased to 5×10^8 copies/ml in urine at his discharge.
Results: No advanced GVHD occurred when leflunomide was administered and no significant side effects were observed during leflunomide treatment in both of two cases.
Discussion: Based on the satisfactory effects of leflunomide in treating BKV-associated nephropathy after renal transplantation, it is inferred that it might be effective for treating BKV-HC. Prospective, randomized control studies are needed to confirm its efficacy, but it may worth trying leflunomide in pediatric HSCT recipients, especially who are refractory to first line treatments.
Disclosure of Interest: None Declared.

[PH-P582]



PH-P583

A TOTAL LYMPHOID IRRADIATION (TLI) BASED REDUCED INTENSITY CONDITIONING REGIMEN FOR CHILDREN AND ADOLESCENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANT FOR HIGH-RISK NON-MALIGNANT DISORDERS

A. Ahmed Hussein[1,], A. Al-Mousa[2], A. Al-Zaben[1], H. Frangoul[3]*
[1]Bone Marrow and Stem Cell Transplantation, [2]Radiotherapy, King Hussein Cancer Center, Amman, Jordan, [3]Pediatric Blood and Marrow Transplant, Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville, United States

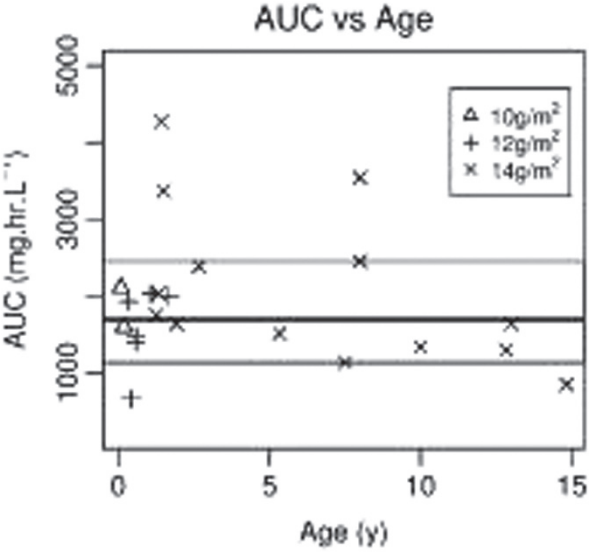
Introduction: Hematopoietic stem cell transplant (HSCT) can benefit with various non-malignant diseases but is limited by regimen-related toxicity especially in high risk patients, graft-versus-host disease (GVHD), donor availability, and graft rejection. To overcome some of these barriers, we adopted a novel conditioning strategy for these patients.
Materials (or patients) and Methods: Between 2004 and 2013, 37 patients with high-risk non-malignant hematological disorders underwent allogeneic HSCT using a standard institutional protocol. All patients received preparative regimen consisting of oral busulfan 2 mg/kg given every 12 h for two days from days -8 to -7, fludarabine 35 mg/m^2 infused over 1 h daily for 5 days from days -6 to -2, horse antithymocyte globulin (ATG) 30mg/kg infused over 12 h daily for 5 days from days -6 to -2 was used in the first 10 patients, while thymoglobulin at 2.5 mg/kg once daily for 3 consecutive days was used in the rest, and total lymphoid irradiation administrated as a single fraction of 500 cGy on day zero prior to stem cell infusion for all patients. Cyclosporine and mycophenolate mofetil were used for GVHD prophylaxis. All donors were fully HLA-matched, 33 (89%) were HLA identical siblings and 4 (11%) were other family members (3 parents, and one cousin). Mobilized peripheral blood stem cells through 5 days of subcutaneous injections of GCSF at 5µ/kg/dose twice daily was used in all donors
Results: Thirty-seven consecutive patients with a median age of 13 year (range, 0.3-25) were treated. Twenty-nine patients had high risk class 3 thalassemia major, 3 congenital pure red cell aplasia, 3 sickle cell diseases, one infantile osteopetrosis, and one autoimmune lymphoproliferative syndrome. All patients had significant morbidity before transplantation, including 10 patients with hepatitis C infection. All patients received peripheral blood stem cells, with median CD34 count of 4.9 x10^6 /kg recipient weight (3.5-8.2). All patients achieved neutrophil and platelet engraftment at a median time of 15 days (11-24) and18 days (15-74). There was no regimen related mortality. The median hospitalization period was 35 days (23-81). No patient required intensive care unit admission in the first 100 days following transplantation. Sinusoidal obstruction syndrome (SOS) developed only in 2 patients (5%), both with thalassemia major and hepatitis C infection. Both were mild and treated with conservative and supportive measures. None of our patients developed CMV disease. A total of 4 patients (11%) developed grade 2 acute skin GVHD, all were responded to first line therapy. Chronic GVHD developed in 3 patients (8%), all were limited and resolved with first line treatment. Four patients (11%) had secondary graft failure (GF) between 9-60 months post HSCT, all have thalassemia major. Three of them are doing well now following second RIC HSCT. At a median follow up of 24 months (range 3-110) the probability of overall and event-free survival is 100% and 89%, respectively.
Discussion: We conclude that this TLI based regimen was well tolerated and resulted in excellent overall and event free survival in high-risk patients with non-malignant diseases.
Disclosure of Interest: None Declared.

