Cellular and gene therapies

R1242
Immunosuppressive modulation of T-cells by 5-azacytidine treatment
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Background: Demethylating agents like 5-Azacytidine (5-Aza) have entered treatment for myeloid dysplastic syndrome (MDS) and acute myeloid leukaemia (AML). Beyond the effect of differentiation, one suggested mechanism is that 5-Aza demethylates antigens such as Cancer-Testis Antigens (CTA) which will then be presented in order to induce immune response by immunocompetent cells. While a lot of attention was paid to the effects of 5-Aza in the myeloid cells, less is known about the impact of 5-Aza on immune cells. We therefore investigated the effects of 5-Aza on T cells.

Materials and methods: T-cells were isolated from buffy coats using magnetic beads. Cells were stimulated and cultured for 1 week in the presence of IL-2. Thereafter cells were treated with or without 5 μM 5-Aza or 20 μM for 48h. mRNA was isolated and used for cDNA synthesis. qPCR was done under standard conditionings for expression of IL-10, FoxP3, and TGF-beta normalized for GAPDH. T cells were analyzed by flow cytometry using the following antibodies: CD3, CD4, CD8, HLA-DR, FoxP3, CD 127, and CD25. Functional killing of an AML cell line (HL60) was measured using a LDH release assay.

Results: We observed a significant (P=0.03) growth delay of the 5-Aza treated T cells compared to untreated control cells. Only the highest dosage (20 μM) of 5-Aza increased the fraction of apoptotic cells. 5-Aza did not influence the expression of CD3, but caused a change in the distribution of T cell sub-populations: percentages of CD8+ T cells decreased from 46% to 35% whereas CD4+ T cells increased from 57% to 68%. When stained for activation marker HLA-DR, a reduced expression was observed after 5-Aza treatment (57%, 48%, and 44% respectively). By screening different mRNA levels we found that the mRNA expression of IL10, FoxP3, and TGF-beta was upregulated (P=0.02, P=0.003 and P=0.02 respectively). Strikingly, we also found an upregulation of CD4+FoxP3+ Treg cells from 4.8% to 9.6% to 10.9% respectively. In a cytotoxicity assay we observed a significantly reduced capability of Aza-treated T cells to lyse target cells (P=0.02).

Conclusions: Our data indicate that 5-Aza inhibits the activity of T cells by: 1. reducing the number of CD8+ T killer cells, 2. upregulating the subpopulation of Tregs as well as increasing the expression of inhibitory cytokines like IL-10. We therefore conclude that 5-Aza treatment may constrain the distribution and function of immune cells.

R1243
Dendritic-cell based tumour vaccination in prostate and renal cell cancer: systematic review and meta-analysis
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Background: So far more than 200 clinical trials have been performed using dendritic cells (DC) as cellular adjuvants in cancer. Yet the key question whether there is a link between immune and clinical response remains unanswered and debates about optimal DC type, antigen delivery, or route of vaccination are ongoing. Prostate and renal cell cancer are considered susceptible to immunotherapy and have been extensively studied as targets for DC-based immunotherapeutic interventions. We therefore identified them as an ideal scenario to address the above question by means of a systematic review.

Methods: We conducted a systematic literature search in the Medline database. Clinical trials using dendritic cells in prostate and renal cell cancer (RCC) that enrolled at least 6 patients were included. If available and possible, individual patient data were collected and systematically analysed.

Results: We identified 24 trials in prostate cancer (15) and RCC (9) involving 445 adult patients. DC vaccination led to a tumor antigen-specific cellular immune response in 78% of patients with prostate cancer, and 55% of patients with RCC. In prostate cancer, 7.7% of the patients had an objective response (CR, PR, and MR). The combined percentages of CR, PR, MR, and SD for all prostate cancer patients summed a clinical benefit rate (CBR) of 54%. In RCC an objective response rate of 9.9% and CBR of 42% were detected. Meta-analysis of available individual patient data (n=348) revealed the cellular immune response to have a significant influence on CBR, both in prostate cancer (OR = 10.6; 95% CI = 2.5 to 44.1) and in RCC (OR = 8.4; 95% CI = 1.3 to 53.0). Furthermore, DC dose was found to have a significant influence on the CBR in both tumor entities. In prostate cancer patients, a positive influence on CBR was identified: first, for a non-intravenous (i.d., i.l., s.c., i.n) compared to intravenous administration; and second for, the use of mature monocyte-derived DC compared to immature monocyte-derived DC.

Conclusion: The systematic review found a strong heterogeneity regarding vaccine dose, DC type, antigen delivery, route of vaccination and quality controls. Nevertheless, we were able to demonstrate a significant dose-dependent effect of DC-mediated cellular immune response on CBR both in prostate and renal cancer, proving the concept of DC based vaccinations.
Both umbilical cord blood transplantation (UCBT) and T-cell depleted haploidentical peripheral blood stem cell transplantation (PBSCT) may be associated with an increased risk of graft failure. Mesenchymal stromal cells (MSCs) have been used to overcome delayed engraftment and rejection in these procedures. MSCs do not constitutively express MHC class II or co-stimulatory molecules and were once considered non-immunogenic. In contrast MSCs may elicit an immune-mediated rejection in a murine model of allogeneic hematopoietic stem cell transplantation (HSCT)(1). This experimental observation raised clinical concerns as to whether repeated MSCs infusions may trigger a similar immune-mediated rejection in allogeneic HSCT recipients.

In Leiden and Pavia, children undergoing UCBT are co-transplanted with haploidentical bone marrow-derived, ex vivo expanded MSC with the aim of improving hematological engraftment. Similarly, children requiring haploidentical PBSCT receive MSC derived from the PBSCT donor to reduce the risk of graft rejection. (CME approved protocols).

We found evidence of low immunogenicity of haploidentical MSCs in two children that underwent an initial unrelated UCBT and later following rejection, a haploidentical PBSCT according to above protocols.

Patients' details and HCST characteristics are summarised in Table 1.

Engraftment (i.e. leucocytes > 1.0, neutrophils > 0.5 and platelets > 20 × 10^9/L and 100% donor chimerism) was rapid in both patients (+12 and 19 days resp.) and sustained following co-transplantation of haploidentical PBSCT and MSCs as previously reported by our group.

Although more research is required to determine the exact nature of MSC initiated immune responses, clinical trials need to consider the potential immunogenicity of multiple MSC infusions. We were able to demonstrate that, despite previous exposure to the hematopoietic stem cell donor derived MSC's, further exposure to same donor MSC's and hematopoietic stem cells did not result into rejection of the donor graft. However, in contrast to the experimental model of Nauta et al., (1) our observations were made following successive myeloablative conditioning regimens, which may have abrogated any immunogenicity subsequent to the MSCs exposure.

As such we feel caution should be exercised in patients transplanted with reduced intensity conditioning and in immune competent patients treated with allogeneic MSCs (i.e. for autoimmune disorders).

Reference: (1) Nauta et al. Blood 2006; 108: 2114–20.

### Table 1

| Patient | Patient |
|---------|---------|
| Age at 1st HSCT | 10 years | 2 years |
| Indication for HSCT | Hemoglobinopathy | EBV induced HLH |
| Cord blood stem cells | HLA match (2 low resolution) | 0/6 |
| MSC harvested at 1/kg | 4.0 | 3.6 |
| CD34+ infused at 1/kg | 8.1 | 1.3 |
| MSC donor | Father | Father |
| MSC infused at 1/kg | 9.3 | 1.5 |
| UCBT | 4 | 24 |
| CDS - cells infused at 1/kg | 40.2 | 40.1 |
| MSC infused at 1/kg | 9.0 | 1.5 |
| UCBT | 3 | 24 |
| CDS - cells infused at 1/kg | 40.2 | 40.1 |
| MSC infused at 1/kg | 9.0 | 1.5 |

Oncologic and hematologic patients treated by chemotherapy and possibly stem cell transplantation usually are poly-transfused and therefore exposed to multiple donor-derived blood products. Multi-component collection (MCC) in apheresis enables to produce various standardized blood components during one donation session and may help to reduce the donor exposure to poly-transfused patients. In this paired prospective study we prepared platelet concentrates (PCs) by MCC using two different cell separators to compare metabolic, functional and activation parameters of platelets (PLTs) immediately after apheresis and during storage.

Twenty-four donors underwent MCC donation on two different cell separators (Fenwal Amicus® and Caridian BCT Trima Accel®) within an interval of at least two months where one double dose of PLTs and one unit of packed red blood cells were collected. These two devices differ in the mode of PLT collection as in the Amicus® separator centrifuged PLTs remain highly concentrated as so-called “dry PLTs” within the collection chamber and are manually re-suspended in plasma at the end of apheresis. In contrast, in the Trima Accel® separator, PLT rich plasma is continuously collected outside the cell separator during centrifugation. On days 0, 2 and 7, PCs were tested for metabolic parameters and PLT function by aggregometry, rotation thrombelastometry and hypotonic shock response. PLT activation was analyzed by flow cytometry.

Until day 7, metabolic parameters were well maintained in both groups. PLTs collected by the Amicus® device were significantly more activated as evidenced by higher CD62P and CD63 expression as compared to Trima PCs. This was observed in parallel to an impaired in vitro PLT function revealed by aggregometry, hypotonic shock response and also partly rotation thrombelastometry. In multicomponent apheresis, standardized PC collection is effective and well tolerated. The higher activation of PLTs derived from the Amicus® separator may be due to the distinct modality of PLT collection. However, the causes for impaired
PLT function and possible consequences for the clinical outcome have to be evaluated in further studies.

R1246
Activated B-cells as cellular adjuvants in immunotherapy: preclinical data and future applications
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B cells are key players in inducing immunity not only as antibody-producing cells, but also as antigen-presenting cells (APC). Depending on activation stimulus (CD40 vs. LPS), antigen-loading (peptide vs. Protein) and route of injection in vivo (subcutan vs. intravenous), they can exert a more tolerogenic or immunogenic function. CD40-activated B cells (CD40-B) express MHC and costimulatory molecules (CD80, CD83, CD86) on their surface as well as adhesion molecules (CD54, CD58) in a density comparable to mature dendritic cells (DC) and efficiently present antigen to T cells (Schultze, J Clin Invest, 1997). Due to these qualities CD40-B cells have been investigated as alternative APC for clinical vaccination studies. Upon activation via CD40 and IL-4R > 95% pure CD40-Bs can be generated from small amounts of peripheral blood within 14 days (Liebig, J Vis Exp, 2009). They can be expanded over several months with stable phenotyp and stable function (Wiesner, 2008), resulting in big amounts of clinical applicable CD40-activated B cells that can be generated from small amounts of peripheral blood. Upon activation via CD40 and IL-4R > 95% pure CD40-Bs can be generated from small amounts of peripheral blood within 14 days (Liebig, J Vis Exp, 2009). They can be expanded over several months with stable phenotyp and stable function (Wiesner, PLOS, 2008), resulting in big amounts of clinical applicable CD40-Bs (CD40-Bs) per patient (von Bergwelt-Baldwin, Blood, 2002). CD40-Bs up-take antigen independently from BCR via endocytosis, process antigen via classical and at least one alternative MHC class II cascade and prime naïve T cells (Kondo & von Bergwelt-Baldwin, unpublished results). Furthermore they can be loaded efficiently with peptide, RNA, DNA, retrovirus, lentivirus and tumor lysate (Lapointe, Cancer Research, 2003, Kondo, Blood, 2004, Coughlin, Blood, 2004). Those antigen-loaded CD40-B cells expand antigen-specific CD4+ and CD8+ T cells in vitro. (Kondo, J Immunol, 2002, Lapointe, Can Res, 2003). Based on this preliminary findings a CD40-B based in vitro T cell expansion system had been established, to characterize antigen-specific T cell responses and identify new and at least in part “universal” tumor antigens. (Vonderheide, Immunity, 1999, Maesker, Blood, 2003, Hirano, Blood, 2003, Kondo, Clin Can Res, 2008, Kondo, Int J Can, 2009, Kondo, Leukemia, 2009). In addition first results allude to the fact that regulatory T cells can be expanded as well (Zeng, J Immunol, 2009, J Immunol, 2008, Shimabukuro-Vornhagen, Blood, 2009). Due to this preliminary work with human CD40-B cells conducted in vitro, a murine in vivo model (Liebig, submitted to J Vis Exp) to preclinically evaluate characterization and development of CD40-B cells as cellular adjuvant for tumor patients has been established. Preclinically findings and planed clinical application will be presented at the meeting.

Cytokines

R1247
Addition of plerixafor to G-CSF alone or combined with chemotherapy in poor mobilizers yields sufficient numbers of CD34+ cells to offer patients high-dose therapy
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PHase IIa study of addition of plerixafor to G-CSF alone or combined with chemotherapy in poor mobilizers yielded sufficient numbers of CD34+ cells to offer patients high-dose therapy. Tolerance of combined treatment was good. A rapid and sustained mobilization after high dose therapy, a minimal number of 2 × 10^6 CD34+ cells/kg are desirable. However, in 15–20% of the lymphoma and myeloma patients insufficient numbers of CD34+ cells are harvested. These patients, characterized as poor mobilizers, will either be intended to be remobilized, or can not be offered high dose therapy even if it can be curative. Recently, new mobilizing agents including plerixafor, a CXCR4 antagonist, have been developed. A limited number of patients have so far been studied in individual transplant centres and the recommendation of how to implement this drug in poor mobilizers is currently not clear. In this study we have examined if administration of plerixafor, in addition to G-CSF ± chemotherapy used upfront or following remobilization, would improve yield of CD34+ cells in patients characterized as poor mobilizers. Here we report the preliminary experiences of plerixafor in poor mobilizers.

Phase-Ib study of addition of plerixafor to G-CSF was performed in poor mobilizers. Addition of plerixafor to G-CSF alone or combined with chemotherapy in poor mobilizers yields sufficient numbers of CD34+ cells/kg: range: 0.88–7 × 10^6 CD34+ cells/kg). Five patients were remobilized using G-CSF + chemotherapy + plerixafor. Two patients were given plerixafor during the primary stem cell mobilization using G-CSF and chemotherapy, due to delay in estimated increase in CD34+ cell concentration (3.9–9.5 × 10^6 CD34+ cells/kg). Our findings show that with a low concentration of CD34+ cells (5–10 cells/μL) and a total white cell count > 10 × 10^9/L, addition of plerixafor resulted in successful stem cell harvesting in these patients. In contrast, in patients with very low or no CD34+ cells present following standard mobilization, addition of plerixafor did not further improve mobilization of stem cells. In conclusion, our experiences, like others, show that remobilization with G-CSF and plerixafor can be an alternative approach to obtain sufficient CD34+ cells in poor mobilizers. However, to avoid delay of induction chemotherapy prior to high dose therapy our preliminary data indicate that plerixafor can be successfully added upfront to mobilize sufficient PBPCs in poor mobilizers.

Stem cell source

R1248
Patient satisfaction in therapeutic apheresis: a simple evaluation system
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Introduction: Patient/Donor Satisfaction is a key performance indicator and quality measure for any intervention. We describe a minimal-resource system for assessment of patient satisfaction in apheresis including PBSC and DLI harvesting. Method: An SOP and a single evaluation form were introduced to the apheresis unit. Patients and donors completed the same evaluation form at the end of the last procedure and, by post, 30 days later. PBSC/DLI harvests, plasma and red cell exchanges and allogeneic donor counselling were all included. Responses to 7 questions were graded from 1 (poor) to 5 (good). These included: preparation, comfort, problem handling, staff treatment, perceived benefit, discharge, overall service provision. Results: 108 patients were approached and 85% responded. 79% of responders had PBSC/DLI harvests. Average of all responses was 95% positive (graded 4.5/5). This included percentage positive responses to individual questions as follows: Preparation 94%, comfort 94%, problem handling 97%, staff treatment 99%, perceived benefit 90%, discharge 95%, overall service provision 97%. Post procedure and 30 day responses were similar. 40% of forms included extra comments, 7% negative comments, 5% mixed and 80% positive.
Discussion: This simple protocol involves minimal staff time or expense. It is part of routine practice. The post procedure form ensures a reasonable response rate and the 30 day form controls for bias. It confirms confidence in procedures and staff and highlights minor problems supplementing adverse event reporting. It provides an objective quality measure enabling continuous improvement.

R1249
The effect of CD34+ cell and lymphocyte subgroups in the donor apheresis product on allogeneic peripheral haematopoietic stem cell transplantation
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Acute leukemia is a malignant disease of the bone marrow results from neoplastic transformation of hematopoietic progenitor cells. Symptoms like bleeding, infections and anemia are the results of bone marrow infiltration by leukemic cell. Besides the cytotoxic chemotherapy in the patients with remission, allogeneic bone marrow transplantation consisting of chemotherapy and immunotherapy is an alternative method for the therapy.

The relationship of CD34+ and T cell in the donor apheresis product with engraftment time, graft versus host disease and relapse respectively has been shown in some studies. However the relationship between the cell content of graft and neutrophil, platelet engraftment time and clinical progress is not known clearly in peripheral hematopoietic stem cell transplantation.

In this retrospective study, we included 20 subjects attempted to Baskent University Haematology Department between January 2004-November 2008 who had a HLA identical sibling donor and underwent an allogeneic stem cell transplantation because of an haematologic malignancy. The means of mononuclear cells, CD34, CD3, CD8, CD4, CD19, CD16 positive cells in donor apheresis product were compared with neutrophil, platelet engraftment time, graft versus host disease, relapses, disease free survival overall survival time. A positive correlation between active or minimal residual disease at the time of transplantation and relapse and mortality was found. The disease-free survival and overall survival was longer in the cases with myeloablative condition as compared to nonmyeloablative. Statistical analysis showed although CD34+ component of the donor apheresis product affected the platelet and neutrophil engraftment positively, it increased the mortality when given in large doses. It observed that increase CD 19+ cells affects the engraftment time negatively and fastens the relapse. No relationship was observed that increase CD 19+ cells affects the engraftment time negatively and fastens the relapse. No relationship was observed that increase CD 19+ cells affects the engraftment time negatively and fastens the relapse. No relationship was observed that increase CD 19+ cells affects the engraftment time negatively and fastens the relapse.

As a result, we concluded that cell populations and their ratios in the donor apheresis product can be predictive for a successful engraftment and clinical progress after the transplantation. Nevertheless, further investigations are needed.

Graft engineering

R1250
Pre-cryopreservation manipulation of bone marrow: use of an automated system.
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The stem cells bone marrow (BM) transplantation is used in patients with neoplastic or other diseases, both in autologous than in allogeneic transplants. When we need to cryopreservation unit of BM, it is necessary to obtain a reduction of volume of the sample, actually it is used a completely manual method with cellular separatory or semi-automatic instruments.

In our experience, before cryopreservation the volume was reduced from 47 units of BM, using a completely automated system (Sepax S100-Eyvis Biosafe SA, Switzerland) with the “GENERIC PROTOCOL FOR THE REDUCTION OF VOLUME” and the kit 490-S, allowing to process for each cycle a maximum volume of 880ml; so in 15 cases we divided the units in two aliquots and in 1 case in three parts. The units were from 30 subjects (6 healthy donors, 5 NHL, 4 beta-thalassemia, 4 SCA, 3 ALL, 2 AML, 1 MM, 1 HL, 1 MDS, 1 SLE, 1 NBL, 1Mucopolysaccharidosis patients), submitted to bone marrow harvesting, with a mean of 28.7 (1–64) years and weight 42.17 (11–104) kg.

To standardization the protocol we settled final volume considering the HT unit; in particular we processed 30 different units: 10 units to set a final volume corresponding to 50% of initial HT, 10 units to the 40% and 10 units to 35% of initial HT.

By the results show in tables 1 and 2, we performed a new experimental condition with a final volume obtained from the 42% of initial HT and we processed 17 units.

In all cases cell viability was higher than 99%.

In our experience, in all cases the volume reduction with automatic system has obtained excellent recoveries of nucleated and CD34+ cells when we settled a final volume of 40% initial HT and the better condition is the 42% of HT with an improvement of amount cells.

To establish the better parameter to set the strument we value another factor: the number of red blood cells, a critical aspect when there is ABO incompatibility between donor and recipient. The best reduction of contamination of red blood cells was obtained by setting the final value of 35% HT, but in this case we had a decrease in cellular recoveries.

In conclusion, the automated system used by us it is an excellent procedure for reducing the volume of BM before cryopreservation, whereas it is necessary to evaluate a large number of samples in order to define the right condition for reducing the contamination of red blood cells without the use of HES, it isn’t requested by Sepax protocol, but only with a closed system in accordance to GMP lines.

Stem cell donor

R1251
Outcome of allogeneic stem cell transplantation from advanced age (>60 yr.) donors
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Background and aims: Several characteristics of the donor (gender, parity and serological and virologic status) have a strong impact on outcome after allogeneic stem cell transplanta-
tion (SCT). The age of the donor has not been evaluated as a prognostic factor. To evaluate the impact of this in the outcome of aSCT procedures in terms of complications and mortality (TRM), engraftment, disease free survival (DFS) and overall survival (OS).

Patients and methods: From March 2001 to August 2009, 132 consecutive SCT were performed to 122 patients in a single center. Median age was 46 yr (range 14–69) and 74 (61%) were males. Reasons for transplant were AML (45 procedures, 35%), ALL (13, 10%), lymphoma and lymphoproliferative diseases including myeloma (35, 29%), myelodysplastic syndrome/secondary acute leukemia (20, 15%), myeloproliferative disorders (13, 10%) and others (2, 1%). Myeloablative SCT was performed in 46 procedures (35%) and non-myeloablative from a fully matched relative was employed in 63 procedures (48%), 20 procedures (15%) were from an unrelated donor (14 myeloablative and 6 non-myeloablative) and the remaining 3 (2%) were syngeneic. In 25 procedures (19%) the age of the donor was over 60 yr. The outcome in terms of transplant related complications, TRM, DFS and OS between procedures with donors >60 yr vs. younger donors were compared.

Results: Out of 9 cases of lack of engraftment (n=6) or slow recovery (n=3) one had a donor over 60 yr. Acute GVHD occurred in 52/104 (47%) in younger donor procedures and 49/104 (47%) in younger donor procedures (P=0.014). Chronic GVHD was observed in 2/17 (12%) with advanced age donor vs. 26/81 (32%) for younger donors (P=0.077). The incidence of severe (grades III-IV) aGVHD was 1/5 (20%) vs. 23/49 (47%) (P=0.25), and the incidence of extensive cGVHD was 1/2 vs. 13/24 (54%) (P=0.72). CMV infections were more frequent in procedures from younger donors (58/107 [54%] vs. 8/25 [32%]).

No statistically significant association was found between donor age (>60 yr vs. ≤60 yr) and TRM (19% vs. 22%) (P=0.738), relapse incidence (41% vs. 40%), 5 yr DFS (31% vs. 32%) and 5 yr OS (30% vs. 34%).

Conclusions: Patients submitted to SCT from advanced age donors (>60 yr) showed a significantly lower incidence of GVHD and CMV infections. However, these differences did not translate to different outcome in terms of TRM, relapse incidence, DFS and OS.

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R1253
A comparative study between peripheral blood stem cell collections using the Cobe spectra or the Optia cell separator
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Successful short and long-term engraftment after Stem Cell Transplant depends on the number of progenitor cells collected. Peripheral Blood progenitor cell collection is related with a number of factors including the type of mobilization or the technique of collection employed. The goal of our study has been to compare the characteristics of the product obtained using a new cell separator (OPTIA, version 3) that uses centrifugation for blood separation and a unique optical detection technology for interface management with other cell separators routinely used such as the COBE Spectra (version 6.1, autoPBSC).

Methods: A total of 23 subjects were included consecutively, 3 normal donors and 20 patients with hematological diseases, with a median age of 59 years old (20–98). G-CSF 5 μg/Kg/12 h in 15 patients and QT+G-CSF 5 μg/Kg/d in 8 was used for mobilization of progenitor cells. Apheresis was started after the 8th doses of G-CSF or with >5 CD34/μ, respectively. In every patient 2 apheresis were performed, one with each cell separator. The first apheresis was assigned randomly to each cell separator. Two blood volumes were processed with each one and ACD ratio was 1:12–14. Comparison between apheresis 1 and 2 in each patient was performed using the Wilcoxon test and paired samples t-test.

Results: The number of CD34/ul pre-apheresis was not significantly different with each cell separator COBE Spectra and Optia, respectively (26 vs. 25 ul). The processed volume and ACD volume used was similar in both separators. Nor CD34 either CMN efficiency was different between the COBE and Optia separator. However, the process duration, volume product and the amount of red cells was higher in Optia than COBE processing. Otherwise, platelet efficiency and performance was lower in the COBE cell separator. See Table 1.

Conclusions: Peripheral blood progenitor cell collection with the Optia cell separator results in a similar efficiency in the CMN and CD34 + cell collection, however improvements should be made for better purity, decrease the product volume and the process duration.

R1252
Transplant glomerulonephritis as a side effect of peripheral blood stem cell mobilization with granulocyte colony-stimulating factor in the healthy donor
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The use of peripheral blood stem cells (PBSC) following granulocyte colony-stimulating factor (G-CSF) administration for allografting has increased in the last decade. PBSC donation is preferred from RETICC.

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S385
Case report: a highly immunized candidate for bone marrow transplantation

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Unrelated Bone Marrow Donor Search histocompatibility assessment of a 61 year old female MDS (Myelodysplastic Syndrome) patient who had received multiple platelet transfusions revealed a 97% panel reactive antibody (PRA) status when tested by the lymphocytotoxicity assay (LCT). Antibody specification by Luminex technology (One Lambda) showed antibodies against all HLA-A specificities except her own antigens. HLA of the patient: HLA-A*3004,*3201, B*3503,*5701, Cw*0401,0602, DRB1*0701,*1601, DQB1*0303,*0502. Donor search was difficult and only 2 donors with HLA-A differences could be found. In both instances the mismatch was an HLA-A*02 (vs. A*30), and as expected the LCT crossmatch result was positive. Antibody reduction treatment by plasmapheresis (PA) and B-cell-depletion with Rituximab was attempted. Semi-quantitative monitoring of antibody levels in serial dilutions by a Luminex technique (Labscreen mixed beads, normalized with Quantiplex beads, One Lambda) was performed before and after plasmapheresis sessions. Figure 1 and 2 show as a snapshot a comparison of antibody levels before and after one session (t1 pre, t1 post) and before one and the next session (t1 pre and t2 pre) respectively. Values are given in median fluorescence intensity (MFI). Intriguingly, this patient showed the prozone-phenomen, which is characterized by rising antibody binding capacity in serial dilutions through better crosslinking of antibodies in lower concentration, and is described as a sign of very high antibody titres. The patient showed maximum antibody binding at a dilution of 1:100. Antibodies were reduced by PA, however, antibody levels rose during the intervals between PA sessions and returned almost to baseline. It was not possible to reduce the antibody levels significantly throughout the therapy and BMT had to be postponed. Rituximab effectively depletes the B-cell population, but does not affect the plasma cell subset. Our results suggest that after effective antibody reduction by PA, antibodies may rebound into the serum either from extravascular compartments or are newly generated by long lived plasmacells. This impairs desensitization regimens. This case highlights the relevance of antibody testing and crossmatching in bone marrow transplant candidates, in particular those who have been multiply transfused, and therefore are in high risk of developing HLA antibodies.

Peripheral blood progenitor cell collection efficiency is not related to either clinical or technical variables when a Cobe Spectra blood cell separator is used

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Objectives: To analyze variables related to collection efficiency with a continuous-flow blood cell separator (Cobe Spectra) under manual control.

Methods: We analyzed data prospectively recorded, since January 2000, from 393 consecutively procedures. All procedures were performed in the Transfusion Service at Hospital Infantil Universitario Niño Jesús (Madrid). There were 143 aphaeresis performed to hematopoietic progenitor cell donors, and 250 performed to patients diagnosed with: acute leukaemia (12,7%), central nervous system tumours (13,5%), Ewing sarcomas (11,9%), neuroblastoma (8,9%) and others (16,5%). Median age of the series was 10 years (1 – 54), and median weight 36 kg (5 – 111). Mobilizations regimens include: G-CSF 12 mcgr/kg/12 h (45%), 10 mcgr/kg/24 h (34%), 12 mcgr/kg/24 h (16%), 10 mcgr/kg/12 h (3,5%), others (1,5%). Median blood volumes processed were 3,5 (0,7 – 9,5). Median duration was 230 minutes (61–406), and median inlet/flow was 45 ml/min (5–100). Acid citrate dextrose (ACD-A) was used as anticoagulant for whole blood: anticoagulant ratio 14:1 (94 cases ~24%~), or a solution of 500 ml ACD-A and 5000 IU of preservative-free heparin with a 1:30 ratio (299 cases ~76%~). Collection efficiency was calculated as follows: (CD34 + cells collected/mean CD34 + cells in peripheral blood ((CD34 + cells before the procedure + CD34 + cells after the procedure)/2) × blood volume processed) × 100.
Correlations were determined using linear regression. Non-parametric Mann–Whitney U-test, and Fisher exact test were also applied for continuous and categorical variables, respectively, on the univariate analysis. Results: Median CD34 + cells collected were $4.06 \times 10^6$/kg (0.04–54.4). Median collection efficiency in this series was 50.5% (8.5–142%). Collection efficiency was not related to either blood volumes processed, or duration of the procedure, or inlet/flow, or type of anticoagulation (variables of the procedure). It was also not related to either diagnose, cell blood counts (leukocytes, platelets or hematocrit) or CD34 + cells in peripheral blood before the procedure (variables of the patient/donor). Conclusion: Collection efficiency is not related to either clinical or technical variables. Even very low flow rates, usually used in low weight children, do not affect collection efficiency. Since these procedures are performed by a semi-automative device, it is crucial to have well trained nurses to achieve the expected results.

R1256
HLA-haploidentical peripheral blood stem cell transplantation in second relapse of AML with Gi tract chloromas after two HLA-identical sibling transplantations and pre-emptive DLT
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A now 34-yr old male patient was diagnosed with AML FAB M0/1 with complex karyotype in May 2005. First complete remission was achieved after chemotherapy. Peripheral blood stem cell transplantation was performed from the HLA identical brother in July 2005. He developed acute graft-versus-host-disease (GVHD) of the skin Grade II resolving after high dose IVIG. The patient received three preemptive donor lymphocyte transfusions (DLT) until April 2006 and remained in complete remission until January 2009. Then he relapsed with 50% AML blasts in the bone marrow and loss of chimerism. Low-dose cytarabine induced a second complete hematological remission. In March 2009 a second stem cell transplantation was performed using the same donor without a conditioning regimen and without immunosuppression. GVHD grade I of the skin occurred. In June 2009 the patient was readmitted with massive diarrhea. Gastroscopy and coloscopy revealed multiple tumorous lesions. A CT scan revealed multiple enlarged abdominal lymph nodes. There were no AML blasts in the peripheral blood, bone marrow and spinal fluid. The intestinal lesions were histologically classified as AML chloromas. Relapse treatment consisted of cytarabine and gemtuzumab. Repeated gastroscopy and CT scans showed no response. As the patient was clinically in a very good condition a third allogeneic transplantation was considered. The HLA haploidentical father was chosen as the alternative donor. The conditioning regimen consisted of clofarabin (30mg/m²) on day -15 to -11, fludarabine (30mg/m²) on day -6 to -2, cyclophosphamide (14.5mg/kgBW) on day -6 and -5 and TBI (2Gy) on day -1. G-CSF was administered, starting on day +1. Immunosuppression consisted of cyclophosphamide (50mg/kg BW) on day +3 and +4, mycophenolic acid (4x500mg/day) and tacrolimus (blood level: 5–15ng/ml). Granulocyte take took place on day +19, the patient developed a mucositis grade III and aGVHD grade I of the skin. Gastroscopy on day +46 showed a good remission of the chloromas. On day +48 the patient was discharged in good clinical condition.

Conclusion: In a chloromatous second relapse of AML after two HLA-identical stem cell transplantations and preemptive DLTs the treatment options for this patient were extremely limited. The unmanipulated stem cell transplantation from a HLA haploidentical donor using cyclophosphamide after transplantation proved a feasible therapeutic choice and induced a third response with low toxicity.

R1257
GVHD-triggered, spontaneous induction of remission after early relapse from infant acute lymphoblastic leukaemia following SCT
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Introduction: To the best of our knowledge spontaneous remission of full blown bone marrow (BM) relapse after stem cell transplantation (SCT) has never been described in acute lymphoid leukemia (ALL). We here report a case of infant ALL experiencing early relapse after SCT. Development of graft versus host disease (GVHD) resulted in complete molecular remission (cMMR) without any additional anti-leukemic treatment.

Case report: A diagnosis of common-ALL was made in a 6 mo old boy (skin infiltrates, CNS-negative, pB blasts: 160.000/μL, MLL-AF4 rearrangement, no myeloid markers, CD7-coexpression). He was treated according to protocol “BFM Interfant 06 – medium risk”, responded well to steroids (day 8 pB: 368/μL blasts) and achieved BM remission at day 33. First relapse (skin, BM: 15% blasts) had to be diagnosed 3 mos later, however, 2nd remission was achieved by protocol MARMA (HD-MTX, HD-Ara-C, 6-MP, Asparaginase). While SCT was scheduled 2nd relapse (BM: 80% blasts) occurred 2 mos later. Treatment with HD-cyclophosphamide (2×3 g/sqm) induced 3rd remission followed 2 weeks later by HLA fully-matched unrelated SCT (conditioning: Treo 3×14 g/qm; Fludara 5×30 mg/qm; ATG 3×10mg/kg; GVHD prophylaxis: MTX + CSA). Prompt engraftment at d +17 was followed by skin GVHD grade I resolving without treatment at d+25. At d+67 BM chimerism was 100% donor, and cMMR was confirmed by negative PCR for MLL-AF4 (threshold of detection: <1×10E-4). However, isolated testicular relapse (BM: 98% donor chimerism, MLL rearrangement negative) had to be diagnosed at d + 71. CSA was tapered over 2 weeks, bilateral orchectomy was performed at d + 78, but home-care palliative treatment was initiated when - without signs of GVHD- 3rd BM relapse (BM blast: 10%, pB blasts: 2000/μL, chimerism 70% donor) occurred at d +96. The boy was readmitted at d +135 with overall grade III GVHD (skin, gut). Surprisingly, he had not been transfusion-dependent during the previous weeks and blasts had cleared from pB. BM puncture confirmed remission (no blasts; chimerism: 100% donor; PCR: cMMR). After 2 weeks of steroid treatment GVHD resolved. As of writing (> d + 400) the boy is well with limited skin cGVHD and low transfusion rates, usually used for continuous and categorical variables, respectively, on the univariate analysis. Results: Median CD34 + cells collected were $4.06 \times 10^6$/kg (0.04–54.4). Median collection efficiency in this series was 50.5% (8.5–142%). Collection efficiency was not related to either blood volumes processed, or duration of the procedure, or inlet/flow, or type of anticoagulation (variables of the procedure). It was also not related to either diagnose, cell blood counts (leukocytes, platelets or hematocrit) or CD34 + cells in peripheral blood before the procedure (variables of the patient/donor). Conclusion: Collection efficiency is not related to either clinical or technical variables. Even very low flow rates, usually used in low weight children, do not affect collection efficiency. Since these procedures are performed by a semi-automative device, it is crucial to have well trained nurses to achieve the expected results.

R1258
Low cell viability in frozen samples from the graft may imply increased incidence of GVHD
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The cell viability was tested in frozen samples from peripheral blood stem cell grafts given to 26 unrelated HSCT patients. Most grafts came from Germany (n = 18) or other European countries (n = 4) while four came from USA. Time from harvest to transfusion was between one (n = 5) and two (n = 18) days. There were 17 males and 9 female recipients with a median age of 47 years (4–65). Most patients had acute myeloid (n = 9) or lymphoid (n = 7) leukaemia, and 10 patients had other haematological malignancies. Thirteen patients were in CR1 and 13 in later stages. All donors were HLA-A, -B and -DR high resolution matched, GVHD prophylaxis consisted mainly of
CsA and MTX. Conditioning were conventional myeloablative in 14 patients and reduced intensity (RIC) in 12. All patients received ATG before transplantation. Median nucleated cell dose was 12.3 × 10^8/kg (5.8–34.0) and median CD34 cell dose was 8.8 × 10^6/kg (0.6–14.9). A small portion of the cells were frozen at the time of the transplant for quality control and future donor lymphocyte infusions (DLI).

Results: Viability using 7-AAD on thawed DLI vials for quality control differed considerable, median 55% range 24–79%. There were no correlation between time from harvest to transfusion and viability. All patients engrafted, at a median of 17 days (10–21) for neutrophils (>0.5 × 10^9/L) and 13 days (9–20) for platelets (>30 × 10^9/L). Time-to-engraftment was not affected by cell viability. Viability did not affect incidence of bacteraemia, but patients with CMV infection (n = 11) had lower viability than those without CMV infection (P = 0.05). The incidence of acute GVHD grades II–IV was 47%. Patients receiving a graft with low viability in frozen control vials (<55%, n = 14) had significantly more GVHD, 71%, compared to those with a higher viability in frozen control vials (n = 12), 12%, P < 0.01. No other factor (NC and CD34 cell dose, conditioning, disease stage, age and sex of the patient and donor) affected the incidence of GVHD in this small material.

Conclusion: The viability in frozen samples of the graft may affect the development of acute GVHD. This needs to be studied in larger materials.

Graft-versus-host disease

R1259
Synthetic kynurenine derivative reduces acute graft-versus-host disease
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We have developed a novel immunosuppressive compound based on the physiological immune tolerance mechanism. Kynurenines, produced by indoleamine 2,3-dioxygenase (IDO), preferentially promote apoptosis in activated T cells without any functional defect in resting T cells. It gives us an expectation to remove pathogenic T cells selectively for preventing transplant graft rejection. To develop bioactive derivatives, we generated a focused library of kynurenines from a chemical library. Using high-throughput flow cytometry, we screened a focused library for inhibitors of alloreactive T cell expansion, simultaneously determining compound efficacy and toxicity. We obtained the lead compound K106 and confirmed its suppressive activities on in vivo allogeneic T cell transfer model. In addition, we evaluated the therapeutic effects of K106 on acute graft-versus-host disease (GVHD). The administration of K106 markedly improved the recipient survival through reduction of alloreactive T cell expansion and proinflammatory cytokine production. This novel immunosuppressive compound provides a remarkable opportunity to treat GVHD.

R1260
Soluble human leukocyte antigen G in haematopoietic cell transplantation is associated with several clinical complications after transplant
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HLA-G is a non classical class I HLA molecule with low level of polymorphisms and with 7 isoforms, 4 are membrane bound and 3 are soluble. Another differential characteristic is it is tissue restricted and have been described in adult thymic medulla, cornea, pancreatic islet and endothelial cell precursors. Several immune modulatory functions have been attributed to this molecule such as the interaction between B, T, NK and antigen presenting cells. Due to their immunomodulatory properties we investigated the possible role of soluble HLA-G (sHLA-G) in the allogeneic hematopoietic cell transplantation (HCT) setting. A cohort of 37 patients, who underwent HCT, were studied, 13 patients had acute myeloid leukemia, 8 patients had myelodysplastic syndromes (preleukemia disease) while the rest of the patients had non myeloid malignancies. Twenty eight patients received reduce intensity conditioning regimen while the rest of the patients received myeloablative conditioning treatment. Plasma samples from all patients were obtained before the conditioning regimen and after transplant at different timepoints. Soluble HLA-G concentration was measured in duplicates of plasmas by a specific enzyme-linked immunoabsorbent assay (ELISA) using the MEMG/9 as the capture antibody. Pre transplant variables were age, gender, disease, type of transplant (related, unrelated), infused marrow cell dose and donor gender. Post transplantation variables were graft versus host disease (acute/chronic, grade, day of onset, affected organ), infections, survival, relapse, day of bone marrow regeneration and immunosuppression therapy. We found a statistically significant association between rising levels of HLA-G during transplantation and clinically relevant (grade II–IV) acute GVHD, infectious events after transplantation, in particular fungal infections, and development of chronic GVHD. Our preliminary data shows that sHLA-G molecules are involved in several complications after allogeneic hematopoietic cell transplantation.

R1261
Autologous peripheral blood stem cell transplantation with induction of autologous graft-versus-host disease in acute myeloid leukaemia – long-term follow-up data
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We previously published data that suggested that induction of an autologous graft-versus-host disease (GVHD) has an anti-leukemic effect, consequently increasing the survival rate of patients who undergo autologous peripheral blood stem cell transplantation (PBSCT). Here we report the long term follow up data regarding the overall survival (OS) and disease free survival (DFS).

In total, 22 acute myeloid leukemia patients with favorable and intermediate cytogenetic risk, in their first complete remission, were administered cyclosporine c.i.v. from day 0 to day +28 at a dose of 3.0 mg/kg per day and interferon-gamma (IFN-gamma) at 0.026 mg/m² s. every other day from day +14 to day +42 following autologous PBSCT. Natural-killer (NK) cell activity assays and skin biopsies were performed. Engraftment was successful in all patients at a median of 13 days without any significant additional toxicity. Histologically confirmed that cutaneous GVHD had developed in 12 patients, and NK-cell activity was significantly augmented after the autologous PBSCT in those patients (P = 0.03). After a median follow-up duration of 117.6 months (range, 87.3–152.8), the 3-year DFS and OS rates were 68.2% and 72.7%, respectively, and the 5-year DFS and OS rates were 63.6% and 73%, respectively. They were without significant correlation with GVHD status or augmentation of NK-cell activity. The median OS was unreached yet.

This data suggests that the administration of cyclosporine and IFN-? following autologous PBSCT improves OS and DFS, which may be attributable to the antileukemic effect, although no difference in survival rates could be demonstrated between cutaneous GVHD-positive and -negative groups.
Influence of high hepatic artery resistance index by liver Doppler ultrasonography on hepatic graft-versus-host disease
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Hepatic artery resistance index (HARI) is known to reflect portal venous blood pressure and resistance in several diseases of the liver. This study was tried to investigate whether preconditioning HARI values would predict hepatic complications, such as hepatic graft-versus-host disease (GVHD), venoocclusive disease, and drug-and sepsis-related hepatotoxicity after allogeneic stem cell transplantation (SCT).

Fifty-nine patients who received allogeneic SCT were analyzed whether pre-SCT HARI would predict post-SCT HC for 59 patients who received allogeneic SCT. Twenty-six patients (44.1%) of 59 showed high HARI (≥ 0.74) prior to allo-SCT. A high HARI value correlated with incidence of hepatic GVHD when compared with low HARI. However, other Hepatic complications were comparable between two HARI groups. Several factors that have a major impact on hepatic GVHD were noted: higher CD 34 cell dose (P<0.001), unrelated donor type (P=0.017), sex incompatibility (female, donor; male, recipient) (P<0.001), ABO incompatibility (P=0.023), and high HARI (P<0.001). Multivariate analysis revealed that a non-myeloablative regimen (P<0.001), infused CD 34 cell dose (HR = 3.315, P=0.011), and high HARI (HR = 2.817, P=0.017) were independent predictors associated with hepatic GVHD. However, high HARI did not correlate with non-relapsed mortality (P=0.402) and overall survival (P=0.607).

In conclusion, it appears that a high preconditioning HARI might be an important predictor of the significant hGVHD for patients receiving allo-SCT.

Intra-arterial methylprednisolone plus methotrexate injection therapy to treat hepatic GvHD
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Severe GVHD is responsible for morbidity and mortality in HSCT. Moreover, the systemic treatment of GVHD with high dose steroids (HDS) leads to higher rates of infections and malignancy recurrence. The development of G III to IV hepatic aGVHD in association with mild cGVHD of other organ systems, beyond the D + 100 of HSCT, is currently treated with systemic HDS by several weeks with all side effects of this therapy. Trying to rescue steroid refractory patients with severe hepatic and intestinal aGVHD Shaphira M, et al., and Nakai K, et al. published the results of a new strategy of local GVHD treatment with intra-arterial steroid-injection therapy (IASI) with acceptable rate of success and without serious complications related to the new strategy. Based on this, we treated six patients that were submitted to identical sibling allo-HSCT (4 for AML, 1 for PNH and 1 for MDS) that developed NIH score 3 to 4 hepatic aGVHD plus mild cGVHD of other organs sites beyond of D + 100 with IASI (methylprednisolone, 75 mg/m² plus methotrexate, 10 mg/m²).

Patients were resistant to HDS (2 patients), or were at high risk for disease recurrence (4 patients). The infusion day ranges between D + 190 to D + 321 after the HSCT. On the day of the infusion all patients had high levels of hepatic and canaliculare enzymes while 4 of them had high levels of bilirubins too. The response to therapy was variable in all 6 treated patients (Table 1). Bilirubins levels reached a 100% to 70% reduction in relation to the previous treatment levels in all 4 patients around D + 60 after infusion. Hepatic enzymes levels reached a 90% to 30% reduction in 5 patients after D + 60 of the infusion. Only 2 patients presented reduction in the levels of canaliculare enzymes (60% and 30%) after D + 60 of the infusion. One patient that persisted with high levels of canaliculare and hepatic enzymes after D + 60 of the first infusion was submitted to a second injection with complete long term response. One patient died 2 years after the infusion secondary to a relapse of the AML. The rationale to this IASI is the administration of high dose of immunosuppressor agents direct to the site of the body accomiited by the GVHD, diminishing undesired systemic toxic effects of immunosuppression and the probability of malignant disease recurrence. This is the first data regarding the application of the IASI to treat hepatic GVHD beyond D + 100 in a scenario of overlap syndrome were systemic immunosuppression was inefficient or undesired.
R1264
TNF antagonist therapy for gastrointestinal acute graft-versus-host disease refractory to steroid in allogeneic hematopoietic cell transplantation
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Background: Steroid-refractory gastrointestinal acute graft-versus-host disease (aGVHD) has remained a serious complication, which contributes significantly to treatment-related morbidity and mortality. TNF antagonists have recently been reported to be effective salvage therapy.

Objective and method: Nine patients were treated with TNF antagonists (infliximab/etanercept) against steroid-refractory gastrointestinal aGVHD following allogeneic hematopoietic cell transplantation (allo-HCT) at Toranomon Hospital from April 2005 to March 2009. Doses of TNF antagonists used were 5 mg/kg of infliximab once a week and 0.4 mg/kg of etanercept once or twice a week. gSteroid-refractory h was defined as persistent symptoms even after 5 day- or progression after 3 day-steroid administration.

Result: Four had grade III and five had grade IV gastrointestinal aGVHD before TNF antagonist therapy. CR was achieved in 4 (57%) out of 7 infliximab-treated patients. On the other hand, etanercept in 2 patients were not effective and was changed to infliximab, but failed to improve symptoms. Infliximab was ineffective in all 5 patients of Grade IV gastrointestinal aGVHD and 5 out of 6 patients developed thrombotic microangiopathy. Eight patients had CMV infection and all cases had bacterial or fungal infection during and after TNF antagonist therapy.

Conclusion: Earlier introduction of TNF antagonists in less severe grade GVHD may be beneficial for steroid-refractory gastrointestinal GVHD. The effect of TNF antagonists for grade IV gastrointestinal aGVHD was limited. We must be cautious for preventing concurrent bacterial, fungal or viral infections. Infliximab may be preferable as second-line therapy for aGVHD when compared with etanercept.

R1265
Usage of monoclonal antibodies in steroid resistant acute graft-versus-host disease
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Acute graft-versus-host disease (aGVHD) is the most important causes of morbidity and mortality in patients (pts) who fails to respond to first-line steroid treatment after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Aim: To estimate efficacy of monoclonal antibodies (Mab) (daclizumab, infliximab, etanercept) in children affected by steroid resistant aGVHD.

Patients: Between July 2001–October 2009 Mob used in 19 pediatric pts (age - 2–21 years) suffering from ALL–8, AML–6, bAL–1, MDS–1, AA–1, lymphoma–1, Ewing’s sarcoma–1 (remission/reapse–13/6) underwent allo-HSCT: 6 pts–MUD HSCT, 7 pts–mismatched UD HSCT, 6 pts–haplo-HSCT with conditioning regimen MAC–10 pts, RIC–9 pts plus antilymphocyte globulin (ALG–60.0 mg/kg). aGVHD prophylaxis was CSA + short course of MTX or tacrolimus + MMF. According to published criteria aGVHD were graded: II–6, III–7, IV–6. Non-responders after 1st -line treatment by methylprednisolone (MPR) (1–2 mg/kg/day) received 2nd–line treatment by daclizumab (13 pts) [1 mg/kg in D + 1, 4, 8, 15, 21, (28)] or infliximab (5mg/kg/wk iv, median–4 doses)/etanercept (25mg/kg/2wk, sc)–6 pts in median 10 days after start of MPR(range 2–15).

Results: Among 13 pts who received daclizumab complete response was achieved in 2 pts (15%), partial responses in...
5 pts (38%), stabilization in 1 pt (8%) (total response rate – 61%). Responses were seen in 1 pts (34%) with skin aGVHD, II-III st, 4 pts (50%) with gut aGVHD, II–IV st and of 2 (40%) with liver aGVHD, II-III st. After infliximab/ etanercept complete response was achieved in 2 pts (33%), partial responses in 1 pts (17%) (total response rate 50%). Responses were seen in 3 pts (60%) with skin aGVHD, II-IV st, 1 pts (50%) with gut aGVHD, II st. Nine pts (47%) are still alive at a median of 21.5 months (range 1–111), 7 pts from them developed chGVHD (limited–7 pts, extensive–1 pt). Overall response after Mab treatment in MPR resistant pts was 58%. Ten pts (53%) died at a median of 3.5 months after alloHSCT (range 1–13) from aGVHD, IV stage–7pts, relapse–3pts but not infection. Overall 1-year survival in pts affected by steroid resistant acute GVHD who received daclizumab, infliximab, etanercept was 60%.

Conclusion: The usage of Mab was effective for steroid resistant aGVHD but require the additional options for consolidation of effect. The efficacy of daclizumab vs. infliximab/etanercept is comparable (61% vs. 50%) and more effective in a case of skin and gut aGVHD.

Infectious complications

R1266
A case of H1N1 influenza A virus-associated acute respiratory distress syndrome in the aplasia period following autologous stem cell transplantation
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We describe a patient who developed a rapidly progressive pneumonia and influenza A pandemic (H1N1) 2009 virus infection leading to acute respiratory distress syndrome (ARDS) during the aplasia period following an autologous stem cell transplantation. A 48-year old man with a Large B-Cell Lymphoma in second complete remission was admitted in good clinical conditions to the Transplant Unit on October 30, 2009, to perform a planned autologous stem cell transplantation. At that time the vaccination against influenza A was not yet available in Italy. He began to complain of cold and hoarseness during the course of the BEAM conditioning regimen, but he remained persistently afebrile. On day 0, 2.4 × 10^6/Kg CD34 + cells were reinfused. On day 0, 2.4 × 10^6/Kg CD34 + cells were reinfused. On day +2, as WHO grade IV neutropenia developed, the patient presented high persistent fever, cough, chest radiograph was normal. On day +4 the patient developed progressive dyspnea and hypoxemia, bilateral massive infiltrates at Computed Tomography and was admitted to the Intensity Care Unit (ICU) requiring intubation and mechanical ventilation. On day +5 he was started on extracorporeal membrane oxygenation (ECMO). All the blood and sputum cultures were negative for bacteria and fungi, while H1N1 influenza A infection was diagnosed by testing nasopharyngeal swab specimens with RT-PCR. He was treated with oseltamivir at the dose of 300 mg/day, meropenem, capsofungin, acyclovir and filgrastim. The fever and the respiratory failure persisted for the following 2 weeks without any substantial change and influenza A tests performed every other day remained positive. After the achievement of neutrophil engraftment (a neutrophil count higher the 1 × 10^9/L on day +19) a progressive clinical and radiological improvement were presented, influenza A test was negative on day +20, ECMO was discontinued on day +27 and mechanical ventilation on day +40. A discharge from ICU was planned in the following days. No other patient of the Transplant Unit developed Influenza A infection in the same period.

This case-report suggests the following considerations:
– a vaccination against influenza A should be administered in all patients at least 2 weeks before a planned stem cell transplantation;
– the neutrophil count seems to be critical for the evolution of the pulmonary involvement of influenza A infection;
– ECMO should not be discontinued precociously.

R1267
H1N1 pneumonia in a patient with graft-versus-host-disease
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H1N1 infection can be a serious complication in severely immunodepressed patients. We describe the evolution of pneumonia in a patient (pt) on corticotherapy for graft versus host disease (GVHD).

A 43 years old pt with acute myelomonocytic leukemia diagnosed in December 2008 with cutaneous infiltration, invasion of central nervous system, gingival hypertrophy and hepatosplenomegaly. In first complete remission after one cycle of induction therapy and two consolidations, a peripheral blood stem cell transplantation of a HLA matched sibling was performed on May 2009, after myeloablative conditioning regimen (intravenous (IV) busulfan and cyclophosphamide). GVHD prophylaxis was done with cyclosporine and methotrexate. Acute grade 2 GVHD occurred on day (D)40 (stage 1 digestive with histological proven disease) and corticotherapy was prescribed associated with posaconzol for fungal infection prophylaxis. Intraesploric immunoglobulin was begun due to hypogamoglobulinaemia. Due to CMV reactivation on D146 gancyclovir was used. At the beginning of November, immune suppression was: prednisolone 1mg/Kg each other day and cyclosporine; the GVHD was well controlled. At this time low grade fever (38–38.5°C) begun, with no other symptoms, blood culture obtained through the catheter showed a bacillus spp., for what IV ambulatory antibiotherapy was prescribed; one week later (D170) pt has high grade fever (39.5°C), with slight hypoxia (pao2 82 mmHg) and hypocapnia (paCO2 30 mmHg); the pt was admitted and a swab of oropharynge was sent for H1N1 PCR; chest xRay showed a scanty interstitial pulmonary infiltrate. Two days later there was a gradual deterioration of respiratory function and pt was admitted in the intensive care unit with SAPS II 42, SOFA 7 and needed ventilatory support; oseltamivir was prescribed and corticotherapy was tapered; refractory ARDS persisted in spite of prone position and nitric oxide inhalation and pt died on D20 of ventilatory support. PCR for H1N1 maintained positive during all time, in spite of oseltamivir 150 mg 2 id.

This clinical case demonstrates the difficulty of diagnosis in these complex pts, the severity of H1N1 infection in imunosuppressed pt and the need for adequate preventive measures in this particular cohort. We must obtain more data of response to immunization after allotransplant with GVHD. Other difficulty is the scanty data of natural history optimal and treatment in these pts.

R1268
Invasive aspergillosis increase the risk of death in acute myeloid leukemia patients
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Objectives: To study the impact of invasive aspergillosis (IA) incidence during induction therapy for acute myeloid leukemia (AML) patients on short and long term overall survival.

Methods: A retrospective cohort study was performed in the Hematology department of the Edouard Herriot Hospital, Lyon (France). Patients with newly diagnosed AML between...
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01/01/2004 and 31/12/2007 were retrospectively included and the follow-up was censored at 30/06/2009. Data were extracted from medical charts and from the prospective surveillance of IA (EORTC diagnosis criteria). The patients with IA after post induction evaluation were excluded (N=5). A Cox proportional hazard model with diagnosis of IA and post induction evaluation (complete remission [CR] of AML or failure of chemotherapy) as the main exposure besides age, year of inclusion, WHO status, cytogenetic group, kind of induction chemotherapy, and hematopoietic stem cell transplantation, was fitted.

Results: Overall, 262 patients counting for 149,370 patient-days were analysed, the median age at diagnosis was 56.6 years (47.9–64.2 years), and 196 (75%) had CR. There were 58 (22%) IA cases with a median interval between induction and IA of 30 days (range, 16–27 days); 29 (50%) IA were possible, 24 (41%) probable, and 5 (9%) proven. At the last follow-up, 165 (63%) patients died with a median overall survival of 18 months (95% confidence interval [95% CI] 14–23 months). The 4 year-survival of patients having had IA was 14%, and without IA 32% (P=0.01). The 2 year-survival of patients achieving of CR was 54% vs. 5% for patients with failure of chemotherapy was 5% (P<0.001). Cox multivariate analysis showed that patients in CR with IA presented a higher risk of death compared to patients in CR without IA (Hazard ratio=1.66, 95% CI 1.05–2.65, P=0.031). In addition, IA was associated with a higher risk of death in patients with failure of chemotherapy compared to patients in CR without IA (Hazard ratio=6.43, 95% CI 3.72–11.10, P=0.001) (Figure). The WHO status, cytogenetic group and kind of induction chemotherapy were associated with lower survival.

Conclusion: IA was associated with a high risk of death in AML patients whether they were in CR or failure after induction chemotherapy. Cytogenetic group or WHO status are not modifiable risk factors for death in this population while prevention of IA with environmental procedures or using individual prophylaxis will improve survival outcome.

R1269

Successful transplantation despite cerebral and pulmonary invasive aspergillosis using combination antifungal therapy and granulocyte transfusions in refractory AML.

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Introduction: Historically diagnosis of active, invasive aspergillosis was considered as a contraindication to alloSCT. New antifungal agents like improved azoles and echinocandines have opened novel treatment options with reduced side effects. Additionally, combinations of antifungal agents employing different modes of action become increasingly attractive.