PH-P584

PHARMACOKINETICS OF HIGH DOSE INTRAVENOUS TREOSULFAN IN CHILDREN PRIOR TO ALLOGENEIC HCT

R. Chiesa[1,], R. Winter[2], Z. Nademi[3], J. Standing[4], P. Amrolia[1], P. Veys[1], J. Silva[1], O. Nikolajeva[1], H. Prunty[2], S. Heales[2], A. Gennery[5], M. Slatter[5]*
[1]Bone Marrow Transplantation Department, [2]Chemical Pathology Department, Great Ormond Street Children Hospital, London, [3]Bone Marrow Transplantation, Great North Children's Hospital, Newcastle upon Tyne, [4]Pharmacy Department, Great ormond Street Children Hospital, London, [5]Bone Marrow Transplantation Department, Great North Children's Hospital, Newcastle upon Tyne, United Kingdom

Introduction: Treosulfan is a bifunctional alkylating drug with a structure similar to Busulfan. It is increasingly used in children prior to allogeneic haematopoietic cell transplantation (HCT). Pharmacokinetic (PK) data on Treosulfan in children is limited: we describe the PK profile of Treosulfan in 22 children undergoing HCT in the UK.
Materials (or patients) and Methods: Twenty-two children who underwent HCT in Newcastle and London between 2012 and 2013 were analysed as part of a pilot Treosulfan PK study. Treosulfan was administered iv over 2 hours at a dose of 10 (age < 3 months), 12 (age 3 months-1 year) or 14 (age > 1 year) g/m^2/day for 3 consecutive days. Treosulfan concentration in plasma was determined at different time points by a validated RP-HPLC method with refractometric detection. The non-compartmental AUC (0-∞) was calculated for each subject, and the data was modelled with the population PK software NONMEM.
Results: Median age at HCT was 18 months (range 1-170). Indication to HCT was: primary immunodeficiency (15), inflammatory bowel disease (4), relapsed JMML (2) and osteopetrosis (1). Median weight was 10 kg (range 3.76-55.5). Conditioning was Treosulfan and Fludarabine 150 mg/m^2 (22) + Thiotepa 10 mg/m^2 (2). Alemtuzumab was given to 18 children. Donors were: matched unrelated (8), 1-antigen mismatched unrelated (7), matched sibling (5), matched/mismatched family (2). Median CD34+ cell dose was 10.1 x 10^6/kg (range, 0.37-30.8). Median follow-up was 7 months (range, 1-12). Neutrophil and platelet recovery occurred at a median of 19 (range, 8-38) and 18 (range, 5-41) days, respectively. All patients achieved donor engraftment; 4 children had mixed donor engraftment in their T-cells (range: 41% to 73% donor) while one child had low level donor engraftment in the myeloid compartment (9% donor). 20/22 patients are alive at last follow up; 2 children died of viral pneumonitis. Ten patients developed grade II-IV acute graft-versus-host disease (aGvHD: *n*=8 grade II, *n*=2 grade III-IV) and one patient limited chronic GVHD. Regimen-related toxicity (RRT) was low, with the exception of severe



dermatitis (1), VOD (1), cyclosporine-induced neurotoxicity (2) and severe mucositis (1). The 2-compartmental model provided best fit with a clearance of 12.6 L/h/70kg and a Vss of 34.5 L/70kg. Terminal half life of Treosulfan was in the range of 0.89-2.16 h (median= 1.46 h). The median Treosulfan AUC was 1694 mg.h/L and 75% patients had an AUC in the range of 1130-2451 mg.h/L. Notably 2/3 patients with the highest AUC were affected by HLH, with previous hepatomegaly and/or liver derangement. A higher Treosulfan AUC did not seem to translate into increased RRT. One child who underwent a second HCT for relapsed JMML experienced VOD; his Treosulfan AUC was at the higher limit of the range (2381 mg.h/L). Interestingly, the child with the lowest AUC (659 mg.h/L) experienced the lowest level of donor myeloid engraftment.
Discussion: Treosulfan-based conditioning in children undergoing HCT is characterized by low RRT and good donor engraftment. Treosulfan AUC seems consistent across age related dose range, supporting a dose reduction in younger children. A higher AUC did not correlate with increased toxicity, while a low systemic exposure to Treosulfan might negatively impact on myeloid engraftment. This data needs to be confirmed in a larger population of children, within the ongoing prospective UK Treosulfan PK study.
Disclosure of Interest: None Declared.

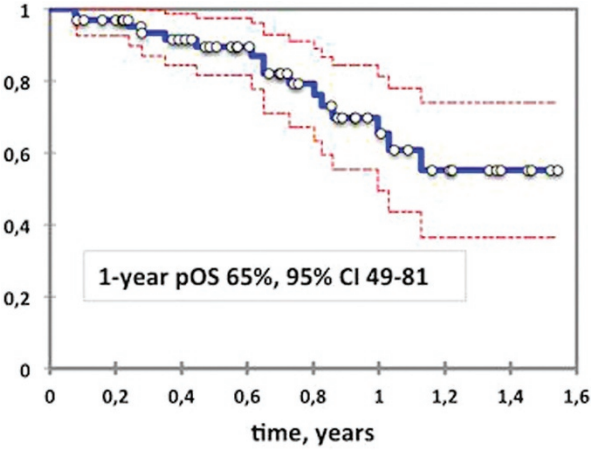
PH-P585

TCR-ALPHA/BETA+/CD19+-DEPLETION IN HEMATOPOIETIC STEM CELLS TRANSPLANTATION FROM MATCHED UNRELATED AND HAPLOIDENTICAL DONORS IN PEDIATRIC ACUTE LEUKEMIA PATIENTS

M. Maschan[1,], L. Shelikhova[2], O. Tatarinova[1], D. Balashov[1], E. Kurnikova[3], E. Boyakova[1], V. Bobrynina[4], Y. Skvortsova[1], M. Ilushina[1], I. Shipitsina[1], D. Shasheleva[1], Z. Shekhovtsova[1], G. Novichkova[5], A. Maschan[1]*

[1]Hematopoietic stem cell transplantation, Federal Center for Pediatric Hematology, [2]Hematopoietic stem cell transplantation, Federal Center for pediatric hematology, [3]Transfusiology, [4]Molecular biology, [5]Hematology, Federal Center for Pediatric Hematology, Moscow, Russian Federation