Method: We report on a seven year old girl with acute myeloid leukemia (AML M2)–relapse, who developed biopsy-proven cerebral and pulmonary aspergillosis during relapse treatment (BFM relapsed AML 01). At both sites of fungal infection surgical excision was performed and A. fumigatus could be detected. Despite FLAG and FLAG-DNX reinduction treatment the patient did not reach remission. Therefore, SCT was carried out from her HLA-identical brother after additional chemotherapy using low dose ARA-C and CD33-antibody mylotarg. Conditioning regimen consisted of reduced FLAMSA, Bu and CYC. The transplanted BM contained 6 × 10^6/kg CD34+ cells. CSA and MMF were employed for GvHD-prophylaxis. After onset of grade III aGvHD, steroids and extracorporeal photopheresis were added. Two granulocyte transfusions (GT) were administered until early neutrophil engraftment on day +12. Immunosuppression was stopped on day +160.

Antifungal treatment was carried out with voriconazole and caspofungin before and after SCT and caspofungin monotherapy during conditioning. Combination therapy was continued until day +131 and then switched to oral voriconazole, when Aspergillus-specific T-cells became readily detectable in peripheral blood.

Result: The child is alive and in remission ten months after SCT. Hemilobectomy was carried out on day +70. The final eradication of the cerebral infection site is planned.

Conclusion: Combination therapy including azoles and echinocandines may lead to synergistic effects because different fungal structures are targeted. GT up to the engraftment may be helpful in patients suffering from aspergillosis undergoing SCT. There is evidence that voriconazole achieves high drug levels.
in central nervous system and in the lung. However, it remains unclear, whether azoles alone or in combination with echinocandines and/or the addition of GT were the critical agents providing treatment success in this patient suffering from cerebral and pulmonary aspergillosis. Nevertheless, this regimen might prove highly effective in aplastic patients during SCT, thus warranting prospective evaluation in clinical trials.

R1271
Invasive pulmonary aspergillosis in allogeneic stem cell transplant recipients
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Background: Invasive pulmonary aspergillosis (IPA) remains one of the main causes of morbidity and mortality in patients undergoing allogeneic haematopoietic stem cell transplant (alloHSCT).
Aims: To determine the incidence and risk factors for IPA in patients receiving alloHSCT.

Patients and methods: Descriptive, observational and retrospective study which includes 61 consecutive patients who underwent alloHSCT between January 2005 and December 2008 in our institution. IPA was defined as proven or probable according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria. Statistical analysis: qualitative variables were analyzed using CHI² test and Student›s t-test to compare qualitatives vs. quantitative variable. Risk factors to develop IPA were identified by univariate and multivariate logistic regression models.

Results: The median age of the 61 patients was 42 years (range, 10–67). The median follow-up was 832 days after transplant (range, 159–1566). 21 (34%) transplantations were performed in patients who had early disease (first complete remission, first chronic-phase or untreated), 74% of them were from related donors and conditioning regimen was myeloablative in about 47% of the cases. According EORTC/MSG criteria, 9 patients had proven or probable IPA and the median time to diagnosis was +261 days after infusion (range, 32–1020). 50% of them were transplanted on complete remission and received a myeloablative conditioning regimen. At the time of IPA diagnosis, 6 patients were receiving double immunosuppressive therapy for graft-versus-host disease (GVhd) which included steroids in 5 of them. Two patients were neutropenic and on relapse after HSCT and 3 patients had a Cytomegalovirus reactivation concomitant to the IPA. In the univariate analysis, no risk factors significantly influenced the development of IPA. Five patients were receiving antifungal prophylaxis when IPA was diagnosed and none of them had previous fungal infection history. IPA-related mortality was 11% (1 patient). All patients were treated with Voriconazole alone or in combination with other antifungal drugs and the outcome were positive in all but one patient.

Conclusions: In our series the incidence of IPA was similar to the one reported by the literature, although the IPA-related mortality was much lower. We could not identify as risk factors for IPA those reported by others, probably due to the number of cases.

R1272
Importance of plasma concentration monitoring of itraconazole in prophylactic oral administration for allogeneic haematopoietic stem cell transplantation
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Objectives: Oral itraconazole (ITCZ) solution has been expected to prevent invasive fungal infections (IFIs) more effectively than capsule among allogeneic hematopoietic stem cell transplantation (HSCT) recipients, because of the superior bioavailability. However, it remains unclear whether reliable serum concentrations are maintained by the oral ITCZ solution in all patients at all times after HSCT. Therefore, we investigated the inter- and intrapatient variability by serial evaluation of the plasma ITCZ levels.

Patients and methods: Prophylactic administration of oral ITCZ solution at a once daily dose of 200 mg started more than 10 days before HSCT in 19 allogeneic HSCT recipients. Eleven patients received cord blood as the stem cell source, six grafts from unrelated donors and two from related donors, respectively. Sixteen patients received fludarabine-based reduced intensity conditioning regimen and the remaining three received the combination of TBI and cyclophosphamide (Cy) for conditioning. Patients continued prophylaxis with intravenous ITCZ at 200 mg/day, if oral medication was intolerable. Plasma samples were obtained within three days prior to initiating calcineurin inhibitors before HSCT, a week after HSCT and a month after HSCT. Trough levels of ITCZ and hydroxy-ITCZ in plasma were measured by using high-performance liquid chromatography.

Results: Median trough concentrations of ITCZ before HSCT, a week after and a month after HSCT were 275.23 ng/mL (range 8.00 to 2376.53 ng/mL), 297.86 ng/mL (range 37.90 to 1371.99 ng/mL), and 492.89 ng/mL (range 53.04 to 1185.17 ng/mL), respectively. Marked variations of ITCZ concentrations were observed among individuals at different periods after HSCT (Figure 1). The concentrations of >250 ng/mL and >500 ng/mL were achieved in 52.6% and 28.3% of patients before HSCT, 52.6% and 42.1% a week after, and 66.7% and 58.5% a month after HSCT. No serious adverse effect was observed in all three patients who received the Cy-included regimen while on ITCZ prophylaxis. One patient developed probable invasive aspergillosis 23 days after HSCT, with the lower concentration of 112.38 ng/mL one week after HSCT in spite of 438.77 ng/mL before HSCT.

Conclusions: Plasma concentration monitoring of ITCZ warrants consideration in allogeneic HSCT recipients, because of the marked inter- and intrapatient variability.

R1272
Cefpodoxim reduces febrile episodes in patients treated with stem cell transplantation during an aplastic period after transplantation
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Introduction: Antibacterial prophylaxis after stem cell transplantation is still controversial and it vary depends from local microflora from center to center. Introduction of chinolones during a first, aplastic period significantly reduced number of febrile episodes and mortality due to Gram negative bacilli. Emerg-
R1274 Early infectious complications in children after auto and allo-haematopoietic stem cell transplantsations – A single-centre experience

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Infections are important cause of morbidity and mortality after haematopoietic stem cell transplantation (HSCT). The aim of the study was analysis of frequency and severity of infections in auto and allo-HSCT settings in the first 30 days after HSCT.

Methods: Between 2002 and 2009 a group of 83 children were transplanted (56 auto, 27 allo-HSCT) and non malignant diseases in 11 cases (allo-HSCT). Conditioning and immunosupresion regimens were chosen according to EBMT protocols. In allo-HSCT myeloablatives protocols were used in 23 cases, 4 children were treated with reduced intensity conditioning. Nine pts from them had match unrelated donor transplantation. Stem cells (SC) were collected from peripheral blood in 58 pts (52 auto-HSCT, 3 allo-HSCT) and from bone marrow in 26 (2 allo-HSCT, 24 allo-HSCT). Two sources of SC were required in 5 pts. CD34+ cells were given in average of 3.9 × 10^6/kg in auto-HSCT and 4.07 × 10^6 in allo-HSCT.

WBC > 1000/ul was noticed in average after 12 and 16 days, respectively. During HSCT procedure all children were located in isolation rooms with laminar air flow. Infection prophylaxis composed of amoxicillin/clavuloniac, acyclovir, flukonazol (in auto-HSCT) + colistine (in allo-HSCT). CMV and EBV detection was performed every week after allo-HSCT.

Results: Fever of unknown origin was detected in 85% of auto-HSCT and 50% of allo-HSCT. Blood cultures were positive in 7% of auto-HSCT and 56% of allo-HSCT. One pts in both group (2% and 4%) was diagnosed with septic shock. Pneumonia was found in 18% and 7% respectively. Two pts in allo-HSCT group required mechanical ventilation. Positive urine culture was found in 7% and 11%, respectively. Fungal infections were recognized in 13% (auto-HSCT) and 48% (allo-HSCT). In allo-HSCT group preemptive CMV and EBV therapy was introduced in 26% and 4% respectively. CMV pneumonia was diagnosed in one pts (4%). Parasitic infection was found in 5% of auto-HSCT pts. We do not observed deaths due to infection in studied group.

Conclusion: FUO in the first 30 days was detected more often after auto-HSCT (engraftment syndrome?). Bacterial, fungal and viral infections were diagnosed mainly in allo-HSCT group (prolonged immunosupresion). As there was no infectious morbiti the management was satisfactory.

R1275 A comparison of the efficacy prophylactic ceftriaxone in patients managed at home after autologous stem cell transplantation using either BEAM or melphalan conditioning: a single-centre experience

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Background: Bacterial infections remain the major cause of morbidity in patients undergoing autologous stem cell transplantation (ASCT). In this study we analyze the efficacy of prophylaxis with ceftriaxone in patients managed at home receiving the two most commonly preparative regimens used in our institution.

Patients and methods: All patients were managed at home since day +1. We analyzed all patients treated with either BEAM (mg/m²) (BCNU 300, etoposide 800, cytarabine 800 and melphalan 140) or melphalan 200 mg/m². Antibacterial prophylaxis included levofloxacino 500 mg/24h p.o. and ceftriaxone 1 g/day i.v. started on day +1 and maintained until febrile

ing of Gram-positive cocci, especially Staphylococcus coagulasa negative, and multiresistant strains of Streptococcus was cause to add Beta lactams antibiotic in antibacterial prophylaxis in our center.

Patients and methods: During a nine years period we have treated 178 patients with different hematological malignancies. Autologous: 128 Allogeneic, from HLA identical donor: 50. All patients were treated in sterile room, conditioned with HEPA filters, low bacterial diet, and antibacterial prophylaxis consisted Ciprofloxacin 1,0gr divided in two doses, antymycotic prophylaxis with Flucunozol 400 mg, and virostatic Acyclovir 750 mg/day. Prophylaxis with Ceftriaxon 2.0gr. was started during conditioning period and was stoped if the patients became febrile, or until engraftment. The aim of this study was to evaluate incidence and duration of fever, and to evaluate bacterial infective complications in our patients.

Results: Incidence of fever in our group of patients was 56,1%, with duration of 3 days. The most frequent bacterial infective complications were: CVC-related infections (11,8%), neutropenic enterocolitis (8%), pneumonia (8,4%), perianal absces-sus (2,8%).

Conclusion: The incidence of fever in our group is significantly lower, compared with other transplant centers. This might be due to addition of cephalosporines during an early aplastic period after SCT.

R1273 Itraconazole versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patients receiving haematopoietic stem cell transplants

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Itraconazole is an azole antifungal that has activity against invasive fungal infections (IFI). However, the efficacy and safety of itraconazole in preventing IFI for patients undergoing hematopoietic stem cell transplantation (HSCT) is unclear. Especially, oral itraconazole was reported that was often discontinued due to intolerance such as diarrhea or vomiting. We performed a single-center prospective randomized trial on patients who received allogeneic and autologous HSCT to determine whether itraconazole prevents IFI better than fluconazole, which is standard medication for antifungal prophylaxis in early phases of HSCT. A total of 76 patients receiving HSCT were randomized to receive fluconazole (oral or intravenous 400 mg/day) or itraconazole (oral or intravenous 200 mg/day) from the day of HSCT until day 28. And, serum concentrations of cyclosporin A and tacrolimus were evaluated under the influence of itraconazole or fluconazole therapy on patients receiving allogeneic HSCT. Success was defined as the absence of proven or probable IFI through the end of the 4-week period after HSCT. After enrollment of the 76 patients, we evaluated 36 patients of the itraconazole arm and 37 patients of fluconazole arm, respectively. Ten of 73 patients developed severe hepatotoxicities (itraconazole:13.9%, fluconazole:13.5%) and no patient was discontinued from both itraconazole and fluconazole due to intolerance. No patient developed proven or probable IFI on both itraconazole and fluconazole during the intended study period. On the patients undergoing allogeneic HSCT (n=48), there were no difference in the concentration/dose ratio (median) between itraconazole + cyclosporin A (n=15) and fluconazole + cyclosporin A (n=12) (2.59 vs. 2.28, P=0.36), itraconazole + tacrolimus (n=8) and fluconazole + tacrolimus (n=13) (15.9 vs. 18.53, P=0.21) on prophylactic therapy for IFI. The overall efficacy and safety of itraconazole was similar to that of fluconazole as antifungal prophylaxis during the neutropenic phase after HSCT. This randomized trial demonstrated the efficacy and safety of itraconazole for antifungal prophylaxis in early phase of HSCT. It was shown that itraconazole was able to become one of choices for antifungal prophylaxis in early phase of HSCT.

S394
neutropenia or granulocyte count over 1 × 10⁹/l. Febrile neu-
tropenia was treated with piperacillin-tazobactam 4.5 g/8h i.v.
using a portable intermittent infusion pump. Teicoplanin was
added if oral mucositis of WHO grade 2–4, signs of infection at
the catheter insertion, or Gram-positive infection. Amikacin was
started if fever persisted more than 3 days or in case of Gram-
negative infection.

Results: Forty-five patients were included in this study: BEAM
(n=24, group A) and Melphalan (n=21, group B). Median
(range) peripheral CD34+ cell dose (×10⁶/kg) was 3.8 (1.5–
10.3) in A and 3 (1.9–5.8) in B (P=0.02). Median day (range)
before start of conditioning and recovery (days) of granulocytes
>0.5 × 10⁹/l was significantly faster in group A [11 (9–13) vs.
12 (10–26); P=0.004]. Recovery days of granulocytes
(0–6) in B (P=0.002). Recovery days of granulocytes
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(0–6) in B (P=0.002).

Fungal infection remains a severe complication despite the use
of new agents and the role of antifungal drug combination is
under consideration. We retrospectively studied 43 infections in
41 patients treated with combined treatment in order to evaluate
both efficacy and toxicity. The patients, aged 40 [12–63] years,
suffered from Acute Leukemia 33, MDS 2, Aplastic Anaemia 2,
NHL 1, CLL 1, H.D. 2. Twenty out of 41 underwent allogeneic HCT
(sibling 14, MUD 5, haploidentical 1) and 2 autologous HCT. Dur-
ing infection sixteen patients had refractory disease, 30/43 neu-
tropenia, 12/20 aGVHD, 13 extensive cGVHD and 1 graft failure.
Two patients developed concomitant viral infections. According
to EORTC criteria 2 patients had proven, 4 probable and 37/43
possible infection. The main sites involved were: 39/43 lungs,
3 CNS and sinuses, 1 skin. All patients had received antifun-
gal prophylaxis. The preceded antifungal treatment was admin-
istered at least 21 days without any response. The antifungal
combinations used were: voriconazole and caspofungin [V + C]
18, voriconazole and amphotericin [V + A] 15, amphotericin
and caspofungin [A+C] 10. Twelve of the patients without any over-
all response were further treated with another drug combination.
The total response was 63% [(V + C) 10/18, (V + A) 10/15, (A + C)
4/10] for the drug combinations used. The treatment lasted from
3 to 30 weeks. There was not significant toxicity, not even in the
long term treatment and no patient stopped the treatment. Nine
patients with resistant fungal infection died, 1 of pulmonary hemo-
rhage after lung fungal infection, 1 of CNS while on CR from
the original disease. The others had either refractory disease or
another infection. According to these data it seems that com-
bined antifungal therapy might be an effective option for severe
immunosuppressed patients with resistant fungal infection, since
63% responded with acceptable toxicity. The infection resolution
to patients allowed them to undergo HCT without any further
delay. The relatively small number of evaluated patients in this
retrospective study does not allow to come up with safe conclu-
sions. For the evaluation of efficacy of the combined therapy,
prospective studies are needed to establish its role.

R1276
Influence of intestinal bacterial decontamination using
ciprofloxacin plus metronidazole or moxifloxacin alone on the
incidence of acute GVHD and blood stream infections
after allogeneic HSCT
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It is well documented that bacterial decontamination with Cip-
rolfloxacin (2 × 750 mg/d) plus Metronidazole (3 × 400 mg/d)
reduces the severity of acute GVHD and supports the theory
that the intestinal anaerobic bacterial microflora is involved in
the pathogenesis of acute GVHD. Therefore, this decontami-
nation regimen became standard in our center. However, this
decontamination regimen is not feasible for patients receiv-
ing Treosulfan based conditioning. Since the combined use of
Treosulfan and Metronidazole results in a higher incidence of
skin toxicity. Thus, we used Moxifloxacin 400 mg daily alone
as decontamination regimen in 152 patients receiving Treo-
sulfan 42 g/m² and FluDarabine 150 mg/m² based conditioning
and compared the results to 228 patients conditioned with TBI
10 Gy and FluDarabine 150 mg/m² receiving standard bacte-
rial decontamination. Analysed patients were transplanted
between 03/2003 and 12/2007 and base line patient charac-
teristics including diagnosis, disease stage, age of patient and
donor, HLA-matching, donor type and stem cell source were
equally distributed in the two groups. Primary endpoints of
analysis were incidence of acute GVHD grade II and higher,
break-through infections, and CDC defined toxicities. The
incidence of severe acute GVHD, break-through infections
as well skin and other toxicities did not differ between the two
arms as revealed by uni- and multivariate statistical analysis.
In conclusion, Moxifloxacin alone is feasible for bacterial gut
decontamination in patients receiving a Treosulfan based con-
ditioning regimen.

R1277
Combined antifungal treatment in patients with haemato-
logical disease and haematopoietic cell transplantation
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Haematopoietic progenitor cell transplantation is the treatment
of choice for patients with malignant haematological diseases.
Neutrophils (NEUT) and platelets (PLT) counts are used to
evaluate the haematological engraftment. Last generation haematology analyzers offer an alternative way to
evaluate the peripheral blood (PB) immature cell fractions, which
may also give indications of haematopoietic recovery. Amongst
these, the immature reticulocyte fraction (IRF) and immature
platelet fraction (IPF), in PB samples, could play an important role
as early indicators of NEUT and PLT engraftment, respectively.
In the present study we evaluated the predictive value of IRF and
IPF, in the haematological recovery of 118 adult patients
undergoing autologous and allogeneic PB progenitor cells
(PBPC) transplantation. Patients were followed since pre-condi-
tioning and throughout all hospitalization period.

Early side effects / Late effects and quality of life

R1278
IRF and IPF as predictors of haematopoietic recovery
after autologous and allogeneic stem cell transplantation
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Comparing median days to recovery for classical parameters and new parameters, in autografted patients, we noticed an anticipation by one day in both immaturity parameters studied: 10 days for NEUT recovery vs. 9 days for IRF and 11 days for PLT recovery vs. 10 days for IPF. We observed significant correlations ($P<0.05$) between NEUT and PLT recoveries, but also between NEUT and IRF recoveries, PLT and IRF, PLT and IPF and between the new parameters studied (IRF and IPF).

For allografted patients we detected an anticipation of IRF recovery by 4 days in comparison with NEUT recovery (11 vs. 15 days), and by one day of IPF in comparison with PLT (10 vs. 11 days). Within this group, we found that the recovery of the parameters was different for patients submitted to non myeloablative regimens (NMA), since we observed an early IRF recovery by 5 days in comparison with NEUT (10 vs. 15 days) and a IPF recovery by two days (9 vs. 11 days), in comparison with PLT. We also detected significant correlations between NEUT and PLT recovery and between these and the new parameters: NEUT and IRF, NEUT and IPF, PLT and IRF, PLT and IPF recoveries along with the correlation between the new parameters, IRF and IPF.

With this study we conclude that IRF and IPF can predict with some anticipation the haematopoietic recovery. For allografted patients following NMA regimens that prediction is even more noteworthy and clinically appears relevant. These immaturity fractions open new perspectives in monitoring patient transplantional support through post-transplant recovery.

R1279
A study of pain, peripheral neuropathy and psychosocial late effects in patients with intensively treated advanced multiple myeloma
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Background: Whilst modern treatments have significantly extended life expectancy in myeloma, there is accumulating evidence that patients are at risk of physical and psychosocial consequences following completion of treatment leading to a reduced quality of life (QoL).

Aim: The aims of this study were to define quantitatively and qualitatively, the spectrum of pain, peripheral neuropathy and psychosocial issues affecting patients living with advanced relapsed myeloma.

Methods: We recruited patients with symptomatic myeloma who had initially received a transplant and at least one subsequent treatment for progressive disease, and were in plateau phase to a prospective, cross-sectional, exploratory study. All patients completed measures of general and myeloma specific quality of life, pain and neuropathic symptoms. Semi-structured interviews were conducted to understand patients’ experiences.

Results: To date, 21 patients’ data (median age 59; 8 males) have been analysed. Median duration in years from diagnosis was 7 (range 2–12) and 18 (86%) had undergone autologous HSCT. From the European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30, amongst the functioning scales, role and social functioning were low. From the EORTC QLQ-MY20, patients reported paraesthesia. This was supported by the neurological examination: 13 (62%) and 9 (43%) had signs of sensory or motor neuropathy respectively. The Self Report-Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS) confirmed probable neuropathy in 10 (47%). Brief Pain Inventory median score for average pain was 4 (range 0–10); pain interfered with work outside the home and housework (median 3.5; range 0–10).

13 patients were interviewed and 7 (54%) reported complications of chemotherapy, one (8%) reported it to be much lower and 5 (38%) reported no change. 12 (92%) patients had experienced anxiety, bitterness, anger or depression since becoming ill. A variety of coping methods were adopted by these patients in order to adapt to their illness include changing expectations, distractions, optimism, and a good social support.

Conclusion: In the modern management of advanced intensively treated myeloma, patients are compromised due to a complex set of pain and psychosocial complications related to myeloma and its treatment. Management strategies should actively address these issues to maintain quality of life in this expanding population of patients.

R1280
Development of squamous cell carcinoma in uncommon oral sites following allogeneic bone marrow transplantation (BMT)
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Objective: This report describes two cases of occurrence of squamous cell carcinoma (SCC) in uncommon oral sites following allogeneic bone marrow transplantation (BMT).

Background: Less than 1.5% of diagnosed oral SCC cancers arise on the vermilion border of the upper lip and the midline dorsum of the tongue, combined. It is considered very rare for SCC to develop in either of these two locations. There is a reported increased risk of oral SCC, especially of the buccal mucosa, in the years following allogeneic BMT. The increased incidence of oral SCC post-BMT has been associated with graft-versus-host disease (GVHD) and treatment with cyclosporine.

Methods: The following cases describe patients with a history of matched-related donor peripheral stem cell transplants. Both patients developed biopsy-proven GVHD and were treated with cyclosporine for 1–2 years. Case 1 involves a 41 year old male with stage II IgA multiple myeloma. He had a long history of tobacco use. One year post-BMT, he developed a painful, ulcerative lesion on the vermilion border of the upper lip that progressed into an indurated nodule. Biopsy of the lesion revealed well-differentiated SCC and the cancer was staged T1N0. The patient’s upper lip was subsequently treated with radiotherapy and the cancer did not recur. Case 2 involves an 18 year old male with AML. The patient had a fair complexion and a history of sun exposure. Nine years post-BMT, he developed a crusty lesion on the vermilion border of the upper lip that progressed into an indurated nodule. Biopsy of the lesion revealed well-differentiated SCC and the cancer was staged T1N0. The patient’s upper lip was subsequently treated with radiotherapy and the cancer did not recur. Case 2 involves a 41 year old male with stage II IgA multiple myeloma. He had a long history of tobacco use. One year post-BMT, he developed a painful, ulcerative lesion on the midline dorsum of the tongue. The lesion was biopsied with histopathology revealing poorly-differentiated carcinoma. The cancer was staged T3N0 and he subsequently received palliative chemotherapy.

Results: Both patients had risk factors for the development of SCC and received immunosuppressive drug therapy for GVHD. In both cases, the initial clinical presentations were similar to oral GVHD and therefore, were not immediately biopsied.

Conclusion: Periodic careful surveillance of the oral cavity should be an integral component of the post-BMT care so that oral SCC can be detected in its early stages. Persistent oral lesions should be biopsied after 2–3 weeks if they do not recur. Case 1 involves a 41 year old male with stage II IgA multiple myeloma. He had a long history of tobacco use. One year post-BMT, he developed a painful, ulcerative lesion on the vermilion border of the upper lip that progressed into an indurated nodule. Biopsy of the lesion revealed well-differentiated SCC and the cancer was staged T1N0. The patient’s upper lip was subsequently treated with radiotherapy and the cancer did not recur. Case 2 involves an 18 year old male with AML. The patient had a fair complexion and a history of sun exposure. Nine years post-BMT, he developed a crusty lesion on the vermilion border of the upper lip that progressed into an indurated nodule. Biopsy of the lesion revealed well-differentiated SCC and the cancer was staged T1N0. The patient’s upper lip was subsequently treated with radiotherapy and the cancer did not recur. Case 2 involves a 41 year old male with stage II IgA multiple myeloma. He had a long history of tobacco use. One year post-BMT, he developed a painful, ulcerative lesion on the midline dorsum of the tongue. The lesion was biopsied with histopathology revealing poorly-differentiated carcinoma. The cancer was staged T3N0 and he subsequently received palliative chemotherapy.

R1281
A study of the endocrine, metabolic and nutritional late effects in patients with advanced intensively treated multiple myeloma
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Background: Modern treatment strategies in myeloma, including haemopoietic stem cell transplantation (HSCT), have increased life expectancy, but late effects of treatment and disease have not been studied systematically.

Objectives: To define and characterise the spectrum of endocrine, metabolic and nutritional issues in intensively treated advanced but stable myeloma.

S396
Methods: We recruited patients with symptomatic myeloma who had received a transplant as part of initial treatment, and at least one subsequent treatment for progressive disease, who were in plateau phase to a prospective, cross-sectional, exploratory study. Patients were extensively screened for endocrine, metabolic and nutritional parameters. Abnormal values were based on locally or published references.

Results: To date, data from 21 patients (median age 59, range 47–71, 13 females) have been analysed. Median duration in years from diagnosis was 7 (range 2–12) and 18 (86%) had undergone autologous HSCT and 3 (14%) allogeneic HSCT. 7 (33%) had 2 HSCT procedures. Other treatments varied between patients: 9 (43%) thalidomide, 11 (52%) bortezomib, 21 (100%) vincristine and 1 (5%) lenalidomide. All patients had received high dose steroids.

Endocrine testing identified 3 pts (14%) were hypothyroid and 4/8 males (50%) hypogonadal. Morning cortisol level and Synacthen tests were normal in all pts. In 4/13 females raised prolactin levels were noted. Body mass index (BMI) was > 25 kg/m² in 7 (87%) males and 6 (46%) in females. In non-fasting state, 8 (38%) had total cholesterol >5 mmol/L. 2 patients (10%) had reduced B12 and 5 patients (24%) borderline/low serum folate. Ferritin levels were raised in 10 (48%) and 9 (43%) had vitamin D insufficiency. All patients had received high dose steroids.

Conclusion: In this relatively small sample of intensively treated (including HSCT) advanced stage but stable myeloma patients, endocrine, metabolic and nutritional abnormalities were common. Screening and interventional strategies should be investigated further in the effective management of the consequences of advanced myeloma and its treatment with the aim of prolonging survival and optimising quality of life.

R1282
Early relevation of body composition changes in paediatric patients following haematopoietic stem cell transplantation using dual-energy x-ray absorptiometry
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Objectives: A rapid decrease in body weight and a change in body composition (BC) following haematopoietic stem cell transplantation (HSCT) in children is a common side effect but there is no single universally recommended method for body composition assessment and nowadays is difficult to carefully evaluate these changes in transplanted children. We investigated early body composition changes in children who received HSCT using a dual-energy x-ray absorptiometry (DXA). DXA combines non-invasive assessment of bone and soft tissue in a three-compartment model: lean tissue mass (LTM), fat tissue mass (FTM) and bone mineral content (BMC). It assesses both whole and segmental body composition (i.e. trunk, arms, legs, ginoid, android) (Figure 1).

Material and methods: Ten patients (pts) affected by hematological malignancies and submitted to HSCT were evaluated before and after a median time of 4 months after HSCT. Seven pts received an allogeneic and 3 an autologous HSCT. Taking a median value of reference, age was 10 years, weight 34.5 kg, high was 143.25 cm and body mass index (BMI) 21.25 with a median BMI SDS of 0.37 (min -0.04 max 1.49) normalized by sex and age. DXA relevation regarded: LTM, FTM, percent of fat and BMC.

Results: 8 patients were evaluable for the analysis (2 pts died before the second evaluation). After a median time of 4 months after HSCT we noted a decreasing in the median LTM from 25.795 kg to 24.375. No substantial variation of median FTM, percent of fat and BMC was revealed, being respectively: from 14,283 to 14,024 kg, 25,1% to 23,7% and 1,321 kg to 1,314 kg. Trunk was the body compartment more involved in LTM impairment, with a median decrease of 5%, while arms and legs seemed to maintain the same composition pre and post HSCT.

Conclusions: In our experience a decreasing of LTM without significant changing of FTM and BMC was detected by DXA after a median time of 4 months after HSCT. In the absence of a ‘gold standard’ to determine body composition changes in children after HSCT, DXA represents an easy non-invasive and safe method that could reveal early modification, especially regarding LTM, of body composition. Furthermore, on the basis of these observations a prospective randomized trial based on a differential nutritional support with an higher protein intake to avoid LTM impairment, started in our Center.
R1283
Prospective single-centre study of oral mucositis incidence and complications in autologous stem cell transplantation

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Oral mucositis (OM) is a frequent adverse effect of stem cell transplantation which induces severe discomfort, malnutrition and increases the risk of infections. A prospective multicentric study of the EBMT Mucositis Advisory Group has recently reported an incidence of OM of 46% and 42% in Multiple Myeloma (MM) and non Hodgkin’s Lymphoma patients respectively receiving autologous stem cell transplantation (ASCT). Objective: primarily we evaluated the incidence of OM in our center. We also investigated clinical factors predictive of OM development. Finally we evaluated the incidence of the main OM complications (total parenteral nutrition – TPN, diarrhea, infections and opioids use) in OM patients.

Methods: Between January 2007 and December 2008 we conducted a prospective study including 99 cases receiving ASCT for Lymphoma (38 cases) or MM (61 cases). The median age of the study population was 53 years old (16–60). Male/Female ratio was 55/44. Conditioning regimen was low dose Melphalan (<120 mg/mq) in 12 patients, High dose Melphalan (>120 mg/ mq) in 46 and polychemotherapy (BEAM or BAVC regimens) in 41; 27 patients received Rituximab during ASCT. We subdivided OM patients in mild form (WHO grade 1–2) and severe form (grade 3–4). Data were analyzed by t test, Pearson correlation test and ANOVA test. Populations were compared by chi-squared test.

Results: OM of any grade occurred in 48.5% of ASCT. Nineteen patients received double ASCT. 84.2% of cases developed at least one episode of OM and 47.4% suffered from OM during both ASCTs. Mild OM and severe OM occurred in 56.2% and 43.8% of cases respectively; median duration of OM was 8 days (2–24). The incidence of OM was not related with disease, gender, preparative regimens and Rituximab use. Duration of neutropenia appeared to influence duration of OM (P < 0.05, r2 0.3). TPN was needed in 62% of patients with OM. The incidence of TPN use was not statistically different in mild and severe OM patients, as well as for fever and diarrhea rate. Opioid analogesics were used in 54% of OM patients; their use was more frequent in severe OM patients (P = 0.02). Finally the infectious rate in OM cohort was not different from ASCT patients without OM.

Conclusions: The incidence of OM in our centre is similar to other recent studies. Age, gender, preparative regimen were not study the OM development. Finally, OM severity did not influence the occurrence of complications except for the use of opioid analogesics.

R1284
The use of spirometry for the diagnostic of pulmonary injury after allogeneic bone marrow transplantation in patients with leukemia

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Background: It is known that pulmonary injury are serious complications after bone marrow transplantation (BMT). According to published date their frequency achieves 40–60%, and LONIPCs (late-onset non-infections pulmonary complications) incidence 10–26%.

The aim: to estimate the frequency, character of spirometry data in different periods of allogeneic BMT and especially in the pts with chronic GVHD.

Methods and materials: The spirometry tests were performed using a national spirometer SMP-21/01 «R-D» with value 15 indexes, such as forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), total lung capacity (TLS), the ratio of forced expiratory flow between 25% and 75% of FVC to FVC (FEF/FVC) and oth. We observed 71 patients undergoing allogeneic BMT from HLA – identical siblings: M/F 37/34, age 17-64 years, AL–39, CML–24, MDS–7 and AA–1. Myeloablative conditioning was used in 54 pts and nonmyeloablative in 17 pts. Before BMT spirometry tests were performed in 37 pts, during of the first year–38 pts and later 45 pts. Total number of tests was 237.

Results: The pulmonary functional alteration seen in BMT recipients were follows: restrictive, obstructive and combined. Before BMT 5 pts (13.5%) had alterations: 2 restrictive and 3 obstructive, main EBMT Myeloablative therapy and infections result. After BMT the alterations were revealed in 27 pts (38%): restrictive in 7 pts, obstructive in 7 pts and both – in 13 pts. After 24 mo we generally observed combined alterations. Constant alterations were predominantly in pts with chronic GVHD (n = 35), combined – in 18 pts (51%). There were no adequate correlations between the spirometry results before BMT, in early period after BMT and development later the severity lung complications. We did not find out significant difference between the pulmonary alterations in pts with myeloablative and reduced intensity conditioning regimens. In most pts with restrictive alterations severity clinical symptoms of pulmonary insufficiency was not observed and intensive respiratory therapy (IT) was successful. Improvement or stabilization of process for obstructive findings were estimated only in part of the patients by combined long IT. Most of severity clinical symptoms were in pts with combinative alterations and the effect of IT was absent.

Conclusion: We believe that spirometry is important method for the detection of pulmonary injury in BMT recipients. This method can be used for strategy of IT definition.

R1285
Hyperfractionate 12 Gy TBI versus no-TBI in myeloablative conditioning regimen: a retrospective analysis on 83 consecutive patients submitted to allogeneic haematoepoietic stem cell transplantation in a single-centre

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Introduction: Total Body Irradiation (TBI) is actually employed in many conditioning regimens for allogeneic Haematopoietic Stem Cell transplantation (HSCT). Particularly, TBI is used in younger patients, in Lymphoblastic Leukemia (ALL) patients, in matched unrelated donor (MUD), cord blood and haploidentical HSCT. Conditioning regimens without TBI are instead preferred in the other remaining situations. Despite this consolidated uses, there are not studies which clearly demonstrate the superiority of TBI in HSCT conditioning.

Aim of the study: We retrospectively analyzed our casuistic of allogeneic HSCTs of the last 6 years in order to compare the outcome of patients who received TBI and patients who did not received TBI in conditioning regimen.

Methods: We analyzed 83 patients who received ablative conditioning regimen for sibling related, MUD, haploidentical and cord blood HSCT. 35/83 patients received conditioning regimen with hyperfractionated TBI 12 Gy, the remaining 48/83 patients did not received TBI. We evaluated age, incidence of mucositis, infections and Graft vs Host Disease (GVHD), Transplant Related Mortality (TRM), late toxicity, relapse incidence and Overall Survival (OS).

Results: Patients who received TBI were younger that patients who did not received TBI (median age 39,1 vs. 47,4). Statistically significant differences have been found in incidence and severity of mucositis (median grade 3,4 vs. 2,5, WHO scale), incidence of infections (37% vs. 17%) and OS (median 881 days vs. 1429 days). There was no difference in relapse incidence, TRM, febrile events and late toxicity and concerning the incidence and severity of acute and chronic GVHD.

Conclusion: Patients who did not received TBI had a better outcome respect to patients who received TBI in term of OS also
if statistically significant difference is not reached in TRM and relapse incidence. Early complications (infections and mucositis) are commonest in TBI patients. Instead no difference has been seen in late complications (GVHD and late effects). These data can be partially explained with the difference in term of disease (TBI patients had more advanced disease) and the kind of transplant (11/35 TBI patients received haploidentical and cord HSCT). Overall TBI inclusion in conditioning regimen seems not useful in improving the outcome of these patients. Large prospective studies need to explore the real necessity to employ TBI in conditioning regimen in allogeneic transplant setting.

R1286
Cyclosporine-induced posterior reversible encephalopathy syndrome after allogeneic bone marrow transplantation: safe reintroduction of cyclosporine at lower dose
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Posterior reversible encephalopathy syndrome (PRES) is a rare central nervous system complication in childhood hematologic-oncologic patients (pts). The precise etiology of PRES is not well known, but the most important reported causes are hypertensive encephalopathy, preeclampsia/eclampsia, immunosuppressive agents such as ciclosporin (CsA) and tacrolimus and uremic encephalopathies.
Allogeneic bone marrow transplantation (BMT) from an HLA identical brother was performed in a 10 years old boy for an acute lymphoblastic leukemia (ALL) in second complete remission (CR). Conditioning regimen consisted in fractionated total body irradiation (12Gy) and cyclophosphamide (120 mg/kg). The patient (pt) was allografted on June 6, 2009 (day 0). Graft versus host disease (GVHD) prophylaxis associated CsA started at d-1to methotrexate given on days 1, 3 and 6. Neutrophile recovery occurred on d21. On d32, the pt was on antinfectious prophylactic therapy per os associating pencillin G, fluconazole, valaciclovir and sulfamethoxazole-trimethoprim, and anti GVHD prophylaxis with CsA. He developed cutaneous grade 3 GVHD. Prednisolone was started on d33. On d34, the pt developed headache and borderline hypertension followed by generalized convulsion which was controlled by phenytoin. Cs A level was high (567 ng/mL). MRI of the brain showed cortical high-signal intensity on fluid attenuated inversion recovery (FLAIR) and T2-weighted images involving the temporal, parietal and occipital lobes, and to lesser extent to the frontal lobes. Elec
troencephalogram showed slowing of the posterior background rhythm and sharp activity in both occipital areas. CsA was stopped. Neurological symptoms resolved. GVHD regressed progressively and prednisolone was tapered. A second MRI of the brain done on d54 was normal. CsA was reintroduced on d64 with a CsA level within the therapeutic ranges. The pt was discharged home on CsA, prednisone, fluconazole, valaciclovir and sulfamethoxazole-trimethoprim. He is alive in CR with no particular complication.
This is a rare case of PRES due to CsA in a boy with ALL after allogeneic BMT. Management of these pts with GVHD and PRES is difficult concerning immunosuppressive therapy. This case proved that the reintroduction of CsA at lower dose is feasible and safe.

R1287
Ultrasonography evaluation with study of vascular hepatic flow before transplantation is useful in liver failure occurring after haemopoietic stem cell transplantation
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Introduction: Hepatic failure is a relatively common problem occurring after haemopoietic stem cell transplantation (HSCT). Veno-Occlusive-Disease (VOD), acute Graft-versus-Host-Disease (aGVHD), mucositis of biliar vessels, viral infections and drug toxicity are the most common ones. Particularly VOD occurs in first days after transplantation and is characterised from the thrombotic occlusion of intra-hepatic venous vessels. VOD determines the reduction or the alteration of the venous haematic flow in the sovra-hepatic veins and if not early diagnosed and treated can be fatal.
Aim of the study: We introduced in our transplantation protocol a basal ultrasonografy evaluation of liver and blood flow in hepatic vessels in order to compare the situation before and after the appearance of an hepatic failure.
Patients and methods: In 79 patients submitted to HSCT we evaluated a basal liver ultrasonografy immediately before the conditioning regimen. Ultrasonography evaluated hepatic and splenic parenchima and blood flow in splancnic vessels (sovra-hepatic, porta and splenic veins and hepatic arteria). 5 of these patients in the first three weeks after HSCT presented hepatic failure, and particularly increase in bilirubin level, so we performed a second ultrasonografic control in order to evaluate differences in the parameters initially considered.
Results: 3/5 patients who were re-evaluated did not presented any significant variation in ultrasonografic parameters: clinical data and this smrutamental evidence were suggestive for an hepatic failure secondary to drug toxicity. Instead 2/5 patients presented significant variations in venous sovra-hepatic flow associated to moderate liver enlargement: these data associated to clin
cial ones were suggestive for the diagnosis of VOD. These two patients were treated for VOD with a favourable outcome.
Discussion: The basal ultrasonographic study of liver and vessel flow results useful in HSCT patients because it give the initial parameters necessary for comparison in case of hepatic failure. Particularly, data about vessel flow are useful to discriminate VOD from toxic hepatic failure.

R1288
Long-term survival after stem cell transplantation for haematological malignancies: single-centre experience
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Stem cell transplantation (SCT) is widely used treatment approach for patients with hematological malignancies. In this study we present our results in 9 years experience in transplantation for long term survival of patients treated with this approach. A total of 172 transplants have been analyzed, 49 allogeneic sibling and 123 autologous SCT. In the group of patients treated with allogeneic SCT, 40% of patients were in active disease prior transplanta
tion, with diagnosis (37 AML; 6 CML; 2 AA; 1 NHL; 1 CLL; 2 ALL); median age 34 years (20–54). Bone marrow (BM) as source of HSC was used in 4 patients and 45 were preformed with periph
eral blood stem cells (PBSC). Conditioning was provided with Bu/Cy (35 pts), Bu/Cy+Mel (7 pts), BEAM (2 pts), hdICE (1 pts), nonmyeloablative FLAG/Ida (4 pts), FluMel (1 pt). The amount of infused fresh bone marrow was 1010ml (950–1100ml) with MNC 3.5 × 10^{10}/kg (2.5–4.5) and PBSC 4.32 × 10^{10}/kg (2.5–6.2). Median number of transplused blood products was for Er median 3 doses (0–6) and Pt 19 doses (5–34). Engraftment for Ne>0.5 × 10^{10}/L and Pt>20 × 10^{10}/L was recognized on day 12 (10–24) and the incidence of acute GVHD gr II and III was 30% and the incidence of chronic extensive GVHD was 20%. In the second group of 123 autologous recipients, 27 patients with diagnosis (19 AML; 4 NHL; 3 HD; 1 ALL) received fresh BM as source stem cells and the other group of 96 patients (15 AML; 3 ALL; 17 HD; 13 NHL; 22 MM) received PBSC previously mobilized with G-CSF chemotherapy. Median age was 35 years (7–63). In 40% of patients with limiproliferative diseases were with refractory/relapsed disease and other patients were in complete remission before SCT. Conditioning regimens consisted of high-dose chemotherapy mainly Bu-Cy, BEAM, ICE high-dose, Mitophalan. The amount of infused fresh BM was 1010 ml (950–1250 ml) with MNC 4.0 × 10^{10}/kg.
(2.9–5.8) and PBSC 3.85 × 10^8/kg (1.71–6,0). Median number of transfused blood products was for Er median 3 doses (0–6) and Plt 35 doses (0–73). Engraftment for Ne > 0.5 × 10^9/L and Plt > 20 × 10^9/L was recognized on + 15 (8–25). Median follow up for both groups was 55 months (3–108), TRM in allogeneic recipients was 28 years (range 20–43) and donors 33 (19–43), 1 from MRD performed for PNH in 2004-2009. Median age of allo-HCT in PNH. We report 15 allo-HCTs: 14 from MUD and 1 from MRD performed for PNH in 2004-2009. Median age of recipients was 28 years (range 20–43) and donors 33 (19–43), median time from diagnosis to allo-HCT was 16 (2–97) months. Median number of transfused RBC and platelets units was 7 (1–12) and 7 (3–15). All pts engrafted, median counts of granulocytes 1.0 G/l, platelets 50 G/l and Hb 10 g/dL were achieved on days 16 (12–27), 18 (9–39) and 30 (16–50). Acute GVHD grade I, II and III was present in 7, 4 and 1 pt, limited chronic GVHD in 4 pts. Complete chimerism was reached at day + 30 in all cases. Two patients were diagnosed of acute GVHD: one grade II cutaneous and the other grade III skin and gut GVHD. Disease status at day +100 was CR in 2 and improved PR in 1, which converted to CR at +180d. One patient (secondary AML) has relapsed after +20m and died in progression. The other 2 patients continue in CR after + 10 and +9 months.

Conclusion: Haploidentical bone marrow transplant with sub-mioablative conditioning and high dose cyclophosphamide post progenitors infusion is a feasible and well tolerated procedure that can lead to disease control in highly treated and selected patients lacking an appropriate donor.

**R1290**

Haploidentical non-myoablative bone marrow transplant with post-infusion high-dose cyclophosphamide as GvHD prophylaxis in high-risk patients

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Introduction: Only 25-30% of patients with hematologic tumours in which an allogeneic transplant is indicated have an HLA identical sibling donor. Around 60–70% of searches find a suitable unrelated donor. Some candidate patients don’t have an appropriate donor and new strategies are needed. Haploidentical family donors have emerged as an alternative with the advantages of rapid availability, donor implication and easier logistic issues in comparison to unrelated donors.

Patients and methods: We report our initial experience with a new haploidential transplantation procedure using bone marrow and post-infusion high dose cyclophosphamide (50 mg/kg iv) as graft-vs-host disease (GVHD) prophylaxis on days +3 y +4 with a combination of cyclosporine and mycophenolate, as previously described by Luzzini L et al (BBMT 2008; 14:641–650). Our sub-mioablative conditioning was slightly modified from the original and consisted of i.v fludarabine 30 mg/m² (days -6 to -2), i.v cyclophosphamide 14.5 mg/kg (days -6 and -5) and i.v busulfan 3.2 mg/kg (day -3) instead of 200 Gy total body irradiation.

Results: Four patients diagnosed of high risk hematologic tumours lacking an alternative donor have been transplanted with this procedure. Main characteristics are displayed on Table 1. They received 0.6 to 4.4 × 10^9 TNC/kg and 1.9 to 4.7 × 10^9 CD34+ kg. Myeloid engraftment occurred between days +13 and +20 and platelets >20fK from + 25 to + 31d. Main toxicities were grade II mucositis in 3 cases and febrile neutropenia in 4. One patient (refractory ALL) died on day +15 due to VOD without engraftment. Complete chimerism was reached at day +30 in all cases. Two patients were diagnosed of acute GVHD: one grade II cutaneous resolved after topic and short course systemic steroids and the other with skin grade II and gut grade III GVHD. Disease status at day +100 was CR in 2 and improved PR in 1, which converted to CR at +180d. One patient (secondary AML) has relapsed after +20m and died in progression. The other 2 patients continue in CR after + 10 and +9 months.

Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN), formerly known as blastic NK cell lymphoma, is a rare CD4 + CD56 + hematopoietic malignancy usually presenting with cutaneous lesions associated with or without a leukemic component. The disease irrespective of the initial pattern has an aggressive clinical course, demonstrating only a brief response to acute leukemia (AL)-type chemotherapy. Long term survival has been documented in sporadic cases following allogeneic stem cell transplantation (alloSCT) and represents the only treatment with the potential for cure.

Aim: To present the outcome of five patients with BPDCN who underwent alloSCT with a myeloablative conditioning regimen.

Patients: Five patients (M =3,F =2), with a median age at the time of transplantation of 27 years (range 15–33 years). Initial presentation of disease involved skin and bone marrow in three patients, isolated cutaneous nodules in one patient, and peripheral lymph nodes and bone marrow in one patient. Four patients were treated with an AL-type induction regimen, while the patient with only primary cutaneous disease was initially treated with involved radiation therapy + CHOP followed by AL-type therapy at relapse.

Results: Median time from diagnosis to transplantation was 7 months (range, 5–21). Three patients underwent alloSCT in CR1, one in CR2 and one in first chemosensitive relapse. All
patients received a myeloablative conditioning regimen (4 = TBI/Cy, 1 = BU/Cy), followed by infusion of PBSCs from a matched unrelated donor. Four patients developed grade II aGVHD. Two patients died of treatment related causes (graft failure/aspergillosis at 6 months, cGVHD/multi-organ failure and sepsis at 16 months) with disease in remission. Two patients died of disease relapse at 5 and 21 months. One patient survives at +41 months in remission without GVHD.

Discussion: BPDCN is an aggressive malignancy with poor long-term response to conventional chemotherapy alone. Although alloSCT seems to offer the only prospect of long-term survival and cure, our limited experience demonstrates that transplant-related mortality and disease relapse remain significant adverse factors for outcome.

R1292
Autologous peripheral blood stem cell transplantation in a patient with POEMS syndrome
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POEMS (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes) syndrome is a rare cause of demyelinating and axonal mixed neuropathy associated with monoclonal plasma cell proliferative disorder. Diagnosis of POEMS syndrome requires two major criteria, including a monoclonal plasma-proliferative disorder and polyneuropathy, in addition to, at least, one minor criterion: sclerotic bone lesions, organomegaly, endocrinopathy, papilledema, and skin changes. Acute ischemic strokes and myocardial infarcts occasionally have been reported. The mainstay of therapy is irradiation of a single, dominant osteosclerotic lesion and alkylator-based therapy, preferably high dose melphalan with peripheral blood stem cell rescue in younger patients with widespread osteosclerotic lesions. We report a 45-year-old female patient, admitted to the hospital with symmetric sensory-motor peripheral neuropathy that began in her toes with a gradual proximal spread to her upper limbs, that confined her to a wheelchair. An IgG lambda monoclonal gammapathy was detected in plasma immunofixation. The skeletal survey showed multiple sclerotic bone lesions (thoracic and lumbar vertebrae, pelvis, humerus, femur, ribs). Additional features included hypogonadotrophic hypogonadism, pleural effusion, acute myocardial infarction and apical endocardial thrombus, hypertrichosis and clubbing. The diagnostic of POEMS syndrome was made by open biopsy of a osteosclerotic vertebral lesion with revealed monoclonal lambda plasma cell infiltration. She was treated with high-dose melphalan (200 mg/m²) rescued by peripheral blood hematopoetic cells, despite congestive heart failure (Ejection Function 40%) complicating myocardial infarction. The patient refers an improvement of the sensory-motor function and waits neurological and endocrine assessment at day 100 of transplant. This report demonstrates that autologous stem cell transplantation is a feasible and effective therapy even in patients with heart failure.

R1293
Life-threatening complications suspected due to clinical pharmacokinetic interaction between cyclosporine A and triazole antifungal agents in allogeneic haematopoietic stem cell transplantation recipients
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Background: It is well documented that the co-medication with the triazole antifungal agents is associated with a flattening of the CSA blood concentration profile via the cytochrome P450 3A4 dependent metabolic pathway in allo-HSCT. Then it raises the question if the therapeutic monitoring of CsA trough levels is sufficient enough to reflect the drug exposure and to assess the transplant-related complications like transplant rejection and drug toxicity.

Objectives: To evaluate the tolerability, toxicity and clinical outcome of the co-administration of CSA and triazole antifungal agents in allo-HSCT recipients.

Patients and methods: A retrospective review of the medical records of 104 consecutive patients undergoing allo-HSCT for hematologic malignancies at our transplant center over past 5 years was conducted. The causality of administration of CSA in combination with triazole in 12 cases with life-threatening complications experiencing supertherapeutic trough levels of CSA were identified and analyzed.

Results: In all these 12 patients, the CSA trough levels remained highly than therapeutic range even after gradual tapering of CSA dosage. Shortly after the engraftment, 6 (50%) developed acute graft-versus-host disease (aGVHD) and non-infectious pulmonary complication, and 2 (16.66%) acute graft rejection. Which indicates the trough plasma concentration might be inadequately reflect CSA absorption profile. Whereas, 4 (33.33%) were evaluated with neurological complications, 1 (8.33%) cardiovascular complication accompanied by acute renal failure and liver dysfunction, that might be ascribed to CSA-related adverse effects. 10 (83.33%) out of the 12 patients eventually died, 2 (16.66%) are still alive after graft rejecting. The major causes of death were GVHD related pulmonary injury with fungal infection.

Discussion: Although preemptive CSA dosage reduction and close monitoring of its whole blood trough levels may minimize the drug-toxicity when co-administration with triazole antifungal agents, the different clinical spectrum and different disease evolution post transplant in this group warn that in the setting of allo-HSCT with CSA as immunosuppression agent the individual variables influence the drug-exposure and drug-toxicity. Therefore, as clinicians we should not be mislead by trough plasma concentrations as a routine therapeutic drug monitoring in terms of CSA exposure related efficacy or toxicity, especially when CSA in combination with triazole.

R1294
Quality of life post blood and marrow transplantation correlates with socio-demographics and pre-transplant expectations
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Objectives: We report a longitudinal study to measure patient health-related quality of life (HRQOL) immediately before and 6 months post- hematopoietic stem cell transplant (HSCT). Differences in HSCT depending on psychological variables (optimism, social support, social comparisons, expectations) were also explored.

Methods: HSCT patients (N = 25) completed standardized questionnaires at pre (T1) and 6 months post-transplant (T2). Information was also taken from medical records.

Results: HRQOL was lower than norms, with the exception of T2 emotional function, global QOL, nausea/vomiting and constipation. There was no change in HRQOL between T1 and T2. Higher HRQOL on certain sub-scales was reported by females, older individuals, non-partnered individuals and those with higher socio economic status. Individuals with lower expectations pre-transplant reported higher HRQOL for some domains post-transplant.

Conclusion: These findings suggest that pre and post-HSCT, patients experience compromised HRQOL, partly dependent on demographic variables. Psychosocial variables may help some patients to cope better than others. These variables should be considered when designing pre-transplant psychosocial assessments. Such assessments may help to identify individuals with unreasonably high expectations, who may
be particularly vulnerable to poor adjustment post-transplant. Interventions designed to target these behaviours may also prove beneficial.

R1296
The evaluation of heart function in patients undergoing peripheral blood stem cell transplantation – preliminary results
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Objectives: Cardiotoxicity is a growing problem among cancer survivors. The aim of the study was to evaluate heart function with a use of electrocardiographic, echocardiographic and biochemical tests in patients undergoing PBSCT (peripheral blood stem cell transplantation).

Methods: The study included 27 patients with a diagnosis of: multiple myeloma (10 patients) – treated with melphalan before PBSCT, non-Hodgkin lymphoma (7) – treated with carmustine, cytarabine, etoposide, melphalan (BEAM); AML (4 patients) and 1 patient with ALL – treated with busulphan and cyclophosphamide, the same, Hodgkin lymphoma (3 patients) – treated with BEAM, aplastic anemia (1) and paroxysmal nocturnal hemoglobinuria (1 patient) treated with – cyclophosphamide and thymoglobulin. The mean age was 44.67 ± 14.06 years, there were 15 men and 12 women, with no strict diagnosis of any cardiological disease. Studies were performed twice in all patients: before the procedure of PBSCT and several days after PBSCT (mean 3.42 days after it) and included 24-hour Holter monitoring, echocardiography, serum troponin and NTproBNP markers of heart dysfunction.

Results: Results showed normal heart function in echocardiography in both tests with a normal ejection fraction of left ventricle and a normal absence of myocardial alterations. Results showed normal heart function in echocardiography in both tests with a normal ejection fraction of left ventricle and a normal absence of myocardial alterations. NTproBNP was present in both measurements. NTproBNP was only insignificantly higher after PBSCT (179.00 ± 141.45 pg/ml; vs: 242.54 ± 208.31 pg/ml).

Conclusion: In patients undergoing PBSCT no evident features of heart dysfunction were present. However, in electrocardiographic analysis the significant increase in heart rhythm after the procedure was observed. Some mild arrhythmias were noted before and after PBSCT indicating that the former cytotoxic treatment should be also taken into consideration.

R1298
The relationship of MTHFR C677T polymorphisms and oral health in the incidence of oral mucositis in haematopoietic stem cell transplantation
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Oral mucositis remains an important side-effect and life-threatening complication of hematopoietic stem cell transplantation. It can occur in 100% of patients undergoing allogeneic stem cell transplantation. The incidence and severity of oral mucositis between allo and autologous transplantation can be due to several factors including chemotherapy, immune suppression and infections. The incidence of mucositis is related to increase hospitalar costs, reduced 100-days survival and occurrence of systemic infections. The last decade was important to the understanding of oral mucositis, including genetics changes knowledge in enzymes responsible to drug metabolism, as the C677T polymorphism in the methylene-tetrahydrofolate reductase gene (MTHFR). We conducted a prospective evaluation of oral mucositis in relation to the C677T MTHFR polymorphism and to the oral health condition (presence of dental plaque and gingival inflammation). A cohort of 97 patients (35 allogeneic HSCT patients – study group and 62 autologous HSCT patients – control group) with median age of 41.5 years was evaluated. The conditioning regimen, in both cohorts, comprising busulfan plus melphalan to leukemia and multiple myeloma and becenum, etoposide,cytarabine,melphalan to lymphoma. GVHD prophylaxis comprised cyclosporine A plus short course of methotrexate in allogeneic transplantation. Oral mucositis was defined as the presence of at least one ulcer per oral site. The incidence of mucositis was defined at 1, 2 and 3 weeks after transplantation. The results showed that C677T polymorphism was not significant in the study group compared with control group.