Introduction: Graft-versus-host disease (GvHD) and GVHD-associated mortality is one of the major obstacles to success of transplantation from unrelated and haploidentical donors. Negative depletion of α/β(+) T cells and CD19+ B lymphocytes is a promising new technology of graft manipulation with a potential to improve GvHD control and immune reconstitution.
Materials (or patients) and Methods: A total of 64 pediatric patients with acute leukemia (33 AML, 31 ALL, 19 female, 45 male, median age 8.8 years) underwent allogeneic HSCT between May 2012 and august 2013. Twenty five - from haploidentical donor and 39 from matched unrelated donor. Preparative regimen included Fludarabine 150 mg/m^2, Treosulfan 42 g/m^2, Melphalan 140 mg/m^2 and ATG (horse, ATGAM) 50 mg/kg. PBSC were depleted of TCR-alpha/beta and CD19+ B-cell according to manufacturer's recommendations. The median dose of infused CD34+ cells was 9.1x 10^6/kg (range 3.9-21.6), TCRa/b 18.6 x10^3/kg (range 1-305). Twelve pts received no GvHD prophylaxis after HCT, tacrolimus (tacro) and short methotrexate (Mtx) were used for 43 pts, tacro 3 pts, Mtx 6 pts. Patients were divided in 3 groups according to remission status: CR1(31pts), CR>1(25pts), active disease (AD) (8pts).
Results: Primary engraftment was achieved in 63 of 64 pts, the median time to neutrophil and platelet recovery was 16 and 15 days, respectively. Cumulative incidence of acute GvHD grade > II was 43% (95% CI: 32-58), grade III – 16% (95%CI: 9-32), no case of grade IV aGvHD was observed. No correlation between graft composition and aGVHD was noted. Early mortality was low with a 100-day pTRM - 1.6% (95%CI: 0,2-10). Cumulative incidence of relapse at 1 year was 33% (95%CI: 21-50), 1-year pTRM – 15% (95%CI: 6-35). After a median follow-up of 6.8 months (0.8–15.4), 1-year pEFS was 52% (95%CI: 36-58), 1-year pOS - 65% (95%CI: 49-81). There was no significant difference in survival and relapse rate according leukemia subtype and donor type. Disease status at HSCT significantly influenced the relapse rate: cumulative incidence of relapse was 17% in CR1 patients, 50% in CR>1 and 55% in AD, Grey test $P = 0.03$.
Discussion: We confirm that depletion of TCR-alpha/beta and CD19 lymphocytes from the graft ensures high engraftment rate and low transplant-related mortality. All major outcomes were equivalent between transplantation from unrelated and haploidentical donor. Improvement of anti-leukemic activity will require further refinement of preparative regimen and post-transplant strategy of disease control.
Disclosure of Interest: M. Maschan Conflict with: Lecturing for Miltenyi Biotec, L. Shelikhova: None Declared, O. Tatarinova: None Declared, D. Balashov: None Declared, E. Kurnikova: None Declared, E. Boyakova: None Declared, V. Bobrynina: None Declared, Y. Skvortsova: None Declared, M. Ilushina: None Declared, I. Shipitsina: None Declared, D. Shasheleva: None Declared, Z. Shekhovtsova: None Declared, G. Novichkova: None Declared, A. Maschan: None Declared.



PH-P586

HAPLOIDENTICAL STEM CELL TRANSPLANTATION IN CHILDREN WITH ACUTE LEUKEMIA – REMISSION STATUS AT TRANSPLANTATION PREDICTS OUTCOME

D. Turkiewicz[1,], J. Dykes[2,3], A. N Bekassy[1], S. Lenhoff[4], J. Toporski[1]*

[1]Department of Pediatric Hematology and Oncology, [2]Clinical Immunology and Transfusion Medicine, Skåne University Hospital, [3]Division of Hematology and Transfusion Medicine, Lund University, [4]Department of Hematology, Skåne University Hospital, Lund, Sweden

Introduction: Allogeneic stem cell transplantation is an established treatment of children with relapsing/refractory acute leukemia. For patients lacking a HLA identical sibling donor, transplantation with a haploidentical donor (haploSCT) is a possible alternative. However, the need for T-cell depletion to prevent GvH disease leads to prolonged T-cell recovery, which in turn may increase the risk of relapse or infectious mortality. On the other hand, HLA disparity between the donor and the recipient may also result in a graft vs. leukemia effect (GvL) mediated by cells other than αβ+ T-lymphocytes.
Materials (or patients) and Methods: We present results of haploSCT in 16 children with either acute lymphoblastic (ALL, *n*=12) or acute myeloblastic leukemia (AML, *n*=4). Twelve patients were in remission prior to transplantation (high risk T-ALL in delayed CR1:1, CR2 after early/very early bone marrow relapse:7, late bone marrow relapse with delayed CR2: 1, CR>3:3). Four patients were transplanted while not in CR. Toxicity-reduced conditioning was used (clofarabine 4x50mg/m^2 or fludarabine 5x25mg/m^2, thiotepa 2x5mg/kg, melphalan 2x60mg/m^2). Rejection prophylaxis consisted of either OKT3 (*n*=7) or ATG-F (3x10mg/m^2 day -11 to -9, *n*=9).To prevent GvHD immunomagnetic depletion of either CD3+

cells (*n*=8) or TCR αβ+ lymphocytes (*n*=8) was used (CliniMACS, Miltenyi Biotec). To prevent EBV-PTLD, a single dose of Rituximab 375mg/m^2 was given on day +1. Eight patients received additional short course of MMF (until day +28). Seven patients received low-dose donor lymphocyte infusion (DLI), either due to mixed chimerism or to boost T-cell recovery.
Results: Graft rejection occurred in one patient (6%), who was successfully rescued by subsequent haploSCT with another donor. GvHD II-IV occurred in 8 patients, however in 4 after DLI. Extensive cGvHD was observed only in 2 patients, in both after DLI. Seven patients are alive in CR with a median follow up of 400 days with EFS of 0.5 (95% CI: 0.3-0.85). All patients transplanted without remission died due to disease progression (*n*=4). None of the patients transplanted in CR has relapsed so far, 3 died due to TRM (AdV hepatitis: 1, post-DLI, GvHD: 2). EFS for patients transplanted in CR was 0.7 (0.45-1). Cumulative incidence of TRM reached 0.2.
Discussion: Haploidentical stem cell transplantation is a feasible treatment option for children with acute leukemia who achieve remission prior to transplantation. Even low-dose DLI after haploSCT may result in severe life threatening GvHD.
Disclosure of Interest: None Declared.