Conclusions: Our retrospective review indicates that fever-deceased HSCT patients are undergoing autopsies compared to the early years of the transplant program. However, high rate of missed diagnosis is still persisting and autopsy is the best and cost effective way to learn our mistakes.
R1299

Does fludarabin increase the incidence of sinusoidal obstruction syndrome when combined with busulfan during conditioning?

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Objectives: Sinusoidal obstruction Syndrome (SOS) is one of the major complications of both alloengenic and autologous hematopoietic stem cell transplantation (HSCT), causing significant morbidity and early mortality. The conditioning regimens, particularly those containing cyclophosphamide, busulfan and TBI, are frequently associated with SOS in HSCT recipients.

Methods: In this study, we retrospectively analysed the data of 50 patients with the diagnosis of ALL, AML, KML, HD, NHL, and MDS who underwent alloengenic HSCT with a conditioning regimen of BuCy (oral busulfan 16 mg/kg on days -7 to -4 days and Cyclophosphomide 120 mg/kg iv on days -3 to -2) or BuCy/Flu (oral busulfan 4 mg/kg/day on days -6 to -5, Cyclophosphomide 350 mg/m²/day on days -4 to -2, and fludarabine 30 mg/m²/day on days -4 to -2) at the Stem Cell Transplantation Unit of Gazi University Hospital between November 2003 through October 2008. Forty one patients were treated Bu/Cy, and 9 patients were treated Bu/Cy/Flu. At the time of transplantation, the median age of patients was 27 years (range 17–53) and, 15 were female and 35 male. The median time from diagnosis to BMT was 295 days (range 38–2430).

Results: Ten (24.4%) of the 41 patients receiving Bu/Cy, and 6 (66.7%) of the 9 patients receiving Bu/Cy/Flu developed SOS (P<0.016). Among these patients 3 (30%) patients in the Bu/Cy group and 3 (50%) patients in the Bu/Cy/Flu group developed severe SOS (P=0.031). Disease activity, age, sex, the number of prior chemotherapies were not found to affect the incidence of SOS (P>0.05). However the majority of patients (6/9) in Bu/Cy/Flu group had active disease when compared to the Bu/Cy group (6/41). And also, the proportions of disease diagnosis were not comparable in both groups, because major of the patients (n:7) in Bu/Cy/Flu group had the diagnosis of lymphoma, while there is no lymphoma patients in the Bu/Cy group which included patients with the diagnosis of AML, ALL, KML, and MDS.

Conclusion: Although fludarabin is not known to contribute to the development of SOS, it might enhance the incidence of SOS when used with busulfan and cyclophosphomide.

R1301

Impact of antithymocyte globulin induced hyperbilirubinemia on outcome of patients undergoing alloengenic hematopoietic stem cell transplantation

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Background: Antithymocyte globulins (ATG) are widely used for prophylaxis of graft-versus-host disease in alloengenic hematopoietic stem cell transplantation (HSCT). By virtue of their mode of preparation ATG contain a broad range of antibodies that may also target liver tissue and thus cause liver function abnormalities. Hyperbilirubinemia is associated with poor outcome of alloengenic HSCT. There are no studies investigating the influence of ATG-induced hyperbilirubinemia on HSCT outcome.

Methods: We performed a retrospective analysis of 176 consecutive patients with various haematological malignancies undergoing alloengenic HSCT at our centre and receiving no ATG (n=24), ATG-Fresenius (n=62) or Thymoglobulin (n=70) on days -3, -2 and -1. Hyperbilirubinemia was considered to be ATG-induced if this occurred during and up to day +2 after application of ATG. Hyperbilirubinemia with onset as from day +3 was considered conditioning-induced. Patients with hemolysis were excluded from the analysis.

Results: Both ATG-Fresenius and Thymoglobulin caused liver function abnormalities as indicated by increase in Bilirubin, AST, ALT, Alkaline phosphatase and gamma GT levels from day -2. Bilirubin peaked on day +1. Kaplan-Meier estimated 2 year overall survival was 62% vs. 55 vs. 37% (P=0.012) for patients with no hyperbilirubinemia, ATG-induced hyperbilirubinemia and conditioning-induced hyperbilirubinemia respectively. Patients with myelofibrosis had higher ATG-induced hyperbilirubinemia compared to other patients receiving the same dose of ATG-Fresenius (3×20 mg/kg), (Mean 3.8 mg/dl vs. 2.3 mg/dl on day 0). In patients with myelofibrosis (n=34) a similar trend was observed with 2 year OS of 67% vs. 67% vs. 29% (P=0.13) for patients with no hyperbilirubinemia, ATG-induced hyperbilirubinemia and conditioning-induced hyperbilirubinemia respectively.

Conclusions: ATG-induced hepatotoxicity appears to have less negative impact on transplant outcome compared to conditioning-induced hepatotoxicity.
Reduced-intensity conditioning

R1302
Allogeneic haematopoietic stem cell transplantation in Jehovah's Witnesses is feasible – a single-centre experience
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Objective: To show if allogeneic hematopoietic stem cell transplantation in Jehovah’s Witnesses is feasible despite their decline of blood products. We have a long expertise in reducing the platelet transfusion trigger in patients (pts.) with acute myeloid leukemia and autologous stem cell transplantation (Wandt H et al. Bone Marrow Transplant 2006;37:387 and ASH 2008; abstract #286). Therefore we have a special interest in treatment of these pts. who otherwise have a desperate prognosis.

Methods: We present our single center experience over the past 6 years. Patient characteristics: total number n=9 (2 male, 7 female), age median 48 years (22–56), diagnoses: 6 acute leukemias, 1 myelodysplastic syndrome, 2 myeloproliferative syndromes, 1 mantle cell lymphoma. 3 patients were primarily untreated, 4 pts. were transplanted in first complete remission (CR) after induction therapy, 2 pts. were in partial remission. Mean blood counts before conditioning were: leucocytes 6.77/ nl (1,1–12.9), hemoglobin 10.8g/dl (4,0–15,1), platelets 218/nl (46-346). Conditioning was performed with treosulfan based protocols in need of cytodestruction and a “protective” conditioning regimen according to Lowsky (NEJM 2005, 353:1321–1331) for patients in stable remission (n=3). The graft was peripheral stem cells in 7 and bone marrow in 2 pts. Prophylaxis for graft versus host disease (GvHD) was performed with cyclosporine and mycophenolate.

Results: The median time of cytopeniae (leucocytes <1/nl 9days (0–18), hemoglobin <8 g/dl 11 days (0–42), platelets <10/nl 3 days (0–14)), the rate of infectious complications, acute and chronic GvHD and the number of treatment related deaths (n=4, 44%) were in the expected range. The overall and disease free survival was 55% (5 out of 9 pts.) at an median observation time of 459 days (71–2275). Patients in CR at time of transplant had a more favorable outcome: overall survival after 2 years was 75% versus 20% for pts. not in CR.

Conclusion: Allogeneic stem cell transplantation with reduced toxicity (treosulfan) or reduced intensity (Lowsky) conditioning is feasible without support of blood products and thus offers a potentially curative approach for patients with hematologic malignancies who do not accept blood transfusions. This approach is feasible in defined settings for patients who are in a good performance status without severe anemia or thrombocytopenia at the beginning of transplantation procedure.

R1303
Intermediate intensity conditioning regimen containing FLAMSA, treosulfan, cyclophosphamide and ATG for allogeneic stem cell transplantation in patients with relapsed or high-risk acute myeloid leukaemia
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Objective: Lower dosages of total body irradiation (TBI) and chemotherapy in reduced intensity conditioning (RIC) regimens have reduced acute side effects of the conditioning and non-relapse mortality. The FLAMSA-RIC protocol introduced 1999 by Schmid et al. for high-risk patients with AML and MDS has shown promising results in refractory disease as well as in first complete remission. Still, the RIC protocol containing 4 Gy TBI/ Cyclophosphamide / ATG implicates acute toxicity mainly due to TBI preventing its usage in elderly as well as in patients with severe concomitant diseases. To further reduce acute toxicity we substituted 4 Gy TBI with Treosulfan.

Patients and methods: During 2009, 11 patients with relapsed or high-risk AML and either advanced age or concomitant disease were included into the following protocol containing a 4-day course of chemotherapy (FLAMSA d-13 until -10 comprised of Fludarabine 30 mg/m², Ara-C 2g/m² and Amsacarine 18mg/m²) followed by RIC comprised of Treosulfan (100 mg/m²) and Cyclophosphamide (40 mg/kg BW d -6 until -4)/Cyclophosphamide (40 mg/kg BW d -3 until -2) and ATG (10 mg/kg BW d -3 until -1). Immunosuppression consisted of cyclosporine A and mycophenolate mofetil.

Results: Median age of patients was 55.4 years (40 to 69 years). 4 patients were included because of first relapse of AML, 7 patients because of high-risk constellation defined as AML secondary to MDS, unfavourable cytogenetics or delayed response to induction chemotherapy. Donors were HLA-identical siblings in 3 and unrelated donors in 8 patients, among them 2 with one antigen mismatch. One patient died due to transplant related mortality. In the remaining patients no graft failure occurred and complete haematological response was documented at d+30 in all cases. Mean follow-up of the surviving patients was 6.1 months (3 to 10 months). One patient relapsed at d +64 and subsequently died. Except for one, all other patients developed 100% donor chimerism at d +100.

Conclusion: FLAMSA / Treosulfan / Cyclophosphamid / ATG is an intermediate intensity conditioning regimen with acceptable acute toxicities for patients with relapsed or high-risk AML. Substituting TBI with Treosulfan provides an alternative opportunity to treat elderly or patients with concomitant diseases when TBI appears not feasible due to possible toxicity.

R1304
Fludarabine and low dose of melphalan as effective conditioning regimen for allogeneic stem cell transplantation in lymphoproliferative diseases and multiple myeloma
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Introduction: Reduced-intensity preparative regimens have been developed to decrease regimen-related toxicities and allow treatment of patients with concurrent medical conditions or older.

Objective: We studied the safety and efficacy of fludarabine (2.5 mg/m² iv daily from day -6 to day -2) and low dose of melphalan (70 mg/m² i.v. on day -1), as conditioning regimen in adult patients who undergo an allogeneic stem cell transplantation (Allo-SCT) because of lymphoproliferative diseases, Non Hodgkin Lymphoma (NHL), Hodgkin disease (HD) and Chronic Lymphocytic Leukemia (CLL), or multiple myeloma (MM).

Patients and methods: Between February 2002 and August 2008, 45 consecutive patients underwent Allo-SCT in our center because of NHL=13 (29%), HD: 7 (15%), CLL=8 (18%), MM=17 (38%). Median patient age was 50 years (range
23–64) and 23 patients were male. Twenty-three patients relapsed following an autologous HSC transplant and 32 patients had received 3 or more lines of treatment. At the time of allo-SCT, thirty of the 45 patients (67%) was in a partial response or activity. Thirty-six patients received HSC from an HLA-identical sibling donor and peripheral blood was used as stem cell source in 19 patients. All patients received GVHD prophylaxis consisting of cyclosporine combined with short course of Methotrexate in 22 patients or Mycophenolate in 23 patients.

Results: All patients engrafted. The median time to achieve an ANC greater than 500 mL was 13 days (range 10 to 16) and to platelets greater than 20,000 mL was 16 days (range 13 to 22). Nine patients experienced grade 2 mucositis and required parenteral analgesics for a short period. One patient developed posterior leukoencephalopathy while on cyclosporine and died. Twenty-five (55%) patients developed acute GVHD, III-IV in 6 (23%). Thirty-nine patients were evaluable for Chronic GVHD. Twenty-eight patients were in complete remission during the last follow-up and in 6 patients disease was always refractory. The 100-day and 2-year mortality related with toxicity was 7% and 16%. The overall survival probability at 5 years was 0.55 (90% CI 0.46–0.63).

Conclusion: The results of this study indicate that fludarabine- and low doses of melphalan-based conditioning is relatively well tolerated, with low toxicity and acceptable rate of survival at 5 years.

R1305
Myelofibrosis, dose reduced conditioning: the Irish experience
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Objectives: Dose reduced conditioning (DRC) regimens were developed for myelofibrosis to treat patients who are older or with comorbidities and are not eligible for a standard myeloablative allograft. Our group evaluated the outcome of DRC allografts in an Irish cohort in terms of toxicity and outcome.

Methods: Patients received DRC consisting of busulphan (10 mg/kg), fludarabine (180 mg/m²) and antithymocyte globulin (Fresenius 30 mg/kg or 60 mg/kg with an unrelated donor). All patients received peripheral blood stem cells as the stem cell source. Cyclosporin and short course methotrexate were used as graft-versus-host disease (GVHD) prophylaxis. All patients received standard supportive care including growth factor (G-CSF) from Day +5.

Results: Between 2005 and 2009, 7 patients with myelofibrosis were treated. Six patients had primary and 1 had secondary myelofibrosis. Four patients received treatment pre-transplant in the form of cytotherapy, splenic irradiation, steroids and antiangiogenics. There were 4 matched sibling allografts, 2 matched unrelated donor (MUD) allografts and 1 single antigen mismatch allograft. The median age was 54 years (range 49–59 years) and the mean cell dose was 5.2 x 10^6 CD34/kg. At transplantation, all had intermediate or higher risk disease according to the revised Dupriez classification and had a Sorror comorbidity score of 0 to 3. Four patients had a transfusion requirement of greater than 6 units per month. Two patients were JAK2V617F positive and one was positive for the MPL W515K mutation.

Post-transplantation chimerism analysis showed greater than 95% donor chimeras in 5 of the 7 patients at 30 days. Subsequently, there were 3 graft failures, 2 necessitating donor lymphocyte infusions and a second myeloablative transplant. Three out of 7 patients died from acute GVHD, infection and graft failure. Two patients died from chronic GVHD. Two patients are alive 6 to 24 months (median 15 months) after allografting.

Conclusion: We report significant treatment related mortality amongst a small cohort of patients with myelofibrosis treated with a dose reduced allograft. A low revised Dupriez and Sorror score correlated with a better outcome. Dose reduced conditioning and the ensuing complications of infection and graft versus host disease are less well tolerated with advancing age. Larger collaborative studies are necessary to further improve transplantation in myelofibrosis.

R1306
Allogeneic stem cell transplantation with a thiotepa-based conditioning regimen for poor prognosis haematological patients: a single-centre experience
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Allogeneic stem cell transplantation (SCT) represents a therapeutic option for many hematologic malignancies. Even if a better outcome after SCT is known to be achieved in presence of a chemosensitive disease, this treatment could be considered in fit patients in whom a donor is available in order to elicit a GVTh effect.

Myeloablative conditioning is known to be associated with a higher transplant related mortality (TRM), especially in previously treated patients, in whom a reduced intensity conditioning (RIC) could be suggested. Thiotepa is a interesting drug with strong anti neoplasm effect, radiomimetic properties and less extra medullary toxicities compared to other drugs.

We proposed a Thiotepa based RIC regimen for high risk patients. From 2007 to 2009 12 patients has been transplanted. Patients characteristic are summarized in Table 1. This cohort comprised strongly pre-treated patients with different hematological neoplasm, with 8/12 of whom had a previous SCT (2 allos) with 4/12 chemoresistant disease at the moment of transplant. 6 patients had a matched unrelated donor (MUD). Conditioning consisted of thiotepa (6 mg/kg day-5), cyclophosphamide (60 mg/kg days -3, -2), melphalan (30 mg/m² day -1). Graft versus host disease (GVHD) prophylaxis consisted in cyclosporine (from -7 1 mg/kg with 200–300 blood level monitored) and methotrexate (15 mg/m² +1, 10 mg/m² +3,6, +11). In MUD SCT ATG was added (Thymoglobine 7.5 mg/kg/die -5,4).
All patients engrafted with a median engraftment (N>500) of 14 days (12-19), all evaluable patients (9/12) had full donor chimerism at day +30. Disease response after transplant (+3 months) showed complete remission in all but 1 evaluable patients (7/8). TRM was 33% (4/12) with 3 sepsis and 1 patient who died for grade IV aGVHD; relapse rate was 12.5% with a death due to progression, with an overall death rate of 45.5%. Post transplant complication were 55% (5/9) aGVHD rate (3 grade II, 2 III-IV), 50% cGVHD (100% limited), CMV reactivation (50%).

With a median follow of 7 months (1-20) seven patients are still alive with 5 in continuous CR. In conclusion this tiotepa-based reduced intensity conditioning seems to be promising with a good anti tumor control suggesting both an anti-neoplastic drug activity and a GVT effect. GVHD rate (both acute and chronic) was average but of limited entity. Unfortunately overall mortality was high but it could be explained by the high risk of these patients.

R1307

Case of IPEX syndrome in which a full chimera was obtained by peripheral blood stem cell transplantation with non-myeloablative conditioning from the same donor after primary graft failure following bone marrow transplantation

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Introduction: IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome is a rare condition that involves various autoimmune and inflammatory diseases resulting from loss of functional regulatory T-cells caused by mutations of the FOXP3 gene. Chronic immunosuppression with cyclosporine (CsA) or steroids is used to control symptoms, but long-term remission is difficult to maintain. Allogeneic hematopoietic stem cell transplantation presents problems such as graft failure and therapy-related deaths. We experienced a case of IPEX syndrome in which a full chimera was obtained by retransplantation with nonmyeloablative conditioning, containing anti-thymocyte globulin (ATG), from the same donor after primary graft failure following bone marrow transplantation (BMT).

Case: The patient was a 5-year-old boy who contracted type 1 diabetes at the age of 4 months. At the age of one year, he developed nephrotic syndrome and had frequent relapses thereafter. Treatment with CsA was initiated. Detailed testing was performed at the time of recurrent sepsis after varicella at 5 years of age, and IPEX syndrome was diagnosed. A BMT was performed after conditioning with total lymphoid irradiation, fludarabine and cyclophosphamide from the mother who was an HLA-A, B, C, DR loci allele compatible carrier. Bone marrow at one month after transplantation showed recovery of autologous hematopoiesis. CsA treatment had been continued, but the patient repeatedly had fungal pneumonia and asthmatic attack-like symptoms. Retransplantation was deemed urgently required. At 4 months after BMT, peripheral blood stem cell transplantation was performed using the same donor after conditioning with fludarabine, melphalan, busulfan and ATG. At 1 month after transplantation, successful engraftment was achieved in the bone marrow. At present, after 6 months, complications other than diabetes have improved and his general condition is good. He was thus placed under observation as an outpatient.

Discussion: Graft failure is apt to occur in allogeneic transplantation in patients with IPEX syndrome, and an appropriate transplantation method has not been established. Our patient showed primary graft failure after the first transplant, but full chimera was achieved after a second transplant with nonmyeloablative conditioning from the same donor. These findings suggest that regimens including ATG may be useful as conditioning in transplantation in patients with this syndrome.

R1308

Allogeneic haemopoietic stem cell transplantation with reduced-intensity conditioning in patients with myelodysplastic syndrome

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Introduction: Allogeneic Hemopoietic Stem Cell Transplantation (allo-HSCT) is the only curative method for patients with myelodysplastic syndrome (MDS). This procedure is associated, however, with grave complications such as graft rejection or failure, relapse and adverse toxicity. Reduced intensity conditioning (RIC) regimens allow performing allo-HSCT in patients with high risk of transplant-related death on standard preparative regimens (elder patients or heavily pretreated patients with low performance index). However, some data suggest an increase of relapse rate in recipients of allo-HSCT with RIC.

Materials and methods: 11 allo-HSCT recipients were included in this study. 4 patients were transplanted from matched related donors, 5 from matched unrelated donor, 1 from haploidentical donor, 1 patient received cord blood stem cells, 3 patients underwent second allo-HSCT from the same donor after relapse or engraftment failure. Median patients age was 24 (7–53) years. According to IPSS scoring system 3 patients belonged to high risk group, 5 to intermediate-1 group, 3 to intermediate-2 group. All patients received fludarabine-based RIC regimens. Most of patients transplanted from matched related donors received graft-versus-host disease (GVHD) prophylaxis with cyclosporin A or tacrolimus and methotrexate. In patients transplanted from unrelated donor prophylactic regimen also included antithymocyte immunoglobulin.

Results: In the observed patient cohort relapse incidence amounted to 32%. 4-year overall survival (OS) was 61%.
4-year event-free survival (EFS) was 42%. Causes of death were: haemorrhage (2), graft insufficiency (1), disease progression (1). In 3 patients was observed grade III-IV acute graft-versus-host disease (aGVHD), in 8 patients grade 0-II aGVHD.

Conclusions: RIC regimens allow decreasing of transplant-related toxicity and infectious complications incidence, our preliminary data doesn’t show an increase in relapse rate in comparison to allo-SCT with conventional regimens.

R1309
Successful second allogeneic stem cell transplantation from the same donor after alemtuzumab containing reduced-intensity conditioning regimen in patient with myelodysplastic syndrome after previous graft rejection
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The only curative treatment available for myelodysplastic syndromes (MDS) is Hematopoietic Stem Cell Transplantation (HSCT). There are many reasons of treatment fail that include graft rejection or failure, relapse and procedure complications. Reduced-intensity conditioning (RIC) regimens were designed to perform allogeneic HSCT in patients who had a high risk of death due to toxicity of standard preparative chemotherapy regimens. However, the use of RIC can increase the risk of relapse after HSCT. We report the case of successful second related DGB1 antigen mismatched HSCT after Alemtuzumab containing preparative regimen in MDS patient with previous graft rejection. The patient is 22 years old male with the diagnosis of MDS. RAEB –II. 47,XY, t(3;21)(q26;q22), +13[18]/46,XY[2] IPSS 3 (January of 2009). One course of Dacogen treatment was performed in January 2009. Only decreasing in transfusion dependence was noted. It was revealed that the patient had an HLA DGB1 antigen mismatched sibling. It was decided to perform allogeneic HSCT due to high IPSS and very poor prognosis without transplantation. HSCT after RIC Fludarabin 5 × 30 mg/m² i.v + Busulfan 8 mg/kg p.o was performed in May of 2009 (BM-HSC 1 × 10⁶ CD34⁺/kg was transplanted). GVHD prophylaxis was CsA+MTX. However, disease relapse was revealed on day +29. On day +38 CsA was stopped. On day +45 Donor Lymphocytes Infusion was performed (CD3⁺5 × 10⁶). Nevertheless, on day +50 graft rejection occurred. It was decided to perform the second allo-HSCT from the same donor. After RIC containing Fludarabin 5 × 40 mg/m² p.o, Thiopeta 3 × 5mg/m² i.v and Alemtuzumab 2 × 20 mg i.v the PB HSCT 10 × 10⁶ CD34⁺/kg was performed. GVHD prophylaxis was Tx and MMF. Engraftment was on day +10. On day +12 the patient developed acute GVHD of gut and skin grade I. GVHD resolved when steroids were added to the therapy. There were no severe infectious complications related to transplant procedure. On day +14 we achieved complete hematological, cytogenetical remission. On day +60 there were no cytopenia, no transfusion dependence. The patient was still in CR with full donor’s chimerism. Alemtuzumab containing RIC preparation regimens may be feasible for MDS patients with high risk of graft rejection. Good engraftment and no severe GVHD were observed on day +60. This case showed that Alemtuzumab may improve engraftment of donor cells and achievement of full donor’s chimerism.

Introduction: Haematological patients are frequently subjected to stem cell transplantation to ensure disease remission. To demonstrate the outcome of the clonal cell administration, and to monitor the follow-up, polymorphisms study in both recipient and donor is indicated. Variable number of tandem repeats (VNTR) and short tandem repeats (STR) can be easily characterized and may display considerable variability due to variation in number of repeats. These microsatellites have often four or more alleles and a Poly-morphic Index level (PIC) >0.7. For this reason they are fully informative in the 50% of Caucasian families. Through the use of these variability among recipient and donor, patients submitted to transplantation could be monitored to verify the complete loss of affected cells and the reconstitution of the donor cells clone.

Objectives: The aim of the present study is to assess the informativity and availability of these VNTR/STR polymorphisms in a group of Sardinian subjects (recipient and donor), in order to give a new informative markers panel to verify the complete loss of affected cells and the reconstitution of the donor cells in Sardinian transplanted patients.

Materials and methods: From 2000 to may 2009, 200 cases were evaluated (98 recipient and 102 donors). Ten polymorphisms in different genes were tested (DYS385, MCT118, PAH, APOB1, APOC1,YNZ-22, IGF1, CAR, 33.6, SE33). Six were STR and four VNTR. Genomic DNA extracted from peripheral blood and bone marrow were assayed with qualitative PCR procedure followed by agarose/acylamides gel electrophoresis.

Results: In the Sardinian group analyzed, we have obtained the following results: 34% were informative for MCT118 gene, 33% PAH, 10% APOB1, 5% YNZ-22, 4% SE33.3, 2% 33.30, 1% APOC1. Moreover, 27 (13%) were informative for one polymorphic gene, while 18 (3%) were informative for two polymorphic gene and only 2 (1%) were informative for three polymorphic gene. In only 8 cases (4%) VNTR and STR were not informative.

Conclusions: In Sardinian subjects we performed the first study with some informative polymorphism genes indicated to detect the full donor (FD) or chimeric state after transplantation. MCT118 on DYS385 locus (1p36p35) and PAH (12q2) resulted the most informative polymorphisms. The present data can be used in diagnostic studies to perform a specific polymorphism panel in chimerism analysis.

R1311
The Jak2 V617F mutation in the follow-up of an allo-transplanted myeloproliferative disorder patient: a diagnostic case study
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Introduction: Myeloproliferative disorders (MPD) are haematopoietic clonal disorders that include polycytemia vera (PV), essential thrombocythaemia (ET) and idiopathic myelofibrosys (MF). The molecular bases of MPDs have been correlated to the presence of a single nucleotide G1849T change in the Janus Kinase 2 (JAK2) gene encoding for the V617F aminoa-cidic substitution. Clinical and genotyping correlation studies have also demonstrated the value of the precise quantification of V617F mutation load in patients, showing the worst prognosis

Minimal residual disease
and relapse risk for homozygotes and heterozygotes patients. Stem cell or bone marrow (BM) transplantation is indicated in few MPD cases.

Objective: The purpose of the present study is to use the V617F mutation as early diagnostic marker to detect MDS-relapse in a patient subjected to allo-transplantation, when associated to VNTR polymorphisms analysis.

Materials and methods: We have analyzed a single case of secondary MPD and PV exordium patient, in blastical crisis that came in January 2009 in our Hospital “A. Businco” Aasl 8 Cagliari. In February 2009 he was subjected to bone marrow allo-transplantation from a familiar donor. Genomic DNA was extracted from granulocytes of peripheral blood. PCR, RT-QPCR, Allelic Discrimination and VNTR-SE33.6 analysis were performed.

Results: The patient demonstrated the presence of Jak2 V617F mutation before allo-transplantation. In the first post-transplantation control (30 days) and in the follow-up at 90 days the patient showed the loss of the mutation as confirmed by the SE33.6 chimerism analysis (Full donor, FD). After 270 days, the Jak2 V617F analysis showed the presence of the mutation in heterozygosis state, subsequently confirmed by morphological analysis (presence of blast cells) and mixed chimerism state for SE33.6 polymorphism. All these diagnostic data demonstrated the recipient MDS-BM reconstitution.

Conclusion: Jak2 V617F mutation analysis have earlier revealed the molecular relapse in this MDS transplanted patient, successively confirmed by polymorphism and morphological study commonly used. This diagnostic relevant finding leads to suggest the power of this mutation as a new marker for secondary MPD and minimal residual disease (MDR).

R1312

The first experience of immunomodulatory therapy after syngeneic BMT

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The aim: It is known that after syngeneic bone marrow transplantation (SyBMT) relapses of leukemia are more frequent than after allogeneic BMT. Earlier we found out that in patients (pts) treated with immunomodulatory therapy after autologous BMT relapse rate decreased twice. In the last 2 years we began the use of the same therapy after SyBMT. In this work we analyzed the results of SyBMT in pts with leukemia and the first results of using of immunomodulatory therapy.

Materials and methods: During 20 years we performed 14 SyBMT in the pts with CML (7), ALL (4), PNG (2), NHL(1) M/F 9/5, age 13–39 years (median 25). We used myeloablative conditioning and in all cases complete preparation of haemopoiesis was observed. In 7 pts (50%) haematologic relapses were estimated in 3–36 mo (median 6) after BMT and 5 pts died. The others 9 pts are alive with complete hematological and cytogenetic remission during 18–213 mo (median 24). The posttransplantation therapy is applied in 4 pts and 3 of them (CML-AML-2) have the markers for the MRD detection: (CML – expression of BCR/ABL gene, AML – inv 16 chromosome). The therapy consist of IL2 infusions 5 days in the patient with ALL, ATRA 5 days+ INF-alfa 3 times in a week in pts with AML and IFN-alfa 3 times in a week in pts with CML monthly. The therapy was started in 3–4 mo after BMT and continue now (maximal number of courses-14).