PH-P587

RETRANSPLANTATION WITH HAPLOIDENTICAL T CELL DEPLETED GRAFTS AFTER 7 GRAY (GY) TOTAL NODAL IRRADIATION (TNI)-BASED RECONDITIONING AFTER GRAFT FAILURE IN PEDIATRIC PATIENTS

H.-M. Teltschik[1,], F. Heinzelmann[2], T. Feuchtinger[1], R. Handgretinger[1], P. Lang[1]*
[1]Pediatric Hematology and Oncology, University Children`s Hospital Tübingen, [2]Radiation Oncology, University Hospital Tübingen, Tübingen, Germany

Introduction: Graft failure is a rare but life-threatening complication after hematopoetic stem cell transplantation. Treatment comprises immunoablative reconditioning regimens and a second stem cell donation as soon as possible to minimize the time of pancytopenia and its sequelae.
Materials (or patients) and Methods: We report a cohort of 19 pediatric patients with acute leukemias (lymphatic *n*=9, myeloic *n*=5), myelodysplastic syndrome (*n*=1), immunodeficiencies (*n*=2), paroxysmal nocturnal hemoglobinuria (*n*=1) and severe aplastic anemia (*n*=1) who experienced graft failure (nonengraftment *n*=2; rejection *n*=17) after TBI, busulphan or melphalan based myeloablative transplantation from mismatched related donors (*n*=16) or matched unrelated donors (*n*=3). All patients were retransplanted with T cell depleted grafts (CD34 positive selection *n*=1, CD3/CD19 depletion *n*=15, TCRαβ/CD19 depletion *n*=3) from haploidentical donors.
Results: Median time from diagnosis of graft rejection to second transplantation was 14 days (range 7-40). Reconditioning regimens consisted of total nodal irradiation 7 (Gy), fludarabine (120mg/m^2) in combination with thiotepa (5mg/kg) or cyclophosphamide (60mg/kg body weight (bw)) and ATG/OKT3. 16 patients received CD3/CD19 depleted (*n*=15) or CD34 positive selected grafts (*n*=1) grafts with a median of 20x10^6 CD34+ cells/kg and 69,5x10^3/kg residual CD3+ T cells. 3 Patients received TCRαβ/CD19 depleted with a median of 16.8x10^6 CD34+ cells/kg and 39375x10^3/kg CD3+ T cells. Mofetilmycophenolat was given as Graft vs. Host Disease (GvHD) prophylaxis, if residual T cells exceeded 25 000/kg bw. Sustained engraftment was achieved in 18 out of 19 patients. One patient died before engraftment. Median time to absolute neutrophile counts above 500/µl was 10 (9-32) days. Independence from platelet substitution was reached at a median time of 10 (8-22) days. 11% developed GvHD °III, 11% developed GvHD °II, 28% of all patients developed GvHD °I and 50% had no signs of GvHD. Severe organ toxicity was observed in 5 patients (bronchiolitis obliterans *n*=1, hemorrhagic cystitis *n*=2, leukencephalopathy *n*=2). Event free survival (EFS) of all patients at 2 years was 63%. 2 year EFS of patients with leukemias in complete remission was 75%. Patients with non-malignant diseases had 5 year EFS of 80%. Transplant related mortality at one year was 11%. Causes of death were: multi organ failure (*n*=2) and 4 patients with acute leukemias died of relapse. None of the patients rejected the second graft.
Discussion: Thus, retransplantation from haploidentical donors with T cell depleted grafts after 7 Gy TNI based reconditioning is a realistic option to rescue patients with graft failure within a short time span and for whom a second stem cell donation of the original donor is not available. The use of TNI before retransplantation from a different donor may help to avoid a second rejection.
Disclosure of Interest: None Declared.

PH-P588

AUTOIMMUNE HEMOLYTIC ANEMIA AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: REVIEW OF NINE CASES

D. Bueno[1], P. M. Rubio[1], B. Gonzalez[1], S. Riesco[1], D. Corral[1], T. Alonso[1], D. Plaza[1], A. Sastre[1,]*
[1]Hematology-oncology, Hospital La PAz, Madrid, Spain

Introduction: Immune-mediated post hematopoietic stem cell transplant (HSCT) cytopenias comprise a rare and severe group of diseases. They are more frequent following allogeneic HSCT, although they are also described in the autologous setting. Immune cytopenias respond poorly to standard treatment and are associated with a high rate of morbidity and mortality. The most common presentation is haemolyitic anemia, followed by Evans Syndrome (immune thrombocytopenia plus haemolyitic anemia).
Materials (or patients) and Methods: we have searched for haemolytic anemia episodes among pediatric patients who underwent HSCT in our center between years 2004 and 2011. Of each patient, data concerning primary disease, type of transplant, stem cell source, conditioning treatment, graft versus host disease (prophylaxis regimen, and clinical features and treatment when applicable), were collected; and laboratory parameters, treatment administered and outcome of each haemolytic anemia episode were also recorded.
Results: Out of 102 allogeneic HSCT performed in our center between 2004 and 2011, 9 cases of haemolytic anemia were found. Ages at the time of transplant ranged from 6 months and 12 years old. Of the 9 patients, 6 had a hematologic malignancy as primary disease (three acute lymphoblastic leukemias, two acute myeloblastic leukemias and one chronic myeloid leukemia); the 3 remaining patients had an immune deficiency. Three patients had an HLA-identical sibling donor, another two an HLA-identical non related donor, three a mismatched unrelated donor, and one patient received a haploidentical transplant. Peripheral blood apheresis was the stem cell source in 4 patients, umbilical cord blood in 3, and bone marrow in 2. Only two patients received a reduced intensity conditioning. Graft manipulation (T cell depletion) was performed in two cases. All patients engrafted, with full chimerism achieved in 8 patients. GVHD prophylaxis was performed with cyclosporin in 7 patients; 2 received also methotrexate, 2 mycophenolate mofetil, and 1 prednisone. Five patients had acute GVHD. Haemolytic anemia was developed in the early postransplant period, with the exception of a patient who had an episode 3 years after HSCT. All of them had increased bilirubin and LDH, decreased haptoglobin leves, and a positive Coombs test, IgG positive in 8 patients (the remaining case was passenger lymphocyte mediated). All patients were treated initially with steroids, adding immunoglobulin in five of them. Three patients received rituximab. Of the nine patients, six required intensive care unit admission. Three patients died due to direct complications of the process; the remaining six patients had a full recovery of the anemia.
Discussion: our experience in post-HSCT haemolytic anemia shows that it is a severe disease. 66% of our patients required intensive care and mortality was of 33%. More than half of our patients required add-on treatment over steroids. All our patients had received transplant-related aggresive therapy, with myeloablative conditioning in 77% patients and immunosuppressive treatment in all of them; these measures often result in the depletion of the T-cell compartment, with a secondary dysregulation of