Results: According to FISH and qualitative PCR results in all 3 pts complete cytogenetic and molecular remission take a place. In the patient with CML after 14 mo the expression of BCR/ABL gene was detected by RTPCR (0,05%). After the start of IFN therapy the expression decreased by 24 mo to 0,01%. In 1 patient with AML inv(16) remained during 4 mo after BMT , disappeared after 2 courses of ATRA+ INF therapy and is absent for 14 mo. In other patient with AML inv (16) is estimated by RTPCR in minimal quantity (0,02–0,03%) after BMT while before BMT inv (16) was 0,7%.

Conclusion: Low number of patients don’t allow to make definite conclusions about the role of immunomodulatory therapy in relapse leukemia prophylaxis. The same time we noted positive results in all cases. We suggest that it is necessary to continue this investigation.

Paediatric issues

R1313

Treatment options for persisting thrombocytopenia after allogeneic stem cell transplantation using romiplostim

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After allogeneic stem cell transplantation (SCT) incomplete engraftment may occur. In addition the development of alloantibodies towards human leucocyte antigen (HLA-) antigens is regularly observed. Both events result in persistent thrombocytopenia as well as long-term transfusion dependency. Treatment options are the administration of additional stem cells or the transfusion of anti-CD19 antibodies. To treat resulting or persisting thrombocytopenia alternative treatment options might arise out of the application of romiplostim. In 2009 romiplostim received clinical approval for treatment of therapy refractory idiopathic thrombocytopenic purpura. The supposed mechanism of function is the increase of megacaryocytes by stimulation of thrombopoetin-receptor and subsequent cytokine release.

We present two patients to whom romiplostim was given either because of persisting thrombocytopenia (#1) or because of a severe thrombocyte-antibody mediated thrombopenia (#2).

Patient #1 with Acute lymphatic leucaemia who received an allogeneic SCT from a 10/10 matched unrelated donor (MUD) remained platelet (plt)- transfusion dependent for one year. A stem cell boost was denied by the donor. Therefore the application of romiplostim was started. The drug was administered subcutaneously over a period of 5 months. 6 days after the onset of treatment plt counts increased and the patient became transfusion-independent for the first time post transplant. When the counts reached 98/ml further administration was stopped. Counts further increased up to 106/ml within the next 12 weeks.

Patient #2 with severe Langerhans cell histiocytosis (Histiocytosis X) was transplanted from a 10/10 MUD. Although megacytopenia was seen (#1), and complete engraftment could be assessed by chimerism analysis in the bone marrow aspirate, the patient needed regular plt-transfusions in three week intervals. Around day +100 platelet counts dropped again and even through high frequency plt-transfusions plt counts could not be elevated above 10/ml. Antibodies against thrombocytes were detected. A treatment with romiplostim was started. Response was prompt. Transfusion independency was reached after 13 days of treatment. Treatment was stopped after 2 months as counts reached 160/ml.

Side-effects were not observed during or after treatment.

Conclusion: Administration of romiplostim might be a promising treatment option for persisting thrombocytopenia or for alloimmune thrombocytopenia after allogeneic SCT.

R1314

Treatment of CNS PTLD with intrathecal rituximab

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Central nervous system (CNS) post transplant lymphoproliferative disorder (PTLD) is a rare but life threatening complication of allogeneic hematopoietic stem cell transplantation. It is usually caused by Epstein-Barr virus (EBV) induced B cell proliferation.
Treatment includes withdrawal of immunosuppression, systemic and intrathecal chemotherapy and more recently rituximab but prognosis remains poor. Here we describe 2 cases of CNS PTLD who were treated with intrathecal (IT) rituximab, following failure of first line treatment.

Case 1: A 5 year old boy was diagnosed with JMML in November 2008. He underwent matched unrelated cord transplant following conditioning with Busulphan, cyclophosphamide and melphalan. Seven months post transplant he presented with gait ataxia and left sided intention tremor. MRI of the brain showed multiple ring enhancing lesions in the basal ganglia bilaterally. Smaller ring enhancing lesions were also seen in left temporal and left occipital lobe, right hypothalamus and right cerebellar hemisphere. Biopsy of these lesions confirmed EBV associated PTLD. Quantitative PCR for EBV in the serum was positive at low level. Initial treatment was withdrawal of immunosuppression and IV Rituximab. The lesions progressed and he received IT rituximab, methotrexate and hydrocortisone. Subsequent MRI scan showed marked improvement of the intracerebral lesions.

Case 2: A 4 year old boy was diagnosed to have X-linked lymphoproliferative disease with EBV driven haemophagocytic lymphohistiocytosis in April 2009. Following treatment with HLH-2004 protocol he underwent matched unrelated donor transplant in July 2009 with reduced intensity conditioning (fludarabine, campath and melphalan). He presented in September 2009 with vomiting and drowsiness due to acute hydrocephalus. Following emergency treatment of hydrocephalus a brain MRI scan showed a lesion in the left cerebellar hemisphere. Biopsy confirmed EBV associated PTLD. He was treated with infusions of third party EBV specific cytotoxic T cells and his neurological normalised. However after 7th treatment he became drowsy and unsteady. Repeat MRI scan showed progression of PTLD with new lesions and he was treated with IT rituximab, in addition to conventional IT and systemic chemotheraphy which resulted in improvement.

Conclusion: Both cases illustrate that use of IT Rituximab can result in reduction of disease in CNS PTLD if standard treatment fails.

R1315
Marked differences in DMSO pharmacokinetics between paediatric and adult recipients of cryopreserved stem cell grafts

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Background: Dimethylsulfoxide (DMSO) is used as an excipient for cryopreserved cell based therapeutics. However, pharmacokinetic data are rare and adverse events such as toxicity affecting the central nervous system do occur. Here we demonstrate that profound differences in DMSO pharmacokinetics exist between adult and pediatric stem cell recipients.

Methods: We prospectively analyzed DMSO metabolism in a cohort of 13 recipients of cryo-preserved peripheral blood stem cells (PBSC) or cord blood (CB, n = 1) at Hannover Medical University between 2008 and 2009. Median age was 4.9 years (range 4.0–9.8 years) in children (n = 7) and 55 years in adults (22–69 years). Median body weight (BW) differed significantly between groups with 19.7 kg (range 12.0–37.0 kg) and 80.0 kg (range 4.0–9.8 years) in children (n = 7) and 55 years in adults (22–69 years). Lithium-heparin plasma samples from peripheral blood were obtained before as well as 5 minutes, 24 hrs, and 48 hrs post infusion and stored at −60°C. Plasma levels of DMSO (lower limit of quantification (LLO) = 0.75 μM) and its oxidative metabolite dimethylsulfone (DMSO2, LLO = 4.0 μM) were subsequently determined by liquid chromatography-tandem mass spectrometry (LC/MS-MS).

Results: Peak levels of DMSO were reached immediately after transplantation and correlated with the applied dose. Within 24 hrs post infusion DMSO levels declined from 1.68–20.34 mM (median: 4.31 mM) to LLQ in all children. DMSO2 rose from 0.01–0.39 mM (median: 0.08) peaking at 24 hrs post infusion (0.26–2.33 mM, median: 0.65) and decreasing to 0.01–0.91 mM (median: 0.37) within the following 24 hrs. In contrast, DMSO2 peak level 2.95–24.4 mM (median: 14.21 mM) remained measurable after 24 hrs (0.03–5.40 mM (median: 2.28 mM)) in adult recipients. Complete clearance below LLQ was observed after 48 hrs in adults as well. Of note, in adults DMSO2 continued to rise to 0.96–5.34 mM (median: 4.25) within the observation time.

Conclusion: Different DMSO metabolism in pediatric and adult patients need to be prospectively correlated with respect to toxicity profiles.

R1316
Results of allogeneic haematopoietic stem cell transplantation in children with acute biphenotypic leukemia

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Background: Biphenotypic acute leukemia (BAL) accounts for only about 5% of acute leukemias based on criteria of European Group for Immunological Classification of Acute Leukemia (EGIL), and usually demonstrate poor treatment outcome. Despite of it still there are no specific treatment regiments for BAL patients, and it is also unclear if allogeneic hematopoietic stem cell transplantation (allo-HSCT) should be an integral part of therapy.

Aim: To evaluate the results of allo-HSCT in children with BAL prospectively recruited for treatment according to recent therapeutic protocols for ALL, AML and infant acute leukemia.

Patients and methods: Analysis was performed in 8 children (2 female, 6 male) with BAL, including 5 in CR1, 2 in CR2, and one in CR3 treated and qualified for allo-HSCT between 2003-2009, according to protocols ALL-IC BFM 2002 (n = 3), IDA-FLAG (n = 3), INTERFANT-06 (n = 1), and ALL-IC BFM 2002/AML-INTERIM BFM 2004 (n = 1). Median age was 9 years (range 1–16). Three patients were transplanted from matched sibling donor (MSD-HSCT), 4 from matched unrelated donor (MUD-HSCT) and one from mismatched donor (MMD-HSCT). In 4 patients preparative regimens for HSCT were based of FTB1, in 3 on busulfan, and in one on treosulfan. Anti-thymocyte globulin was administered prior MUD- and MMD-HSCT. For GvHD prophylaxis cyclosporine (CSA) and methotrexate were given after MUD-HSCT, and CSA alone after MSD-HSCT.

Results: Two non-relapse deaths occurred; both due to severe GvHD grade (day + 35 after MMD-HSCT, and day + 181 after MUD-HSCT). Relapse was diagnosed in 3 patients 6, 9, and 16 months after HSCT, including one after MSD-HSCT in CR1, and 2 after MUD-HSCT (one in CR2, and 2 in CR3). These children are alive in CR after 1, 16, and 28 months, including 2 after MUD-HSCT (one in CR1, 2 in CR2), and one after MSD-HSCT performed in CR1.

Conclusions: According to our preliminary observations in children with BAL undergoing allo-HSCT the transplant related mortality is comparable to that one observed in children transplanted for ALL or AML. The anti-leukaemic effect of allo-HSCT...
in advanced BAL is limited, and therefore it should be performed already in CR1. There is a need for further studies concerning allo-HSCT for childhood BAL to de

R1317 Management of allogeneic haematopoietic stem cell transplantation in children with Philadelphia positive acute lymphoblastic leukaemia: a “twins’ experience”

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Objective: To report our experience with a pair of twins with Philadelphia positive acute lymphoblastic leukemia (Ph-ALL) who differently responded to the conventional and identical treatment, including an allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Results: A pair of monozygotic twins were diagnosed with a common Ph-ALL, at the age of 2 years, 8 months (Twin A) and 3 years, 15 days (Twin B). They were enrolled to the high risk arm of the ongoing AIEOP-ALL 2000 protocol. Conventional cytogenetic analyses showed a 46,XY; t(9;22)(q34;q11). Molecul

R1319 Minimally manipulated haematopoietic stem cell components for allogeneic transplantation in children

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Background: In order to prevent immediate or delayed complications in ABO/HLA incompatible bone marrow transplantation (BMT) minimally manipulated or manipulated HSC components have to be prepared according to type and degree of the incompatibility between donor and recipient. With any of approaches it is important to retain HSC population that are shown to result in acceptable stem cell recovery.

The aim of this study is to analyze the results of recovery of the CD34+ cells after processing ABO and HLA incompatible BM. It is about the initial experience of the national paediatric centre for HSC transplantation in children.

Methods: Between May 2005, and December 2009, 16 children cured due to: AML-9, NHL-1, MDS-1, beta-thalassemia-1, infantile osteopetrosis-1, ALL-1 and FHHL-2 pts received 17 minimally manipulated BM components from: matched related donor-8, partially matched related donor-7 and one patient from matched unrelated donor. Major ABO incompatibility was in 7, minor in 7, and bidirectional in 2 cases. In major ABO incompatibility BM was processed on blood cell separator, in minor donors plasma was removed by centrifugation, and in HLA and ABO mismatched buffy coat preparation and pharmacological depletion of the T cells were conducted. Recovery of the CD34+ cells was determined on FacsCalibur flow cytometer using ISHAGE protocol.
From January 2004 to December 2008, 14 children who underwent unrelated cord blood transplantation in children: single-centre experience in Korea.

We present the case of a 14 month old boy who was diagnosed to have acute bilaenage leukemia in May 2007. He had chemotherapy to induce remission and had no matched sibling donors. In September 2007 he underwent myeloablative conditioning with fludarabine, intravenous busulphan and cyclophosphamide followed by infusion of a 5/6 matched cord unit. He had brisk engraftment on day 14 and was discharged. Follow up revealed loss of graft with only 1% donor on day 60. His marrow however was in remission and he was taken off all immunosuppression. He remained well until January 2009 when he suffered a systemic relapse. He underwent reinduction with cytarabine and etoposide and went into remission. The second cord was again a 5/6 matched unit which was infused following a myeloablative conditioning of total body radiotherapy at 12 Gy, cyclophosphamide and ATG at 15 mg/kg/day for 2 days. Both units had a cell dose of over 3 × 10^7/kg total nucleated cell count and were thawed at the bedside in a waterbath prior to infusion. He engrafted on day 17 and but grade IV graft versus host disease of the gut which settled with steroids and etanercept. He remains in remission with full donor chimerism and no active GVHD over 8 months post transplantation.

Unrelated transplantation has been performed in India only since April 2007. Cords have been the preferred graft source at our centre. Performing second transplants in children carries a high mortality of over 50% even with sibling donors. Our case demonstrates that complex procedures such as administration of total body radiotherapy to a 3 year old child and taking them through a second unrelated transplant is feasible in a country with resource constraints. The total cost of both procedures has come to 80,000 US dollars including the cost of both cords!
Despite our serie is small we show that in a developing country like Mexico, a HSCT program is feasible also for non-malignant disorders.

**Tolerance and chimerism**

**R1323**

*Case report: association of imatinib plus donor lymphocyte infusions as treatment of relapse after allogeneic stem cell transplantation in a young Ph1+ CML patient*

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Here we describe a case of a 14 year old boy who was diagnosed chronic phase CML with low euro score since 2001. At presentation blood count revealed leukocytosis (WBC = 155000/ul), anemia (Hb 9.7 g/dl), PLT 250000/ul and there was II degree splenomegaly. Oncocarbose therapy was then started until October 2001 when he underwent allogeneic bone marrow transplantation (BMT) from HLA identical sibling (female); conditioning regimen consisted of busulfan (16 mg/kg) and cyclophosphamide 120 mg/kg. Total infused CD34+ cells were 1.3 × 10^6/kg. GVHD prophylaxis consisted of methotrexate (MTX) and cyclosporine (CSA). After 6 months karyotype and FISH analysis for BCR-ABL were negative, while FISH for XY showed mixed chimerism along with persistence of BCR-ABL transcript at the molecular level; albeit prompt CSA tapering, residual molecular disease and mixed chimerism persisted, so DLI at the dose of 1 × 10^7 CD3/kg was scheduled. 5 months later, the patient obtained molecular remission while XY chimerism was still mixed (95% donor) and this situation lasted until 2005. Subsequently (November 2006), molecular, cytogenetic and haematological relapse (leukocytosis) occurred; then therapy with Imatinib 400 mg/die was started, but interrupted after 5 months because of uretral stenosis. Nevertheless, the patient achieved haematological but not molecular remission of disease. On July 2007 the patient received further DLI (still 1 × 10^7 CD3/kg), obtaining within a month complete molecular remission and, notably, full donor chimerism. To date, the patient achieved haematological but not molecular remission of disease and exhibits full donor chimerism. We conclude that treatment of relapse and complete chimerism could be obtained only with association of DLI plus TKIs, being DLI alone capable to give rise only to molecular remission without full chimerism; therefore, in the era of TKIs, treatment of relapse after BMT in CML, classically based on DLI infusion, could be integrated by TKIs for better results.

**Experimental stem cell transplantation**

**R1324**

*Effect of hypoxia and metabolic starvation on murine bone marrow cells*

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Background: Bone marrow (BM) transplantation is a standard therapeutic method widely used for the treatment of hematopoietic disorders and some other diseases. Hematopoietic stem cells (HSCs), responsible for the engraftment of donor BM, are also known for their resistance to disadvantageous conditions, particularly to hypoxia. This study is focused on effects of hypoxia and metabolic starvation on the HSCs and BM cells.

Methods: Murine congenic transplantation model Ly5.1/Ly5.2 mice was used in the experiments. Mice were sacrificed and BM was left in intact femurs at 37°C for different time periods ranging 0 to 6 hours. Bone marrow cells were harvested and analyzed for changes in cell cycle distribution, apoptosis and necrosis in subpopulations, i.e. erythropoietic, B-lymphopoietic, granulopoietic and monocytes. mRNA was isolated from the BM cells and gene expression study was performed with Illumina MouseRef8 BeadChip. Finally, BM cells were transplanted to sublethally or lethally irradiated recipients to determine short-term and long-term repopulating cells.

Results: BM subpopulations show different sensitivity to hypoxia and metabolic starvation. HSCs are significantly more resistant to these conditions than BM cells in the process of differentiation. BM transplantability is not influenced for at least 1 hour. HSCs are still present and able to engraft even after 4 hours of hypoxia and starvation. In the BM, many changes in gene expression is induced (e.g. in apoptotic pathway, energetic metabolism etc.). The number of differently expressed genes increases as the period of hypoxia becomes longer.

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Comparison of transplant related mortality between the first and second high-dose stem cell transplantations – the data from the Czech registry

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Introduction: We submit a comparison of transplant related mortality (TRM) between first and second high stem cell transplantation (HSCT). There are 5473 fulfilled transplantations in ProMiSe database (1st HSCT - 4388, 2nd HSCT - 839, 3rd - 177, 4th - 56, 5th - 8, 6th - 5).

1st autologous stem cell transplantation (alloSCT) was performed on 18th June 1986, 1st autologous stem cell transplantation (ASCT) on 9th January 1990.

Methods: We compare TRM between first and second transplantations in alloSCT and ASCT and the differentation of number of acute GvHD in alloSCT.

Results: There are 1359 females (46%) and 1569 males (54%) who underwent first ASCT (n = 2928). For the first ASCT were indicated patients with the following diagnoses: NHL (964, 33%), multiple myeloma (MM-902, 31%), solid tumours (381, 13%), Hodgkin’s lymphoma (HL-329, 11%), other diagnosis were below 5%. 965 (34%) patients of this group died. Cause of death: relaps/progression (24%), transplant related mortality (TRM, 5%), other cause (2%), secondary malignancy (1%), unknown (1,5%).

The 2nd ASCT were done in 526 patients (217 females 41%, 309 males 59%) with the main diagnoses: MM (311, 59%), solid tumours (108, 21%), NHL (50, 10%), HL (42, 8%) and other rare below 2%. Cause of death: relaps/progression (40%), TRM (9%), other cause (4%), secondary malignancy (1%), unknown (1%).

The total number of 1460 patients passed 1st alloSCT (583 females 40%, 876 males 60%). The main diagnoses were: AML (344, 24%), CML (259, 18%), ALL (237, 16%), bone marrow failure (90,6%) and other below 2%. Acute GvHD: gr.I–177 (12%), gr.II–382 (26%), gr.III–81 (6%), gr.IV–42 (3%), no aGvHD 728 (50%), unknown 50 (3%). Cause of death: relaps/progression (14%), TRM (28%), secondary malignancy (0,3%), unknown (0,3%).

Three hundred and thirteen pts underwent 2nd alloSCT. The main diagnoses for this group were: AML (80, 26%), NHL (52, 17%), ALL (35, 11%), MDS (32, 10%). Acute GvHD: gr.I–20 (6%), gr.II–56 (18%), gr.III–34 (11%), gr.IV–15 (5%), no aGvHD –175 (56%), UNK –5 (2%). Cause of death: relaps (25%), TRM (34%), secondary malignancy and unknown below 1%.

Conclusion: The difference between TRM after first and second ASCT wasn’t statistically significant, the difference between first and second alloSCT was borderly statistically significant. Acute GvHD grade III and IV was higher by the 2nd alloSCT as we expected.

Improving outcome in high-risk acute myeloid leukaemia: a comparison of clofarabine and FIA-Ida pre-conditioning prior to full or reduced-intensity allogeneic stem cell transplant

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The prognosis for patients with high-risk AML particularly those with leukaemia present at the time of transplant remains poor even using full intensity conditioning schedules. With the aim of improving outcome we have used pre-conditioning chemotherapy to reduce disease burden prior to the delivery of a full or reduced intensity (RIC) allogeneic transplant during the following period of cytopenia. 14 patients have been treated using two pre-conditioning chemotherapy strategies: Clofarabine-based (n=8) or a Fludarabine, High dose Cytarabine and Idarubicin schedule (FIA-Ida) (n=6). All patients had evidence of disease in the marrow prior to administration of pre-conditioning chemotherapy. In 7 of 8 patients Clofarabine was administered as a single agent (40 mg/m²/day for 5 days) and in 1 in combination with Cytarabine. 3 received RIC Busulphan or Melphalan-containing transplant schedules, 5 full intensity total

Effect of allogeneic bone marrow mesenchymal stem cells on the chemosensitivity of leukaemia cells to antileukaemic drugs in vitro

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Objectives: Human bone marrow mesenchymal stem cells (MSC) are promising candidates for new treatment options in transplant and regenerative medicine. MSC have been shown to display a considerable therapeutic potential in clinical studies for graft-versus-host diseases and support of hematopoietic engraftment. Previous investigations revealed MSC can protect blasts cells from apoptosis and the rate of relapses is increased in patients of MSC co-transplantation.

Aims: To investigate the effect of donor bone marrow MSCs on the patient leukaemia cells chemosensitivity to antileukemic drugs in vitro.

Methods: The sensitivity of blast cells to cytostatics drugs was studied by the MTT-assay with our modifications. LC50 of cytarabine (ara-C), daunorubicine (DNR) and methylprednisolone (MP) for bone marrow leucemic cell (from 25 ALL and 13 AML primary patients), incubated in direct contact with MSCs or alone, was calculated. MSC (from 20 healthy donors) were prepared by seeding bone marrow mononuclear cells in culture flasks in DMEM with 20% FCS. After 72 hours the non-adherent cells were discarded and cells were passed every 7 days by trypsinization and replanting at the density of 0.5 × 10^6 cells/75 cm² flask until 3–4 passages.

Results: Incubation of blast cell with MSC decreased their sensitivity to ara-C and DNR. LC50 for ara-C was 2.5 fold higher and for DNR – 2.7 fold higher for ALL patients; LC50 for ara-C was 1.3 fold higher and for DNR – 3.6 fold higher for AML patients. MSC increased the sensitivity of blast cells from ALL patients to MP: LC50 was 5 fold lower in comparison with blast cells incubated without MSC. MSC did not influence the sensitivity of AML blast cells to MP.

Conclusion: Bone marrow allogenic MSCs can influence the chemosensitivity and increase the viability of leukemic cells in vitro. For cell therapy with MSC it is necessary to take into consideration the possible changes of blast cells chemosensitivity and its influence on the prognosis of oncohematology patients.
relapses, HD-PAM plus autologous rescue is an effective and
relapses ensuing after HSC transplantation. In case of resistant
term survivors. autologous rescue, 2/11 of allo-transplanted patients are long
regimens (BU-TT-FLU) at a median time of 1.5 months after
sequential allogeneic HSC transplantation using myeloablative
plete remission it was possible to submit 8/11 pts (72%) to a
CR rate was 92% and TRM was 0%. Furthermore, once in com-
cases, also when bone marrow engraftment was massive. Overall
median neutrophil recovery was 15 days (11–27) and to
platelets recovery – 15 days (13–29). The full donor chimer-
ism (DC) in all cell lines was achieved in 24 pts to 30 day after
autologous HSC harvest in patients with a high risk of failure. The cell lines were received by means of specific
magnetic antibodies.

Results: The achievement of engraftment was 100% with a
median time to neutrophil recovery 15 days (11–27) and to
platelets recovery – 15 days (13–29). The full donor chimer-
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autologous HSC harvest in patients with a high risk of failure. The cell lines were received by means of specific
magnetic antibodies.

R1329
Autologous PBSC rescue after HD-PAM or after
aggressive polichemotherapy as treatment of high-risk
leukaemic relapses: high rate of complete remission and
no treatment-related mortality
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Relapses intervening after autologous as well as relapses that show chemo-resistance are at high risk of failure. HD-PAM followed by infusion of autologous PBSC has been reported to be an effective way of treatment for relapses ensuing after autologous HSC transplants in AML patients (Bug Geline, Annals of haematology, 84:748:2005), however, autologous PBSC could be useful also after aggressive poli-chemotherapy (HD-ARA-C plus antracycline) since it could reduce neutrope-
nia length and infectious risks.

We have used a salvage treatment based on PBSC autologous rescue after HD-PAM or after HD-ARA-C + antracycline in high risk leukemic relapses; 12 patients affected with AML (n.8) or with ALL (n.4) were treated, median age was 41.8 years. HD-PAM at the dosage of 140 mg/m² followed by autologous PBSC was used in 6 cases, 5 patients were treated in refrac-
tory relapse intervening during chemotherapy treatment and 1 patient was treated with HD-PAM because of a relapse post autologous transplant. Aggressive chemotherapies based on HD-ARA-C + Antracyclin and followed by PBSC autologous rescue were collected during a previous
conditioning are currently in CR at 2.5–47 months follow up. 4 of 6 patients receiving FIA-Ida pre-conditioning, underwent a Busulphan or TBI-based full intensity transplant, 2 received Busulphan-based RIC transplants. One of 6 patients in this cohort is alive in CR at 92 months (16.7%). 3 of 5 patients died from non-relapse causes at 1.3–12.5 months and 2 from relapsed disease at 5 and 6 months.

The use of Clofarabine as a pre-conditioning agent prior to the administration of the transplant conditioning schedule is well tolerated with both full and reduced intensity protocols. This approach to allogeneic transplantation shows promise for the therapy of patients with high risk, refractory AML.

R1330
Line-specific chimerism in patients with acute myeloid
leukaemia
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Despite of the impressive progress in the treatment of acute
myeloid leukaemia (AML) a by autologeneic hematopoietic stem

cell transplantation (HSCT) the early detection of the patients
(pts) with high risk of relapse remains the serious problem par-
cularly for pts with AML without cytogenetic abnormalities. We investigated chimerism in CD34+, CD3-, CD56- and CD15-cell lines to define its value in the prevention of relapse.

Patients and methods: We reviewed the records of 28 AML
patients (20 m/8 f) who underwent HSCT at the Russian
Children Research Hospital between December 2006 and Sep-
tember 2009. The median age was 7 (1–17) years. Status of
disease: complete remission I (CRI) – 11 pts, CR>1 – 8 pts,
non-CR – 9 pts. Fifteen kids were transplanted from MSD, 7 pts
– from MUD, 6 pts – from MMFD with CD3+ /CD19± depletion of the graft. The cell lines were received by means of specific
magnetic antibodies.

Results: The achievement of engraftment was 100% with a
median time to neutrophil recovery 15 days (11–27) and to
platelets recovery – 15 days (13–29). The full donor chimer-
ism (DC) in all cell lines was achieved in 24 pts to 30 day after
HSCT (Group A). Three pts from group A had mixed chimer-
ism in CD34-cell line to day 60. The reduction of immunosup-
pressive therapy resulted with the achievement of full DC to
100 day. Three pts (Group B) had full DC in CD3-, CD14-, CD15-cell lines and mixed DC in CD34- and CD56-cell lines to
30 day but then they had full DC in all cell lines to 60 day. Two
pts from Group A relapsed to 100 day. The interval between the
decreasing of CD34-cell chimerism and the morphological
relapse was 11 and 14 days. Seven pts relapsed after + 100
day. The median time of relapse was 239 days (157-302). The median time between decreasing of CD34-cell chimerism and the morphological
relapse was 60 days (25–120). Two pts received DLI+s IL-2 but
relapsed after 87 and 120 days.

Conclusion: Our results show 1) the monitoring of ND34-cell
chimerism had the important meaning for the prevention
of relapse. 2) the mixed DC to 30 day does not affect on probabil-
ity and time of relapse. 3) CD3-, 14- and CD15 –cell chimerism
does not affect on outcome in pts with AML.
Background: Acute lymphoblastic leukemia (ALL) in first complete remission (CR) with high-risk prognostic factors (Ph+, i(4;11), i(1;19), B-lineage >30 × 10⁹/l WBC, T-lineage >100 × 10⁹/l WBC at diagnosis, pro-B-ALL, late achievement of CR and complex aberrant karyotype) is indicated for allogeneic stem cell transplantation after myeloablative conditioning. Older patients (pts) or pts with comorbidities can rarely benefit from this strategy due to high risk of transplant-related mortality (TRM). Reduced-intensity transplantation (RIT) could potentially improve outcome of these pts but the experience in RIT in ALL is limited. To evaluate the potential role of RIT for high-risk ALL in 1st CR we retrospectively analysed the outcome of such pts undergoing RIT in our centre.