B lymphocytes, which is the most commonly accepted pathogenic mechanism of immune cytopenias.
Disclosure of Interest: None Declared.

PH-P589

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOLLOWING CONDITIONING WITH BUSULFAN, CYCLOPHOSPHAMIDE AND MELPHALAN IN CHILDREN WITH THERAPY RELATED MYELODYSPLASTIC SYNDROME

A. Yoshimi[1,], R. Ammann[2], W. Truckenmüller[3], B. De Moerloose[4], M. Dworzak[5], H. Hasle[6], M. Schmugge[7], J. Starý[8], M. van den Heuvel-Eibrink[9], M. Zecca[10], R. Masetti[11], P. Noellke[3], F. Locatelli[12], C. Niemeyer[3], B. Strahm[3] and of the European Working Group of MDS in Childhood (EWOG-MDS)*

[1]Department of Pediatrics and Adolescent Medicine, University of Freiburg, Freiburg, Germany, [2]University of Bern, Bern, Switzerland, [3]University of Freiburg, Freiburg, Germany, [4]Ghent University Hospital, Ghent, Belgium, [5]Medical University of Vienna, Vienna, Austria, [6]Aarhus University Hospital Skejby, Aarhus, Aarhus, Denmark, [7]University Children's Hospital, Zurich, Zurich, Switzerland, [8]Charles University and University Hospital Motol, Prague, Czech Republic, [9]Sophia Children´s Hospital, Erasmus Medical Center, Rotterdam, Netherlands, [10]Fondazione IRCCS Policlinico San Matteo, Pavia, Pavia, [11]University of Bologna, Bologna, [12]Bambino Gesu' Children's Hospital, Rome, Italy

Introduction: Therapy-related myelodysplastic syndrome (tMDS) is a serious late event for cancer survivors and carries a poor prognosis. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment, but there are limited reports. Here we report the outcome of HSCT with busulfan, cyclophosphamide and melphalan (Bu/Cy/Mel) regimen in 48 children (27 males, 21 females) with tMDS who were enrolled in the studies of the EWOG-MDS.
Materials (or patients) and Methods: The first malignancies were a hematological malignancy in 32 patients (23 ALL, 4 AML, 5 lymphoma) and a solid tumor in 16 patients. The median interval between the diagnosis of the first malignancy and tMDS and between the diagnosis of tMDS and HSCT was 40 (12–133) mo and 5 (1–45) mo, respectively. The median age at HSCT was 11.0 yrs (3.9–18.5 yrs). Chromosomal analysis revealed a normal karyotype (*n*=9), abnormalities of chromosome 7 +/- additional aberrations (*n*=14), a structurally complex karyotype (*n*=7), other (*n*=12) or no result (*n*=6). The highest WHO type before HSCT was refractory cytopenia of childhood (RCC) in 4 patients and advanced MDS in 44 patients. Donors were matched siblings (*n*=22), a matched family donor (*n*=1) or unrelated donors (*n*=25). Stem cell source was bone marrow (*n*=28) or peripheral blood (*n*=20).
Results: Neutrophil engraftment was achieved in 43 patients at the median 17 days (9-44) days after HSCT, one patient failed to engraftment and 4 patients died prior to engraftment (day 6-23). Grade II-IV and III-IV acute GvHD occurred in 20 and 9 patients (cumulative incidence: 48 and 21%) of 43 evaluable patients, respectively. Eleven of 38 patients at risk developed chronic GvHD (cumulative incidence: 30%) that was extensive in 5 patients. Relapse of tMDS and the primary malignancy after HSCT occurred in 15 patients and one patient, respectively.
With a median follow-up time for survivors of 4.9 years (0.9-13 years) after HSCT, the overall and event-free survivals at 5 years were 51% (35-67%) and 42% (27–57%), respectively, with no difference between matched siblings and other donors. The cumulative incidence of relapse and transplant related mortality (TRM) was 32% (21-49%) and 23% (14-39%) at 5 years, respectively. Causes of death (*n*=23) were relapse of tMDS (*n*=11), TRM (*n*=11) and relapse of the primary malignancy (*n*=1). Eight patients received a second HSCT for graft failure (*n*=1) and relapse of tMDS (*n*=7) and three of them are alive in remission. Patients with a tMDS following a solid tumor have a poor prognosis compared to patients with tMDS following a hematological malignancy (EFS: 13% vs. 55%, *P*<0.01) due to the high rate of TRM (cumulative incidence: 56% vs. 6%, *P*<0.01). Patients with a structural complex karyotype have a dismal prognosis (EFS: 0%).
Discussion: The outcome of patients with tMDS following a hematological malignancy is comparable to the outcome of patients with primary advanced MDS. Patients with a complex karyotype or a tMDS following a solid tumor require a novel therapeutic approach for cure.
Disclosure of Interest: None Declared.

PH-P590

A PILOT STUDY OF POST-TRANSPLANT DECITABINE IN CHILDREN WITH HEMATOLOGIC MALIGNANCIES

M. Maschan[1,1,], M. Ilushina[1], L. Shelikhova[2], D. Balashov[1], Y. Skvortsova[1], D. Shasheleva[1], E. Gutovskaya[1], V. Tsetlina[1], R. Khismatullina[1], G. Novichkova[3], A. Maschan[1]*

[1]Hematopoietic stem cell transplantation, Federal Center for pediatric hematology, [2]Hematopoietic stem cell transplantation, Federal Center for pediatric hematology, [3]Hematology, Federal Center for Pediatric Hematology, Moscow, Russian Federation

Introduction: Relapse of malignant disease remains the most important cause of failure after allogeneic transplantation for hematologic neoplasms in children. Hypomethylating agents are being studied as candidate agents for post-transplant maintenance therapy in adult leukemia patients. We report the results of a pilot study of decitabine safety in children with hematologic malignancies after allogeneic HSCT.
Materials (or patients) and Methods: Thirty two patients received 131 course of decitabine between 30.05.2012 and 13.11.2013. Cohort included 20 boys and 11 girls, median age 7,3(0,9-22) years. Thirteen patients had acute myeloid leukemia (CR1-9, CR2-2, AD-2), 12 – acute lymphoblastic leukemia (CR1-5, CR2-3, CR>2-4), 1 –biphenotypic leukemia, 6 – juvenile myelomonocytic leukemia. All patients got uniform conditioning with total doses of treosulfan 42g/m^2, horse ATG (ATGAM) 50 mg/kg, melphalan 140 mg/m^2, fludarabine 150 mg/m^2. Fifteen patients were transplanted from matched unrelated donors, 10 – from haploidentical donors, 6 – from matched sibling donors. Decitabine courses were planned to start at 10mg/m^2 after day +30 as soon as WBC reached >1x10^3/l, ANC >0,5x10^3/l, PLT >30x10^3/l, Hb >95 g/l. Courses were to be administered at 30-45 day intervals with the same starting criteria. Mild aGVHD and basic GvHD prophylaxis did not preclude therapy.
Results: Median time from HSCT to first course was 47 (31-127) days. Median number of decitabine courses was 4 (2-6). Hematologic toxicity included grade IV neutropenia in 35%, grade III in 33%, grade II in 19%; grade IV thrombocytopenia in 7,6%, grade III in 7%. Only 9 platelet and 13 RBC transfusions were required over all courses. Hepatic toxicity included grade II ALT/AST elevation in 16,8%. Renal toxicity – transient grade I azotemia in 13%. Infections complicated therapy in 22 patients. Overall grade I infections developed during 12,2% courses, grade II in 12,2%, grade III (requiring inpatient care) – in 7.6%. Microbiologically documented infections included one case of P.aeruginosae blepharitis and two cases of gram-positive bactremia. Ten of the 32 patients experienced disease relapse, cumulative incidence of relapse being 40% (95%CI: 23-68).
Discussion: Decitabine can be administered to children on outpatient basis post-transplant with mostly moderate hematologic and mild visceral toxicity. Efficacy of post-transplant decitabine can be safely evaluated in a prospective controlled trial.
Disclosure of Interest: None Declared.

PH-P591

A SINGLE CENTER EXPERIENCE WITH ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACQUIRED SEVERE APLASTIC ANAEMIA IN CHILDHOOD

N. Eker[1], F. Tayfun[1], A. Yasar[1], P. Hos[1], Ö. Dogru[1], V. Hazar[2], E. Guler[1,], M. Yesilipek[3], O. Kupesiz[1]*

[1]Department of Pediatrics, Div. of Hematology-Oncology, Akdeniz University School of Medicine, Antalya, [2]Department of Pediatrics, Division of Hematology-Oncology, Medipol University, [3]Department of Pediatrics, Division of Hematology-Oncology, Medicalpark Hospital, Istanbul, Turkey

Introduction: Severe aplastic anaemia (SAA) is a bone marrow failure disorder characterized by pancytopenia and hypocellular bone marrow. SAA is a rare disorder which has a fatal course when allogeneic hematopoietic stem cell transplantation (HSCT) or an immunosuppressive therapy (IST) is not applied. SCT is the only curative treatment.

Materials (or patients) and Methods: This study included 26 patients (18 boys, 8 girls) with SAA and 29 transplantations because three of these patients received second transplantation. HSCT was performed between 2002 and 2013. The median age at HSCT was 11.1 years (range 2.4- 18.6 years). Before HSCT 16 (59.2%) patients received IST with antithymocyte globulin (ATG). The median time from diagnosis to transplantation was 22.7 months (4.8 -119.2 months). Among the 29 donors there were 8 matched sibling donors (MSD), 15 matched unrelated donors (MUD), 6 matched family donors and 1 mismatched family donor. The stem cell source for transplantation included peripheral blood stem cells (PBSC, *n* = 17), bone marrow (BM, *n*= 10) and umbilical cord blood (UCB, *n*= 2).

Results: Twenty four of the 29 transplantation (82.7%) engrafted, with neutrophil recovery occurring at a median of post transplant 14.4 day (8-21 day). Twenty three of 29 transplantation (79 %) engrafted with thrombocyte at a median of post transplant 23.8 day (14-90 day). One patient had neutrophil engraftment, but he died before thrombocyte engraftment. Seven patients (26.9 %) had acute graft versus host disease (GvHD). Immunosuppressive therapy was administered for all patients. Three of them had successful treatment and complete remission without any finding. Two patients (7.6%) had chronic GvHD. Four patients (15.3%) had hemorhagic cystitis and two (7.6%) of 26 patients had veno-occlusive disease as complications of transplantation. Overall survival is 74%.