Results: At last follow up (FU) 7 pts are alive; toxicities of grade >2 (CTC) due to the transplant procedure were observed in 2 cases, 1 cardiac (atrial fibrillation), 1 hepatic (transaminisits), which proved reversible in both cases. Other toxicities and data on haematopoietic recovery are reported in the table. Four (50%) pts are in CR1 after a median FU from autoSCT of 942 days (201–1216), 4 pts relapsed (1 is in second CR after allogeneic SCT; 2 pts have active disease and 1 died). Median EFS from autoSCT is 368 days (92–1216), DFS from CR1 is 430 days (160–1280), OS from diagnosis is 907 days (343–1300).

Conclusions: Our preliminary experience proved that consolidation of CR1 in elderly AML/MDS pts with autoSCT after conditioning with the new FLAT regimen is feasible and well tolerated. Moreover, in this poor prognosis group of pts, prolonged OS from diagnosis, DFS from CR1 and EFS from transplant have been obtained. A phase II study in pts older than 65 is ongoing in our Center to confirm and consolidate these data.
disease. It was also shown that Treg could play a role in preventing graft rejection and regulation of graft rejection. We have studied the CD3 + CD4 + CD25+ T cells level at 1–2 months after hematopoietic stem cell transplantation on 12 consecutive patients with allogeneic HSCT for acute leukemia. Ten patients received related allograft: 8 for acute lymphoblastic leukemia (ALL), and 2 for AML (1 after myeloablative conditioning and 1 after nonmyeloablative conditioning). Other 2 patients received unrelated allograft (1 for ALL and 1 for AML). During the follow-up, the patients with myeloablative allograft had a higher level of CD3 + CD4 + CD25+ T cells (median 77.19%/Th, 69.15–85.23) than the patient with nonmyeloablative allograft who had low level of CD3 + CD4 + CD25+ T cells (15.25%/Th). The level of CD3 + CD4 + CD25+ T cells decreased 7–10 days before the clinical active chronic graft versus host disease (cGVHD) increasing. In conclusion, the level of CD3 + CD4 + CD25+ T cells after HSCT seems to be different with stem cell source and with conditioning regimen. For nonmyeloablative allograft which creates a more immunological than hematological space for hematological stem cells housing the slight suppressive activity of immune cells is the decisive factor for engraftment. In the same time, the CD3 + CD4 + CD25+ T cells level would be a useful indicator for the modulation of the cGVHD therapy.

R1334
Performance status and the time from treatment of previous neoplasia affect the outcome of therapy-related acute myelogenous leukemia and myelodysplastic syndromes receiving allogeneic stem cell transplantation
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Allogeneic stem cell transplantation (alloSCT) can cure few patients with therapy-related myelodysplastic syndromes (MDS) and acute myelogenous leukemia (AML) since mortality by disease or transplant still remains very high. This study evaluated which characteristics may affect the prognosis of MDS/AML patients receiving alloSCT. Overall (OS) and progression free survival (PFS) were analyzed by Kaplan-Meier, non-relapse mortality (NRM) and relapse incidence (RI) by Cumulative Incidence method with competing risks.
Twenty consecutive patients with AML (80%) or tMDS (20%) were allografted in 3 Italian centers between 2001 and 2007. Median age was 53 years (range 24–66), median time from treatment of previous neoplasia was 79 months (range 13-234). Previous treatment included chemotherapy (20%), radiotherapy (20%) or both (80%). Twelve (80%) patients had Karnofsky performance status (KPS) >80%, 8 had KPS ≤80%, 55% of patients had Sorror comorbidity score >1, 75% had high risk and 25% intermediate risk cytogenetics. Median time from diagnosis to alloSCT was 7 months (range 1–25). Patients underwent reduced intensity (55%) or fludarabine-busulfan (45%) alloSCT in complete (CR), partial remission (PR), or minimal residual disease (MRD). The level of CD3 + CD4 + CD25+ T cells decreased 7–10 days before the clinical active chronic graft versus host disease (cGVHD) increasing. In conclusion, the level of CD3 + CD4 + CD25+ T cells after HSCT seems to be different with stem cell source and with conditioning regimen. For nonmyeloablative allograft which creates a more immunological than hematological space for hematological stem cells housing the slight suppressive activity of immune cells is the decisive factor for engraftment. In the same time, the CD3 + CD4 + CD25+ T cells level would be a useful indicator for the modulation of the cGVHD therapy.

R1335
IV busulfan and fludarabine as conditioning regimen in acute myeloid leukemia: low treatment-related mortality and acceptable antileukaemic activity. Analysis of a single-centre experience to date
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Background: The outcome of the hematopoietic stem cell transplantation (HSCT) is largely determined by the effectiveness of the transplant conditioning regimen. Early treatment-related mortality (TRM) remains as a major obstacle, to choose the best preparative regimen. Fludarabine (Flu) is a potent immunosuppresor that enhances engraftment and hematologic recovery and it is known to have a synergistic tumor-killing effect with Busulfan (Bux). Safety and efficacy data regarding once-daily intravenous administration of Bux in combination with Flu as HSCT conditioning regimen have been recently reported. This combination seems to be effective with low TRM and acceptable antileukaemic activity.
Aims: To analyze our experience in terms of feasibility, efficacy and security of Bux-Flu conditioning in Acute Myeloid Leukemia (AML) patients undergoing HSCT.
Methods: From March 2005 to November 2009, 20 patients with high or intermediate risk-AML were transplanted using Bux-Flu as conditioning regimen. The myeloablative scheme consisted of iv Flu 40 mg/m²/day and iv Bux 3.2 mg/Kg/day given on days -6 to -3 (Total dose: Flu 160 mg/m² and Bux 12.8 mg/Kg). The reduced intensity conditioning (RIC) included: iv Flu 30 mg/m²/day on days -7 to -3 and iv Bux 3.2 mg/Kg/day on days -6 to -5 and 1.6 mg/Kg on day -4 (Total dose: Flu 150 mg/m² and Bux 8 mg/Kg). Cyclosporine and methotrexate were used as graft-versus-host disease prophylaxis in allogeneic transplants, adding Thymoglobulin (2.5 mg/Kg × 3 days) in patients receiving unrelated donor grafts.
Results: Main results are summarized in table 1. Neutrophil and platelet engraftment occurred in all patients but 1* RIC patient who died due to disease progression on day +23. The most common conditioning-related toxicity was mucositis, present in all patients. Grade-1 liver toxicity was observed in 20% of autologous and 40% of allogeneic transplants. Relapse or progression occurred in 3 allo-grafted and 2 auto-grafted at 1*, 3, 3 and 9 months and 18.5 and 22 months after transplant respectively. Three of them underwent rescue treatment and remain alive. With a median follow-up of 16 (1.5-57) months, progression-free survival is 60% for autologous and 80% for allogeneic transplant patients. No TRM was observed.
Conclusions: Our preliminary results confirm that Bux-Flu has a low toxicity profile and an acceptable anti-leukemic activity. Longer follow-up and a comparative formal analysis are needed to confirm these results.
Autologous haematopoietic stem cell transplantation as an intensive consolidation therapy for adult patients in remission from acute myeloid leukaemia

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Despite advances in our understanding of its pathogenesis, acute myeloblastic leukemia remains difficult to treat. Although initial complete remission can be achieved in a high percentage of patients, relapse occurs in 70–80% of the patients. Two main approaches have been the attempt to eradicate the leukemic clonal cells population via chemotherapy with or without autologous stem cell rescue or to pursue a combined approach using an antileukemic therapy combined with an antileukemic immune response via allogeneic bone marrow transplantation. Autologous transplantation compares favorably against allogeneic bone marrow transplant in several ways. Autologous transplantation can be used as a consolidation therapy in the older population, and lack of a matched donor does not preclude the patients from this treatment.

We report a retrospective analysis on 48 patients diagnosed with de novo AML, who did not have an available histocompatible donor, and who underwent autologous transplantation between years 2000-2009 at the University Hematology Clinic, Skopje, Macedonia. All patients had ECOG score 1 or less. The patient’s age ranged from 17 to 65 years with the median age 41 years. There were 26 males and 22 females. For stem cell mobilization patients received chemotherapy or chemotherapy plus G-CSF. The preparative chemotherapy regimen prior to autologous transplantation consisted of BuCy in 24 patient, BEAM in 22 and BuCyMel was used in the remaining 2 patients. We used bone marrow as primary source of stem cells in 18 patients, and peripheral blood stem cells in remaining 30 patients.

The five years overall survival was 52% and the 5 years disease progression free survival were 42%. We analyzed several factors that can influence the overall survival and the disease free survival such as: age, disease status, stem cell source, chemotherapy regiments prior to transplantation, conditioning regiments, number of mobilized stem cells. Advanced age and bone marrow stem cell source seems to be more influential factors. We report that the clinical results of autologous hematopoietic stem cell transplantation are sufficiently encouraging to warrant future trials that include autologous transplantation as an option for appropriately selected patients with AML in CR1. We conclude that autologous hematopoietic stem cell transplantation is a reasonable and save intensive consolidation for patients with acute myeloblastic leukemia who do not have a suitable HLA-matched donor.

R1337
What is the best conditioning for auto-transplant in AML patients?

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The conditioning regime more used in bone marrow transplantation, in patient with AML, has been the BuCy-2. Such treatment, mieloablative and immunosuppressive, he favours the engraftment of the marrow in the allogeneic transplant, not clear the advantage in the autologous transplant. In the last years, new conditioning regimes to more intense mieloablation has been used in AML autotransplant patients. We have retrospectively evaluated, in terms of efficacy and results, the classical conditioning BuCy-2 with a conditioning with busulfan (4 mg/Kg from day-6 at day-3) and melphalan (140 mg/mq day -2 (BuMel). From June 2001 to May 2009 we have autotransplanted 42 patients with AML in first CR (18 males and 24 females; median age: 46 years (range 14–73) subtype FAB: M0: 2; M1: 8; M2: 12; M4: 17; M5: 3), 26 patients have been conditioned with BuMel (12 M and 14 F; median age: 46 years (range 18–73) subtype FAB M1: 6; M2: 8; M4: 9; M5: 3) and 16 with BuCy-2 (6 M and 10 F; median age: 41 years (range 14–59) subtype FAB M0: 2; M1: 2; M2: 4; M4: 8). The factors of risk in the 2 groups are similar. High risk: 8 patients (6 in BuMel and 2 in BuCy group), intermediate risk: 28 (15 in BuCy-2 and 13 in BuMel) and low risk: 6 (4 in BuMel and 2 in BuCy group). The PBSC has been the source of the stem cells in all patients, and the median CD34 infused cells has been of 5,15 and 5 × 10⁶/Kg in BuCy-2 and BuMel groups respectively. All patients have achieved a full hematological recovery. The median days to neutrophil > 1000/mm³ and platelets > 20000/mm³ have been of 14 and 12 days in the BuCy-2 and BuMel groups respectively. TRM was 4% and 6% respectively in BuMel and BuCy-2 groups. With median follow-up of 32 months (range 4–102 months), after autotransplant, 14 patients (54%) they are alive (13 in CR) in BuMel group; in the BuCy-2 group the median DFS and OS are 9 and 12 months respectively. The EFS projected to 76 months is 50% and 33% in BuMel and BuCy-2 groups respectively, this difference is not statistically significant (P: 0.08, Cox F-test). Evaluated the AML risk intermediate and high, the DFS projected at 80 months is 53% and 20% in BuMel and Bucy2 groups respectively. This difference is statistically significant (P: 0.01).

In conclusion, even if the number of the patients is small, in High and intermediate risk AML, seems superior the conditioning with BuMel versus BuCy2. Is necessary a large cohort and a randomized study to confirm these data.
R1338
FLAG-IDA regimen as salvage chemotherapy before haematopoietic stem cell transplantation in the treatment of refractory/relapsed acute myeloblastic leukaemia: single-centre experience
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During the past several decades, improvements in chemotherapeutic regimens and supportive care have resulted in significant but modest progress in treating AML. Conventional chemotherapy is highly effective in the treatment of acute myeloblastic leukemia (AML). About 50–80% of adult patients with de novo acute myeloblastic leukemia achieve complete remission (CR) with currently available chemotherapy regimens consisting of antracyclines and cytarabine. However, relapse develops in more than 40% of the cases within two years, and 15–25% of patients fail to achieve complete remission because resistant to treatment or death. The management of cases with primary refractory and/or relapse disease is very difficult and prognosis in this subset of patients after several different chemotherapy combinations is still very poor with a CR rate 33–41%.

We evaluated efficacy and toxicity profiles of FLAG-Ida combination chemotherapy as salvage chemotherapy before hematopoietic stem cell transplantation in patients with refractory/relapsed AML. At the University Hematology Clinic in Skopje, Macedonia, in the period 2006-2008, fifteen patients with refractory/relapsed acute myeloblastic leukemia were treated with FLAG-Ida regiment. Patients were between 16–52 years old, 5 female and 10 male. They were treated with fludarabine 30 mg/m², cytosine arabinoside (AraC) 2 g/m² for 5 days, Idarubicin 10mg/m² for 3 days, and granulocyte colony stimulating factor G-CSF 5 mikrog/kg from day 0 till neutrophil recovery (ANC > 1.0 × 10⁹/l). Complete remission were achieved in 7 patients (47%), there patients (20%) died of post chemotherapy complications, and 5 failed to achieve complete remission. Out of 7 patients who achieved complete remission, 3 went autologous bone marrow transplantation, 3 went allogeneic bone marrow transplantation, and 1 is being evaluated for the same. Major complication encountered were mucositis, transient hepatic toxicity, fungal and bacterial infections.

Our experience confirmed that FLAG-IDA regimen is well tolerated and effective therapy in relapsed/refractory acute myeloid leukemia. FLAG-Ida is a good choice in cases with refractory/relapsed acute myeloblastic leukemia for salvage chemotherapy and it is wise to consolidate it with hematopoietic stem cell transplantation. Those patients included in the hematopoietic progenitor transplant program, clearly benefit from allogeneic or autologous BMT, obtaining a longer disease free survival and overall survival.

R1339
Treatment of relapsed acute leukaemia patients after haematopoietic stem cell transplantation – a single-centre experience
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Purpose: Hematopoietic stem cell transplantation (HSCT) is the curative treatment of acute leukemia. For patients of relapsed acute leukemia after HSCT, the prognosis is poor even after chemotherapy or second transplant. We present our experience to deal with these patients with various modalities of treatment.

Methods: Ten patients of acute leukemia found to have disease relapse between August 2005 and Nov 2008. Eight patients were acute myeloid leukemia and two were acute lymphoblastic leukemia. Previous transplant modalities included 9 allogeneic (7 from matched sibling donors and 2 from unrelated donors) and 1 autologous transplant. These patients underwent various modalities of treatment according to the patient tolerability and availability of donor.

Results: For these 10 patients, the range of elapsed time after transplantation was between 3 months and 40 months, 1 received second allogeneic transplant, 4 received chemotherapy and GCSF-mobilized donor leukocyte infusion, 2 received chemotherapy alone, 2 received immunosuppressant withdrawal and involved field radiation, and 1 received immunosuppressant withdrawal and donor lymphocyte infusion. In the period of 5 months to 43 months of follow-up, seven patients got complete remission achieved again but one of them died of acute graft-vs-host disease and fungemia and another one died of pulmonary tuberculosis. One patients got remission but had residual disease. Two patients who received chemotherapy alone died of refractoriness of leukemia.

Conclusions: For relapsed AML patients, the salvage treatment with chemotherapy plus CD-34 riched donor leukocyte infusion is still hopeful if there is available primary donor to harvest stem cell and lymphocytes again. For relapsed ALL patients, the chance of achievement of next remission is poor which may be related to its worse GVL effect.
**R1340**

Early immunosuppression withdrawal could improve outcome of reduced-intensity transplantation in patients with acute myeloid leukemia beyond complete remission – single-centre experience

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Background: reduced-intensity transplantation (RIT) for acute myeloid leukemia (AML) beyond complete remission (CR) is associated with higher relapse incidence, lower progression-free survival (PFS) and overall survival (OS). But elderly patients (pts) or pts with comorbidities can rarely benefit from myeloablative conditioning due to high risk of transplant-related mortality (TRM). Early immunosuppression withdrawal could potentially enhance graft-versus-leukemia (GVL) effect and improve outcome of RIT in AML beyond CR. To evaluate the role of early withdrawal of immunosuppression after RIT in pts with AML beyond CR we retrospectively analysed the outcome of such pts undergoing RIT in our centre.

Patients and methods: since 2002 19 pts with median of age 58 years (22–66 years) with AML beyond CR underwent RIT. 12 pts were in the 1st or 2nd untreated relaps, 7 pts had chemotherapy-resistant disease, all pts had more than 5% blasts in the blood. Donors were in 47% HLA identical related, 37% HLA matched unrelated and 16% HLA mismatched unrelated. The source of stem cells was peripheral blood and the median age of the patients were 28 years (range, 21–32); female/male were 5/3. Five patients received steroidal and 2 patients received cyclosporin and eculizumab pretransplant. Five of the 8 patients (62.5%) experienced at least one major thrombotic event before transplant. All the patients with thrombosis history were receiving anticoagulants. Just before transplant two patients experienced catastrophic thrombotic events. One patient developed a sudden Budd Chiari Syndrome, which was not responsive to TIPSS and died of respiratory failure. After Hickman like catheter insertion another patient developed catastrophic, extensive upper extremity thrombosis, which ended up with cancellation of AHCT. All but one patients received EBMT (Flu-CY-ATG) protocol. The stem cell source was bone marrow in half. Time from diagnosis to transplantation was 11 mos. (range, 9–27) and median follow-up interval was 10 mos. (range, 0.5–41). Two of the patients died of severe refractory coenucluive disease of the liver and co-infection in aplasia period. The remaining patients engrafted well. Two patients had acute grade 2 graft versus host disease (GVHD) of the skin but none of them developed chronic GVHD. Four of the six AHCT recipients (66%) were alive without any existing PNH clone by flow cytometric analysis. In our single center case series we observed that PNH clone is removed by AHCT with reduced intensity conditioning. Excessive thrombotic events even just before transplant during catheter insertion and peritransplant period were major concerns for the fate of the transplantation in PNH patients.

**Aplastic anaemia**

**R1341**

Allogeneic haematopoietic cell transplantation in paroxysmal nocturnal haemoglobinuria: a therapeutic dilemma from start to discharge

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare and unique disease entity. Allogeneic haematopoietic cell transplantation (AHCT) is the only available curative tool for PNH. The thrombophilic nature of the entity itself has been always a major obstacle for transplant decision especially after FDA approval of eculizumab. In Turkey as a decision of the national health authority, patients having HLA identical sibling donors are directed to allo transplant programs even when responding to eculizumab. We aimed to analyze retrospectively the outcome of our center, which is receiving many referrals and performing more than 50 allogeneic HCT per year. In intent to transplant analysis we have found eight PNH patients who were candidates for AHCT from their HLA identical siblings. Three of them were PNH/AA and 5 of them were in florid form. The median age of the patients were 28 years (range, 21–32), female/male were 5/3. Five patients received steroidal and 2 patients received cyclosporin and eculizumab pretransplant. Five of the 8 patients (62.5%) experienced at least one major thrombotic event before transplant. All the patients with thrombosis history were receiving anticoagulants. Just before transplant two patients experienced catastrophic thrombotic events. One patient developed a sudden Budd Chiari Syndrome, which was not responsive to TIPSS and died of respiratory failure. After Hickman like catheter insertion another patient developed catastrophic, extensive upper extremity thrombosis, which ended up with cancellation of AHCT. All but one patients received EBMT (Flu-CY-ATG) protocol. The stem cell source was bone marrow in half. Time from diagnosis to transplantation was 11 mos. (range, 9–27) and median follow-up interval was 10 mos. (range, 0.5–41). Two of the patients died of severe refractory coenucluive disease of the liver and co-infection in aplasia period. The remaining patients engrafted well. Two patients had acute grade 2 graft versus host disease (GVHD) of the skin but none of them developed chronic GVHD. Four of the six AHCT recipients (66%) were alive without any existing PNH clone by flow cytometric analysis. In our single center case series we observed that PNH clone is removed by AHCT with reduced intensity conditioning. Excessive thrombotic events even just before transplant during catheter insertion and peritransplant period were major concerns for the fate of the transplantation in PNH patients.

**R1342**

Line-specific chimerism in patients with aplastic anaemia

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Allogeneic hematopoietic stem cell transplantation (HSCT) remains the single curative approach to the treatment of severe aplastic anaemia (AA). The rejection is rare but the important complication of HSCT.

Patients and methods: We investigated the chimerism in patients (17, Group A; 23, Group B) who underwent HSCT between November 2006 and September 2009. The median age of pts was 7 (4–15) years. All pts received non-myoeloaibative conditioning regimes. The cell lines were received by means of the specific magnetic antibodies. Results: The achievement of engraftment was 100% with a median time to neutrophil recovery 15 days (12–33) and to platelets recovery – 17 days (11–25). The full donor chimerism (DC) in all cell lines was achieved in 16 pts to 30 day after HSCT. One patient had DC only in CD14- and CD15-cell lines and mixed DC in CD3- and CD19- cell lines. One patient had DC only in CD14- and CD15-cell lines and mixed DC in CD3- and CD19- cell lines. Five patients were mixed DC in all cell lines (Group B). In 1 patient the DC in all cell lines without GvHD. One pt with the decreasing mixed DC from group B achieved the full DC in all lines. Four pts from group B achieved the full DC in CD14-, CD15-cell lines and mixed DC in CD3-, CD19- and CD56 – cell lines and 3 pts had mixed DC in all cell lines (Group B). One patient had DC only in CD14- and CD15-cell lines and mixed DC in CD3- and CD19- cell lines. Five patients were mixed DC in all cell lines (Group B). Three of them were PNH/AA and 5 of them were in florid form. The median age of the patients were 28 years (range, 21–32) and median follow-up interval was 10 mos. (range, 0.5–41). Two of the patients died of severe refractory coenucluive disease of the liver and co-infection in aplasia period. The remaining patients engrafted well. Two patients had acute grade 2 graft versus host disease (GVHD) of the skin but none of them developed chronic GVHD. Four of the six AHCT recipients (66%) were alive without any existing PNH clone by flow cytometric analysis. In our single center case series we observed that PNH clone is removed by AHCT with reduced intensity conditioning. Excessive thrombotic events even just before transplant during catheter insertion and peritransplant period were major concerns for the fate of the transplantation in PNH patients.
Introduction: Shwachman Diamond syndrome (SDS) is a rare autosomal-recessive disorder characterized by exocrine pancreas dysfunction, skeletal abnormalities and bone marrow failure. Bone marrow dysfunction typically develops in childhood including varying cytopenias, aplastic anemia and myelodysplasia, whereas leukemic transformation usually occurs in adolescence. Mutations of the SBDS gene (Shwachman Bodian Diamond syndrome gene, located on chromosome 7q) have been reported in about 90% of all patients with clinical features of SDS. Therefore cytogenetic aberrations like monosomy 7 and isochromosome 7q are frequently found in these patients. Apart from supportive care like G-CSF (granulocyte colony-stimulating factor) administration, transfusion for anemia and thrombocytopenia hematopoietic cell transplantation is the only potentially curative treatment of bone marrow failure. Few transplantations for SDS with aplastic anemia in childhood have been reported, but data in adults are missing.

Case report: A 30-year-old female with SDS (46xx, isochromosome 7q, combined heterozygous carrier of SBDS splice mutation), who had been suffering from exocrine pancreatic insufficiency and growth deficiency since early childhood, was first diagnosed with pancytopenia at the age of 29. A bone marrow biopsy and aspirate showed aplastic anemia without signs of dysplasia or blasts. Due to increasing transfusion frequency and decline of performance status we decided to perform an unrelated donor bone marrow transplantation (BMT) in absence of an HLA-matched family donor. The conditioning regime consisted of total nodal irradiation, 2 x 2 Gy per day (day -10 and -9), fludarabine 30 mg/m² per day (day -9 to -6) followed by 30 mg/kg antithymocyte globulin and 50 mg/kg cyclophosphamide per day (day -5 to -2). Immunosuppression with cyclosporin A started at day -1 and was combined with sirolimus starting at day +1. The patient underwent transplantation ten months after diagnosis of aplastic anemia. She recovered very slowly, supported by daily administration of G-CSF (day 91 to 101) with neutrophil engraftment on day +102 and no evidence of acute Graft-versus-host disease. Bone marrow biopsy and aspirate revealed a hypocellular marrow with normal maturation, no evidence of aplastic anemia and full chimerism.

Conclusion: This case report indicates that BMT may be performed successfully in adult SDS patients with aplastic anemia.

Autoimmune diseases

R1344
Immuno-suppressive high-dose therapy and auto-transplantation in a case of resistant thrombotic thrombocytopenic purpura
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The thrombotic thrombocytopenic purpura (TTP) it is a thrombotic microangiopathy. The plasma exchange (PE), in association with steroids, it currently represents the first-line treatment. Among 11% and 36% of the patients present recurrent episodes of TTP. The high doses immunosuppressive therapy (HDIT), with or without autologous transplantation (ASCT), has been used in different autoimmune disease resistant for, with the immunological reset followed by high doses therapy, to destroy the clones or oligo-clones population of lymphocyte auto-reagents and to restore a functionality immunity normal.

The experience in the TTP with this treatment is limited. 47 year-old woman with recurrent episodes of TTP treated with HDIT and auto-transplant. The patient has presented among 1999 and 2004, 6 episodes of TTP, initially treated with infusion of plasma, steroids and vincristine, subsequently with PE and steroids. The remission of the TTP more than one year has not lasted and the last episode in August 2004 has poor responded to the treatment and has exposed the patient to serious danger of life. In September 2004, after mobilization with high doses of cyclophosphamide (7 gr/m²) the stem cells have been harvested (4 x 10^6/Kg) and in December 2004, after conditioning with Fludarabine (50 mg/m² from the day -6 a day -4); cyclophosphamide (60 mg/Kg day -3); G-CSF (from day +1) and mycophenolate mofetil (600 mg/m² from day -6), the patient has been auto-transplanted. Not has been shown fever in aplasia and the patient has not had necessity of transfusion. The attachment has been documented a day +8 after transplantation. The treatment with mycophenolate mofetil has been continued for two years. Currently the patient is to +60 months from the transplantation and has not had new episodes of TTP. The association HDIT/ASCT has been used to the purpose to eliminate the auto-immunity in the patient through the conditioning and to have a rapidly haematological recovery with the infusion of stem cells. The use prolonged of mycophenolate mofetil has served to control the new immunological order restored after such treatment. Therapy has excellently been supported, have not been documented side effects and the patient has had the longest period of remission. In conclusion such procedure can be an option for the treatment of the patients with resistant autoimmune disease and not curable, also after treatment with rituximab.