Discussion: For children with SAA who do not have a MSD, both IST and alternative donor (AD) HSCT are treatment options. Recently, updated European Group for Blood and Marrow Transplantation reports showed an 83% 5-year acturial survival rate for AD HSCT performed after 2004, even when adult patients were included; 92% 5-year survival was observed if patients received transplant within 2 years of diagnosis. In our study group, overall survival was 74%. Lower overall survival can be related to our small SAA group.

In summary, we think that if a suitable MUD can be found quickly, MUD HSCT may be a curative therapy for many patients with SAA in children.

Disclosure of Interest: None Declared.

PH-P592

HAPLOIDENTICAL HSCT MAY PROVIDE THE POTENTIAL OF ALLOREACTIVE NK CELLS IN NEUROBLASTOMA. RESULTS OF *IN VITRO* STUDIES ON NK SINGLE CELL CLONE LEVEL USING LAN-1 AND LS NEUROBLASTOMA CELL LINES WO/W GD2MAB REVEALING SIGNIFICANT IMPACT OF KIR R/L-MISMATCH

P. Schlegel[1,], M. Matela[1], A. Horrer[1], C. Kyzirakos[1], M. Pfeiffer[1], R. Handgretinger[1], P. Lang[1]*

[1]Hematology/Oncology, University Children's Hospital, Tübingen, Germany

Introduction: The treatment of relapsed high risk neuroblastoma in children is a challenge. Haploidentical stem cell transplantation provides a therapeutical platform for the transfer of expanded allogeneic NK cells. The combination with GD2 monoclonal antibody (CH14.18/CHO) treatment is likely to exceed antitumor activity. To investigate the alloreactive potential of NK cell clones with and without KIR receptor-ligand mismatch, we sorted and expanded defined NK subsets.

Materials (or patients) and Methods: 4 defined NK phenotypes from 3 healthy donors were sorted via FACSAria cell sorter. NK single cell clones were high efficiently expanded (6.0-6.6 logs) using K562mb15 4-1BBL feeder cells and IL-2. After 21-28 days of expansion, phenotype of NK cell clones was confirmed by FACS analysis. Cellular cytotoxicity as well as ADCC-mediating GD2mAB was measured in a 2 hour BATDA release assay against LAN-1 and LS. NK clones were matched by K562 lysis. NK phenotyping included CD56, CD16, CD158a, CD158b and CD158e. CD3 positive clones were excluded. LAN-1 ($bw4^+$, $cw3^+$, $cw4^-$) and LS ($bw4^+$, $cw3^+$, $cw4^+$) were well characterized by flow cytometry.

Results: Phenotype of NK cell clones was reliably defined by sorting procedure. LS showed significantly higher expression of NKG2D ligands and GD2. LAN-1 expressed significantly lower levels of HLA I per cell. $CD158a^+$ (R/L-mismatched) NK clones of all 3 donors lysed LAN-1 significantly higher without (P=0.0047) and with GD2mAB (P=0.0001) than $CD158b^+$ (no R/L-mismatched) clones whereas there was no significant difference in the lysis of LS by CD158a+ clones vs CD158b+ clones in all 3 donors without and with GD2mAB. Median specific lysis for LS 42.1% without and with GD2mAB 56% (E:T, 5:1) was significantly higher than median specific lysis of LAN-1 13.7% and with GD2mAB 35.8% (n=198; for both conditions P<0.0001). GD2 antibody enhanced specific lysis significantly in LAN-1 and LS (LAN-1 P<0.0001; LS P<0.0001) independent from phenotype in CD16+ NK cell clones. Increase of GD2mAB-mediated ADCC was significantly higher in LAN-1 than LS (P<0.0001).

Discussion: Here we could show that NK clones with R/L-mismatch exert a significant better lysis than clones without KIR-mismatch. This model provides evidence for NK mediated alloreactivity not only in leukemias but also in neuroblastoma cell lines. In general NK alloreactivity in neuroblastoma may be significantly influenced by KIR R/L-mismatch and should therefore be taken into account for donor selection strategies. *Ex vivo* expanded, highly activated alloreactive haploidentical NK cells could be used for NK cell transfer posttransplant in combination with GD2mAB to augment antitumor activity. NK cell clones can be expanded high efficiently with predefined KIR-receptor phenotype via FACS sorting. NKG2D expression seems to be superior for the induction of NK cell mediated lysis in this certain range of HLA I quantity, which would explain higher specific lysis for LS than LAN-1. The significantly higher increase of ADCC-mediated lysis may be due to significantly higher expression of GD2 on LAN-1 compared to LS tumor cells. An efficient NK cell clone expansion protocol has been established for further evaluation of NK alloreactivity in different tumor entities.

Disclosure of Interest: None Declared.

PH-P593

HLA MISMATCHED ALTERNATIVE DONOR SCT IN APLASTIC ANEMIAS AND REFRACTORY CYTOPENIAS IS SAVE AND FEASIBLE USING A REDUCED INTENSITY CONDITIONING AND A T- AND B-CELL DEPLETED GRAFT BUT REQUIRES INTENSIVE IMMUNE ABLATION

T. Feuchtinger[1,], H.-M. Teltschik[1], M. Pfeiffer[1], I. Müller[2], A. Schrauder[3], F. Heinzelmann[4], R. Handgretinger[1], P. Lang[1]*

[1]Pediatric Hematology/Oncology, University Children's Hospital, Tübingen, [2]Pediatric Hematology/Oncology, University Hospital Eppendorf, Hamburg, [3]Pediatric Hematology/Oncology, University Hospital Kiel, Kiel, [4]Department of Radio-Oncology, University Hospital, Tübingen, Germany

Introduction: Allogeneic stem cell transplantation is still the curative treatment option of choice for the majority of patients with severe aplastic anemia and refractory cytopenias. However, a HLA-matched donor is not available for all patients. Alternative donor transplantation has been an experimental treatment option, limited by high rejection rates and transplant related mortality.

Materials (or patients) and Methods: We performed a prospective clinical trial to evaluate the safety and feasibility of haploidentical stem cell transplantation, since haploidentical family members are always available donors. We investigated a cohort of 10 pediatric patients with severe aplastic anemia or myelodysplatic syndrome (refractory cytopenia) transplanted with T-cell depleted grafts between 2004 and 2011.
Results: 7 patients had myelodyslastic syndrome with refractory cytopenia (MDS-RC), 3 had severe aplastic anemias (SAA) refractory to immunosuppressive treatment. 3 patients received a 2nd SCT after rejecting the graft from matched donors. Median age was 11.4 years. Standard conditioning regimen consisted of Fludarabin 3-4x40 mg/m^2, Thiotepa 1-3x5 mg/kg, Melphalan 2x70 mg/m^2 (*n*=8) and serotherapy using OKT3 (*n*=5) and ATG (*n*=5). 8 patients received additional total lymphoid irradiation (TLI 7 Gy) to prevent graft rejection. In vitro graft manipulation was carried out by direct depletion using antiCD3/19 magnetic microbeads. A median number of 10.1x10^6 CD34$^+$ progenitor cells and 27x10^3 T-cells/kg body weight (BW) were transfused. Pharmacological GvHD prophylaxis (graft vs. host disease) was carried out with Mycophenolate until day 60, if residual T-cells in the graft exceeded 25000/kg BW. Primary engraftment occurred in all patients Median time to reach 500/µl neutrophiles was 9 days (9-11). Independence from platelet substitution was reached after 13 days (8-16). Three patients rejected the graft later on. 6/10 patients had no signs of acute GvHD or GvHD grade I, 2 patients had GvHD grade II. TRM at day +100 and after 1 year was 0% and 20%, respectively. After TLI no rejection was observed. Event free survival (EFS) at 3 years was 80%.
Discussion: Haploidentical SCT with T-cell depleted grafts is a therapeutic option for refractory cytopenias and severe aplastic anemia after nonresponse to immunosuppressive treatment if no HLA-matched donor is available. Recovery of neutrophiles and platelets were fast and TRM was low, even if retransplantation was necessary. Since spontaneous outcome of these conditions are poor, alternative donor SCT is a realistic option for these patients.
Disclosure of Interest: None Declared.

PH-P594

COMPARISON OF STATIC AND DYNAMIC NK CELL EXPANSION PROCEDURE USING UNTOUCHED AND CD3-DEPLETED PBMCS OF HEALTHY DONORS FOR POSTTRANSPLANT NK TRANSFER TO AUGMENT ANTITUMOR ACTIVITY WITH AND WITHOUT ADCC-MEDIATING ANTIBODY APPLICATION

P. Schlegel[1,], R. Klaffschenkel[2], C. Klose[1], R. Teltschik[1], A. Horrer[1], M. Matela[1], G. Siegel[2], C. Kyzirakos[1], M. Pfeiffer[1], R. Handgretinger[1], P. Lang[1]*

[1]Hematology/Oncology, University Children's Hospital, [2]Transfusion Medicine, Institute for Clinical and Experimental Transfusion Medicine, Tübingen, Germany

Introduction: GMP-grade NK cell expansion for clinical purpose has been demonstrated feasible and safe. In our project we compared conventional flask-based cell culture NK expansion with the NK expansion using an innovative flow-through bioreactor (Zellwerk®) from healthy volunteer donors and produced and applied 7 NK products in 2 patients post allogeneic hematopoietic stem cell transplantation.
Materials (or patients) and Methods: NK cells were expanded using 3 healthy donors' untouched isolated PBMCs and CD3-depleted PBMCs (100ml PB) under static and dynamic expansion conditions. Isolated PBMCs or CD3-depleted PBMCs were pooled with 100Gy irradiated K562mb15 4-1BBL feeder cells 1:10 and divided equally into two portions. One portion was seeded in cell culture flask (175cm^2) at a density of 1.1E6/ml and one portion was seeded in the bioreactor chamber containing an absolute volume of 50 ml. Harvest of cells was performed at day 15-17. Isolated PBMCs and the expanded NK products were well-characterized by FC. 2h BATDA release was performed against various targets including K562, LAN-1 wo/w GD2mAB. Additionally two patients received static expanded NK cell products without and with GD2mAB.
Results: NK cells were expanded 68-688 fold in 14-17 days. Conventional flask-based expansion revealed significant higher numbers of NK cells (350-688 fold) compared to bioreactor expanded NK cells (68-290 fold; *P*=0.0045) and untouched PBMC NK expansion reached significant higher NK cell count compared to CD3-depleted NK expansion of the same donor (*P*=0.008). Static and bioreactor expanded NKs showed high direct cellular cytotoxicity against all cell lines tested and showed excellent ADCC activity using GD2mAB (LAN-1; LS) and CD19mAB (MHH-4; Raji). There was no significant difference in viability of cells and NK phenotype but for functional properties static culture showed significant higher lysis than bioreactor expanded NKs (*n*=35; *P*=0.0178). The autologous NK expansions (*n*=7) post stem cell transplantation for NK transfer in two patients reached 64-5700fold expansion. The NK cell transfer of high numbers of NK cells (150-505E6 NK cells/kgBW) induced transient coughing and elevated temperature. Post transfer isolated PBMCs showed significantly increased direct and ADCC mediated cytotoxicity. CD69+ cells could be traced for several days after NK transfer. Patients' expanded NK cells showed the same phenotype and functional properties like NK cells expanded from healthy donors.
Discussion: The expansion of NK cells under static rather than dynamic culturing conditions seems to be superior in terms of total NK cell count and functional properties. Nevertheless static culturing requires higher effort of maintenance and therefore needs an every-day engagement, whereas the bioreactor facilitates to program feeding rate of expanding cells for several days ahead. Thus we will further optimize bioreactor NK expansion to reduce handcraft during expansion period. NK transfer of autologous expansion NK cells post allogeneic SCT was tolerated alone as well as combined with GD2mAB (single dose 20 mg/m^2) and is likely to enhance NK cell antitumor activity as it has been shown for various targets *in vitro*.
Disclosure of Interest: None Declared.