R1345
Allogeneic haematopoietic stem cell transplantation in a patient with severe treatment-refractory optic neuromyelitis
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Background: Allogeneic Haematopoietic Stem Cell Transplantation (Allo-HSCT) can induce stable remissions in patients with severe treatment-refractory Autoimmune Diseases (ADs). Here we report the experience on Allo-HSCT in a patient (pt) affected by severe Optic Neuromyelitis (NMO) associated with aquaporin-4 antibodies (AQP4), with severe relapsing myelitis. Material and methods: The pt is a 28 years man, who underwent HLA identical sibling Allo-HSCT for severe NMO, positive for AQP4, unresponsive to several treatments as steroids, Cyclophosphamide (Cy), Plasma Exchange, Rituximab, Thiotepa/Cy based Autologous HSCT. Before Allo-HSCT pt showed 6 points in EDSS score with paraparesis, thoracic spinal cord lesion (D4-D8) on MRI, 3 severe relapses occurred in the previous year. Results: The pt received a Treosulfan-Fludarabine based myeloablative conditioning regimen, consisting of Fludarabine i.v. 30 mg/m²/d day -6 to -2, Treosulfan i.v. 14 g/m²/d day -6 to -4, Rituximab i.v. 375 mg/m² day -1. The graft source was peripheral blood stem cells. At day 0 he received 5.98 x 10^{10} infused CD34 + cells/Kg. The prophylaxis for graft-versus-host disease (GvHD) was based on ATG-Fresenius i.v. 10 mg/kg day -4 to -2 and standard cyclosporine plus short course methotrexate. Hematopoietic recovery required 21 days for neutrophil and 6 days for platelet engraftment. During hospitalization the pt developed febrile neutropenia solved with antibiotics and a CMV reactivation responsive to Ganciclovir iv. Twelve days after Allo-HSCT a transient neurologic worsening occurred (paraparesis and MRI enhancing lesion on dorsal spinal cord), rapidly solved with high dose steroids. There were no major adverse events. Three months after transplantation patient developed...
a transient grade I GvHD (for skin grade I) not treated, and an episode of cholecystitis treated with antibiotics. At 30 days after Allo-HSCT pt showed 7.0 points EDSS, chimerism >95% donor on STR analysis. At 60 days: 6.0 points EDSS, absence of AQP4, chimerism >95% donor STR. At 100 days he presented 5.0 points EDSS, absence of AQP4, chimerism 100% donor STR. Conclusions: These findings suggest that Allo-HSCT is well tolerated without any side effects. 17 days after the treatment the dermal ulcer showed some improvement of 2,01*10^6 MSCs / kg and 1,97*10^6 MSCs/kg respectively compared to baseline. Phenotypical and functional properties. An intravenous application of the factor-independent 32Dp210 bcr-abl cell cycle distribution and induction of apoptosis in T315I cells as compared to wt and IM or nilotinib resulted in an increased inhibition of proliferation in wt (P<0.0001) and mutated cells (H396P, T315I, P<0.0001) versus controls. Cotreatment with bcr-abl siRNA or the combination of bcr-abl siRNA with IM and nilotinib significantly induced apoptosis and inhibited proliferation in wt and mutated cells. In T315I cells bcr-abl siRNA with nilotinib dramatically reduced the BCR-ABL expression in wild-type (wt) and mutated bcr-abl cells and increased the lethal capacity. Bcr-abl siRNA significantly induced apoptosis and inhibited proliferation in wt (P<0.0001) and mutated cells (H396P, T315I, P<0.0001) versus controls. Cotreatment with bcr-abl siRNA and IM or nilotinib resulted in an increased inhibition of proliferation and induction of apoptosis in T315I cells as compared to IM or nilotinib alone (P<0.0001). Furthermore, the combination of bcr-abl siRNA with IM or nilotinib significantly (P<0.01) reversed multidrug resistance gene 1-dependent resistance of mutated cells. In T315I cells bcr-abl siRNA with nilotinib showed powerful effects on the cell cycle distribution. Conclusions: Our data suggest that silencing by bcr-abl siRNA combined with IM or nilotinib may be associated with an additive antileukemic activity against tyrosine kinase inhibitor-sensitive and resistant BCR-ABL cells, and might be an alternative approach to overcome BCR-ABL mutations.

R1347
Autologous stem cell transplantation as a treatment option for patients with multiple sclerosis
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Introduction: High dose chemotherapy followed with autologous stem cell transplantation (ASCT) has been proposed in the last few years as a potential treatment option for patients (pts) with secondary progressive multiple sclerosis (MS).
Aim: We analyze retrospectively small cohort of pts with secondary progressive MS treated with ASCT within ESTIMS trial.
Methods: Since 2005. five pts with secondary progressive MS, who have failed to several lines of therapies (Interferon beta, Mitoxantrone...) was treated in our center with ASCT. Median age was 31 (21–52), male/ female 4/1, baseline median disability status scale (EDSS) of 6.0 (3.5–9.0). All of them were mobilized with Cyclophosphamide 4 g/m² and G-CSF 10 microg/kgIT and in all cases conditioning was up to standard BEAM regimen with addition of ATG. EDSS was estimated 3, 6, 9 and 12 months after the procedure.
Results: With the median follow up of 21 months (10–55), 1 pt (20%) remained stable without new lesions on MRI and other 4 (80%) have showed improvement according to EDSS by at least 0.5 points. Also, there were no transplant related mortality and engraftment was observed in each case of this small group of pts.
Conclusion: This modest retrospective analysis shows benefit of autologous SCT in pts with secondary progressive multiple sclerosis. Larger number and adequate pts selection with longer follow up is necessary for more valid estimation of efficacy and safety of this therapy option.

R1348
Small interfering RNA against bcr-abl transcripts sensitized mutated T315I cells to nilotinib
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Background: Selective inhibition of the BCR-ABL tyrosine kinase by RNA interference has been demonstrated in leukemic cells. Therefore, we evaluated specific bcr-abl small interfering RNA (siRNA) silencing in BCR-ABL positive cell lines, including those resistant to imatinib (IM) and particularly those with the T315I mutation.
Design and methods: The factor-independent 32Dp210 bcr-abl oligoclonal cell lines and human IM-resistant bcr-abl positive cells from patients with leukemic disorders were investigated. The effects of bcr-abl siRNA or the combination of bcr-abl siRNA with IM and nilotinib were compared with those of the ABL inhibitors IM and nilotinib.
Results: Coadministration of bcr-abl siRNA with IM or nilotinib sensitively induced apoptosis and inhibited proliferation in wt (P<0.0001) and mutated cells (H396P, T315I, P<0.0001) versus controls. Cotreatment with bcr-abl siRNA and IM or nilotinib resulted in an increased inhibition of proliferation and induction of apoptosis in T315I cells as compared to IM or nilotinib alone (P<0.0001). Furthermore, the combination of bcr-abl siRNA with IM or nilotinib significantly (P<0.01) reversed multidrug resistance gene 1-dependent resistance of mutated cells. In T315I cells bcr-abl siRNA with nilotinib showed powerful effects on the cell cycle distribution.
Conclusions: Our data suggest that silencing by bcr-abl siRNA combined with IM or nilotinib may be associated with an additive antileukemic activity against tyrosine kinase inhibitor-sensitive and resistant BCR-ABL cells, and might be an alternative approach to overcome BCR-ABL mutations.

R1349
Haematopoietic stem cell transplantation in primary myelofibrosis cases: single-centre experience
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Since year 2000, 11 patients with primary myelofibrosis (9 females and 2 males age 29 – 57 yrs median 47 yrs) received allo HSCT (7 sib and 4 unrelated donors matched at allele.
levels). 2 patients were in high, 7 in intermediate and 2 in low risk of the disease (Dupriez score). The patients were in advanced stage of fibrosis, all had splenomegaly and abnormal blood smear with the presence of erythoblasts. The disease was manifested clinically from 7 to 52 months (median 19). Seven patients were transfusion dependent because of anemia or thrombocytopenia, three patients were on steroids and seven on hydroxypropamide. Five patients had JAK 2V617F mutation. Splenectomy prior transplantation was performed in two patients. Two patients received, myeloablative conditioning (Busulfan 16 mg/kg Cyclophosphamide 120 mg/kg) and nine reduced intensity conditionin (Busulfan 8 mg/kg, Fludarabine 120–150 mg/m² or Melphalan 120–140 mg/m² and low dose ATG). All patients were transplanted with PBPC with CD34 dose from 1.8 to 11.7 × 10^6 cells/kg (median 6.63 × 10^6 cells/kg). Two patients died due to transplant related toxicity, one due to severe aGvHD. Two patients transplanted with major blood group incompatibility developed PRCA. Erythropoetin, plasmapheresis and anticy CD20+ antibodies were employed in those patients. Finally all surviving patients were in full hematological reconstitution. Terephine biopsy performed one month post transplant documented bone marrow remodeling with a normal picture six month post transplant. All patients had full chimerism. Five patients with aGvHD positive before transplantation were negative during observation period from 1 to 116 months (median 37). Eight out of 11 patients are alive and well. In conclusion: haematopoietic stem cell transplantation in primary osteomyelofibrosis is associated with rather low risk and results in sustained haematological recovery.

Haemoglobinopathy

R1350
Haematopoietic stem cell transplantation in children with severe beta thalassaemia – Bulgarian experience
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Hematopoietic stem cell transplantation (HSCT) is only one treatment in children with severe beta thalassemia. Allogeneic HSCT from full match sibling donors is preferred because of better results and lower transplant – related morbidity and mortality rate.

We present bulgarian experience on HSCT in children with beta thalassemia. Between June 2005 and November 2009 eight HSCT were performed in seven children with beta thalassemia major in our center. One patient received second transplantation due to graft rejection. Three of our pts were in class 3, two in class 2 and two in class 1. Median patient’s age was 8, 5 (5–14) years. The donors were much sibling donors – 6, much related donor (mother) – 1 and cord blood from sibling (sister) – 1. Two patients received only bone marrow, one patient – bone marrow in first transplantation and peripheral blood stem cells in second, three patients – only peripheral blood stem cells and one cord blood. Median CD34 + cells count transplanted was 3.25 × 10^6/kg. The conditioning regimens were: Bu + Cy – 2, Fludara + Bu + Cy + ATG – 3, Bu + Cy + ATG – 1, Fludara + Bu + ATG – 1 and only ATG – 1 in second transplantation. The Graft versus host disease (GvHD) prophylaxis was given according to EBMT. Engraftment was achieved in 5 pts; in two we observed autologous bone marrow reconstitution. One of engrafted patients developed graft rejection 6 months after transplantation and received second transplantation. He is dead 30 days after second transplantation from serious infectious and hemorrhagic complications, without engraftment. Median engraftment time for neutrophile and platelets were 20 an 23 days, respectively, longer in bone marrow transplantations. Acute GvHD Grade I–IV was seen in 5 pts and cGvHD – in 2 pts. The transplant-related mortality was 28.5% (2 patients, one from graft failure and one from aGvHD and severe infection). The median follow-up was 27 months. The 3–year OS and EFS are 71, 4% and 42, 9% respectively.

We conclude in spite of small number of patients in our center until now, that HSCT in pediatric patients with beta thalassemia major is only one curable procedure, especially in early years and from sibling donors. In the future more thalassemia patients must be transplanted in our country because of high disease frequency in Bulgarian population.

Inborn errors

R1351
Prolidase deficiency may be reversed by HSCT
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Introduction: Prolidase deficiency (PD; MIM + 170100) is a rare hereditary disorder resulting from mutation in the PEPD gene, mapping at 19cen-q13.11. Prolidase hydrolyzes dipeptides containing C-terminal proline and hydroxyproline; PD causes massive excretion of urinary iminodipeptides. The clinical manifestations of PD may be widely variable: affected patients show mainly skin ulcers of lower extremities resistant to various types of treatment, an unusual facies (saddle nose, hypertelorism, exophthalmos, low hairline, micrognathia, mandibular protrusion, high-arched palate, facial hirsutism), ocular abnormalities, deafness, splenomegaly, obesity, often with mental retardation, and a history of recurrent infections (otitis, sinuitis, and cellulites).

Case report: G.C. presented at 18 months with recurrent skin ulcerations, a typical PD face, but normal intelligence. Her disease course was complicated by recurrent deep ulcerations with superinfection, and even sepsis on one occasion. She had to be repeatedly hospitalized and was always on low-dose steroids. The diagnosis of PD was made at the age of 7 years by mean of blood prolidase activity evaluation, and confirmed by mutation analysis.

Given her life-threatening course, we decided to treat her with allogeneic HSCT from her HLA-identical brother, confirmed heterozygous for PD. After a conditioning regimen with BU 16/mg/kg+CY 200 mg/kg, we infused 3.52 × 10^6 CD34/kg. Defibrotide 10 mg/kg was given for VOD prophylaxis from +1; GvHD prophylaxis consisted of MTX 10 mg/sqm iv on day +3, +6, +11 and cyclosporin 3 mg/kg from day −1. The PMN take was recorded on day +16, while no platelet engraftment was observed. Full donor chimerism was repeatedly documented. On day +16 she developed spiking AST/ALT levels, up to 10,460/5,507 IU/L on + 19, with jaundice, hepatomegaly and weight gain. Liver biopsy confirmed VOD. The child died on +92 of invasive Geotrichum capitatum infection. Post-transplant monitoring of blood prolidase activity showed that, in keeping with the full donor chimerism, the child had converted to an heterozygous pattern. CE analysis of the urines also showed a reduction of iminodipeptides peaks.

Conclusion: despite the unfavourable outcome in this case, we provide the first evidence that prolidase deficiency may be reversed by HSCT. The indication to transplant must be balanced against the clinical manifestation of individual patients.

Haemoglobinopathy
On day 0 the patient received an iv dose of 2.7 × 10^6/kg MSC (according to the EBMT MSC expansion consortium guidelines), prior to the initiating of the conditioning regimen. iv MSC were isolated, expanded and cryopreserved (150 mg/m², 3 doses). After initial clinical improvement, the relapse was with two life-threatening HLH episodes, treated with CSA, methylprednisolone (max. 5 mg/kg/day) and anti-thymocyte globulin (ATG, 5 × 10 mg/kg). She received HSCT from an HLA-id sib, after ATG (5 × 5 mg/kg/day), Bu (4 × 4 mg/kg/day), Cy (4 × 50 mg/kg/day). GVHD prophylaxis was CSA and methotrexate. She had neutrophil and platelet engraftment by D+23 & D+32. Whole blood chimerism on D+40 was predominantly donor alleles; by D+100 she had mixed chimerism. Quantitative chimerism at 2.33 years post HSCT showed 30% T and B, and 15% myeloid donor cells. After 6 years she developed fever and cytopoenia with spontaneous recovery after 1 week. Over the next year she had recurrent episodes, lasting 4–5 days, every 5–7 weeks, with occasional abdominal pain, headache, arthralgias and non-specific rash. At 7 years post HSCT she developed hypofibrinogenemia, hypertriglyceridemia, hepatosplenomegaly, respiratory distress and lung infiltrates on CT scan. HLH was seen on lung biopsy, confirming sub-acute relapsing HLH. Chimerism was unchanged.

Genetic studies identified homozygous 1284G>A mutations in PRF1 for which the sibling was heterozygous. She received HLH 2004, was conditioned with Flu (30 mg/m², 5 doses), Melphalan (140 mg/m²) and had HSCT from the same donor. CSA and MMF were used as GvHD prophylaxis. She was 100% donor by D+48 and remains well. Good chimerism is required after HSCT for AR immunodeficiency when the defect is unknown and a sib donor is used, to prevent relapse. This is the latest case reported of relapsed FHLH after transplantation. We discuss the hypothesis that a higher donor chimerism is required in cases without a known genetic defect, when a sibling donor is used.

R1353
Late relapse of FHLH after bone marrow transplantation from a carrier donor
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Familial hemophagocytic lymphohistiocytoses (FHLH) are primary immunodeficiencies cured by HSCT. Mutations in the perforin/granzyme-dependent cytotoxic pathway cause disease. Successful engraftment leads to cure. Mixed donor chimerism is more common recently, likely due to RIC regimens. A minimum of 20% donor cells is considered sufficient to control disease.

We report a girl of consanguineous parents who had late relapse of FHLH after successful sib donor HSCT. She presented at 2 months with fever, lymphadenopathy, hepatosplenomegaly, cytopenia, hyperferritinemia, hypertriglyceridemia and hypofibrinogenemia. Bone marrow aspirate and lymph node biopsy demonstrated HLH. She received HLH 94, (dexamethasone (10 mg/m²), etoposide (150 mg/m², 3 doses). After initial clinical improvement, she relapsed with two life-threatening HLH episodes, treated with CSA, methylprednisolone (max. 5 mg/kg/day) and anti-thymocyte globulin (ATG, 5 × 10 mg/kg). She received HSCT from an HLA-id sib, after ATG (5 × 5 mg/kg/day), Bu (4 × 4 mg/kg/day), Cy (4 × 50 mg/kg/day). GVHD prophylaxis was CSA and methotrexate. She had neutrophil and platelet engraftment by D+23 & D+32. Whole blood chimerism on D+40 was predominantly donor alleles; by D+100 she had mixed chimerism. Quantitative chimerism at 2.33 years post HSCT showed 30% T and B, and 15% myeloid donor cells. After 6 years she developed fever and cytopoenia with spontaneous recovery after 1 week. Over the next year she had recurrent episodes, lasting 4–5 days, every 5–7 weeks, with occasional abdominal pain, headache, arthralgias and non-specific rash. At 7 years post HSCT she developed hypofibrinogenemia, hypertriglyceridemia, hepatosplenomegaly, respiratory distress and lung infiltrates on CT scan. HLH was seen on lung biopsy, confirming sub-acute relapsing HLH. Chimerism was unchanged.

Genetic studies identified homozygous 1284G>A mutations in PRF1 for which the sibling was heterozygous. She received HLH 2004, was conditioned with Flu (30 mg/m², 5 doses), Melphalan (140 mg/m²) and had HSCT from the same donor. CSA and MMF were used as GvHD prophylaxis. She was 100% donor by D+48 and remains well. Good chimerism is required after HSCT for AR immunodeficiency when the defect is unknown and a sib donor is used, to prevent relapse. This is the latest case reported of relapsed FHLH after transplantation. We discuss the hypothesis that a higher donor chimerism is required in cases without a known genetic defect, when a sibling donor is used.
stomatitis and sclerosing cholangitis. Overall, 75% of patients with XHIM develop liver complications which are the main cause of death and also make successful bone marrow transplantation (BMT) more difficult to perform. Here we report successful bone marrow transplantation from HLA identical sibling donor in a case of XHIM with cirrhosis by using new conditioning agent treosulphan together with cyclophosphamide. The patient was the 3rd child of consanguineous parents with no family history of immunodeficiency. He has recurrent upper and lower airway disease and oral aphthous lesions since 6 months of age and diagnosed as XHIGM when he was 1.5 years-old. The diagnosis of XHIGM was confirmed by sequence analysis of CD40L gene showed a hemizygous deletion of a C nucleotide in exon 5 which results in a frameshift and a premature stop codon. He referred to our clinic when he was 3.5 years-old for BMT. On physical examination growth failure, bilateral fine crackles on both lungs and 6 cm hepatomegaly and 6 cm splenomegaly was detected. Results of liver function tests were abnormal and liver biopsy revealed grade III fibrosis and compensated cirrhosis. Following conditioning with treosulphan (12g/m² d × 3) and cyclophosphamide (50 mg/kg/d × 4), bone marrow (6,1 × 10⁶/kg CD34 + cells/kg) from HLA-identical sister was infused. Cyclosporin-A was given for GVHD prophylaxis. Myelo- loid engraftment was achieved on day +13. CD40L expression on activated lymphocytes of the patient was 84% on day + 21. Moderate transaminase elevation and CMV antigenemia were resolved and the patient discharged on day +43. He is now on posttransplant 14 months with full donor chimerism and mild chronic GVHD on his tongue.

**Lymphoma**

**R1355**

Autologous peripheral blood stem cell transplantation for elderly patients with relapsed non-Hodgkin lymphoma: a single-centre experience

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Background: As indicated by IPI-Score and already well proved by many other authors, patients with > 60 years and relapsed non-Hodgkin’s lymphoma (NHL) have a poor prognosis. Despite the effectiveness of standard therapeutic regimens, such as R-CHOP, the percentage of relapsing patients is still high. High dose therapy with rescue of autologous PBSCT has shown to be the best salvage therapy. We report our experience on 20 patients aged >60 years with relapsed NHL treated between February 2000 and June 2008 with autologous PBSCT.

Patients and methods: The median age of patients was 65 years (60–71). Thirteen (65%) patients were male and 7 (35%) female. All had a good performance status according to comorbidities. Twelve patients (60%) had diffuse large B-cell lymphoma (DLBCL), 4 (20%) mantle cell lymphoma, 4 (20%) transformed follicular lymphoma. Adjusted for age IPI at relapse was 0–1 in 4 patients (20%) and 2–3 in 16 patients (80%). At hospital admission, 10 patients (50%) were in complete remis- sion (CR) and 10 patients (50%) in partial remission (PR). 3 patients (15%) were conditioned with TEAM regimen, 3 patients (15%) with BEAM, 13 patients (65%) with BEAM, and 1 patient (5%) with the combination of mitoxantrone and melphalan. The median number of CD34 + cells infused was 3.85 × 10⁶/kg body weight (2.4–6.5 × 10⁶/kg).

Results: The median days for neutrophils (> 500/mmc) and platelets (> 20,000/mmc) recovery was 9 days (6–12) and 10 days (7–12), respectively. The median days of febrile neutropenia was 7 days (–11). The median number of packed red cell units and platelet concentrates were 2 (0–9) and 4 (1–19), respect- ively. The incidence of grade 2–3 and grade 3–4 mucositis was 70% and 30%, respectively. No relevant renal and hepatic toxic- ity was observed and only 1 patient died because of multiorgan failure. One patient died after 30 days after transplant because of unknown cause. After 3 months from transplant 18 patients (90%) showed a CR and at a median follow-up of 78 months (5–108) all patients were still in CR.

Conclusions: In our hands, autologous PBSCs transplantation was found the best salvage therapy for elderly patients with relapsed NHL. The toxicity and the transplant related mortality were showed not relevant and the hematologic recovery the same as in younger patients. However, an accurate pre-trans- plant evaluation of performance status and comorbidities of elderly patients is mandatory.

**R1356**

Successful use of plerixafor on an immediate-rescue basis for lymphoma patients with suboptimal CD34+ counts after salvage chemotherapy regimes for PBSBC mobilization: a single-centre series of 3 consecutive patients

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Plerixafor is usually given following G-CSF without mobilising chemotherapy. This could be a barrier to its use in patients with higher-grade lymphomas, where PBSBC mobilisation is usually carried out using salvage regimes, & where chemotherapy “hol- idays” for purposes of mobilisation are undesirable. We present 3 patients who mobilised transplantable PBSBC doses using plerixafor on an immediate-rescue basis to boost peripheral CD34 + counts & allow next-day apheresis despite suboptimal CD34 + counts during recovery from mobilising chemotherapy. Patient 1: Male; age 58; Stage 4 DLBC NHL; extensive pre-treatment including fludarabine, & 2 failed mobilisation attempts using cyclophosphamide. At Day 13 following 2nd cycle of ESHAP salvage chemotherapy, followed by G-CSF 10 mcg/kg/day for PBSBC mobilisation, his total WCC was 3*10⁹/l with peripheral CD34 + count of 2/microlitre. Plerixafor was given that evening & the following evening with next-day aphereses, yielding peak peripheral CD34 + count of 33/microlitre and total CD34 + dose of 3.64*10⁶/kg. He proceeded to autograft, and is alive in CR at 10 months post-transplant. Patient 2: Female; age 47; relapsed Stage 4B Hodgkin Lymphoma; extensive pre-treatment including chlorambucil & 2 previous failed mobilisation attempts using cyclophosphamide. At Day 6 following Cycle 1B of ABVD salvage chemotherapy followed by G-CSF 10 mcg/kg/day, her total WCC was 6 with a peripheral CD34 + count of 2/microlitre. Plerixafor was given that evening & the next day with two aphereses, yielding peak CD34 + count of 88/microlitre and total CD34 + dose of 6.36*10⁶/kg. A decision on transplant is awaited pending re-staging. Patient 3: Male; age 67; Stage 4 DLBC NHL; extensive prior chemotherapy including fludarabine and considered at high risk of mobilisation failure. Following Cycle 1 of IV E salvage chemotherapy with G-CSF 10 mcg/kg/day, his total WCC was 18.8 at Day 26 with peripheral CD34 + count of 7/microlitre, and he underwent apheresis yielding a CD34 + dose of 0.68*10⁶/kg. He received plerixafor that evening with 2 further aphereses the next 2 mornings, yielding peak CD34 + count of 12/microlitre and total CD34 + dose of 2.9*10⁶/kg; he is scheduled for transplant in the near future. Plerixafor is effective for PBSBC mobilisation on an immediate-rescue basis for lymphoma patients mobilising poorly after salvage chemotherapy regimes. Economic analysis suggests that this is more cost-effective than delayed re-mobi- lisation with plerixafor plus G-CSF.
R1357
Alectuzumab-ESHAP as salvage regimen in refractory T-cell lymphoma patients candidate to intensification with autologous or allogeneic stem cell transplantation (a short single-centre pilot study)
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Introduction: Refractory T cell lymphomas have a very bad prognosis. Intensification with autologous (auto) or allogeneic (allo) stem cell transplantation (SCT) may be proposed to young patients if they reach a partial response. We report here the results of Alectuzumab, an anti CD52 monoclonal antibody and ESHAP in association (A-ESHAP) as a salvage regimen in 5 patients with refractory T cell lymphoma eligible to intensification with SCT.

Methods: Two alectuzumab (10 to 30 mg) IV injections were added to the standard ESHAP cycle. Patients received infectious prophylaxis with valacyclovir and cotrimoxazole or pentamidine.

Results: Five patients received A-ESHAP, 4 males and 1 female. Median age was 56 years (40–61). Four patients had stage IV lymphoma and 1 had stage II bulky disease. LDH was > normal in 4 patients. The patients were refractory to first line chemotherapy. Patients received 1 to 4 cycles of A-ESHAP. One patient received an HLA identical allo SCT. Peripheral stem cell harvest was successful in 2 of 3 patients after G-CSF stimulation and in 1 patient after stem cell factor + G-CSF stimulation. Two patients received autoSCT conditioned with BEAM and engrafted 15 and 20 days after stem cell infusion. Pre transplant toxicities consisted of febrile neutropenia, CMV reactivation and undocumented pneumonia in 1 patient, seizures in 1 patient. Post-transplant infectious events consisted of CMV pneumonia, BK virus cystitis and multiple bacterial cystitis in the allo SCT recipient, herpes zoster infection, s.oralis septicemia and pneumonia imputed to pneumocystis carinii in one of the auto SCT recipient and CMV pneumonia the other one. Two patients died from lymphoma progression: 1 patient neither responded to treatment and 1 patient relapsed 6 months post auto SCT. Three patients were alive complete remission, 32 months post allo SCT, 19 months post auto SCT and 24 months after EBV lymphoproliferation treated with four rituximab injections.

Conclusion: With 3 long-term remitters among 5 patients, A-ESHAP appeared feasible, but very toxic, as a salvage regimen before intensification and auto or allo SCT. In the setting of refractory T cell lymphoma these results merit consideration even if they are counter-balanced by the high infectious rate.

R1359
Autologous bone marrow transplantation with purging in vivo in Burkitt and Burkitt-like lymphoma
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Burkitt and Burkitt-like lymphoma is a highly aggressive lymphoma. In recent years, efforts have focused on improving therapy for this rapidly proliferating neoplasm while minimizing, to the extent possible, treatment-associated toxicity. These efforts have led to the development of high-intensity, short-duration combination chemotherapy that has proven extremely effective for a high proportion of these lymphoma patients. From 2003 in our division have been treated, the patients with these lymphomas, with aggressive treatments, transplantation with purging and with purging and without. From April 2003 to December 2008 we have treated with Autologous stem cell transplantation, purged in vivo with monoclonal antibodies, 7 patients (1 F; 6 M median age: 37 years) with Burkitt (5 patients) and Burkitt like (2 patients) lymphoma. In all patients treatment has been effected according to the scheme R-CODOX-M for 2 cycles alternated to R-IVAC for 2 cycles with intrathecic medication with ARA-C and MTX. After the last cycle of R-IVAC the patients have been mobilized and harvested the PBSC. To the transplantation all patients were in CR. All patients have harvested (median CD34: 4 × 10^6/kg) and minimal residual disease in the harvest has been not detectable to immunophenotype. All the patients have been conditioned with BEAM-R (with infusion of rituximab to the day +1) and the graft are documented in 7/7 patients (any complication before and after treatment) with recovery of neutrophils > 1000 in media to day + 11 (range 8–12 days). After transplantation all patients were in CR. With a median follow-up of 48 months after transplantation (range 16–64 months) 6/7 (86%) patients are in CR (1 patients have relapsed with burkitt lymphoma (is relapsed extra-nodular at months +8 and died for disease a months +10 after transplantation). The EFS and OS projected at 64 months are of the 86%. In conclusion the use of the rituximab and autologous transplantation in this cohort of patients has allowed obtaining very good results with minimal toxicity. Occurred al more number of patients for to confirm such data.
R1360
Good outcome of refractory patients affected by primary mediastinal B-cell lymphoma treated with peripheral blood stem cell autografting
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Objectives: The aim of this retrospective study is to evaluate the outcome of refractory patients (pts) affected by Primary Mediastinal B-Cell Lymphoma (PMBCL) who received high dose therapy (HDT) and peripheral blood stem cells autografting (PBSCA) as salvage therapy.
Methods: Between 3/2003 and 9/2009, 32 newly diagnosed pts with PMBCL have been treated. First line treatment consisted of 6-8 courses of Rituximab in association to CHOP chemotherapy (R-CHOP) and consolidative radiation therapy (RT) on mediastinal bulk. Refractory pts were defined as follows: pts who achieved a response inferior to partial remission (PR) after 3-4 courses of R-CHOP, or pts who obtained a response inferior to complete remission (CR) after the completion of treatment or pts who had progressive disease (PD) during first line treatment. Refractory pts were directed to receive HDT (2 courses of DHAP. Cylophosphamide 7 gr/m², Methotrexate 8 gr/m², Etoposide 2 gr/m²) followed by PBSCA with BEAM conditioning regimen. CR was documented in all cases with PET/CT.
Results: Eight pts were male and 24 female (M/F ratio: 0.33; median age was 35 years (range 14–81). Stage I–II was present in 26 pts and stage III–IV in 6. B symptoms and bulky disease were recorded in 22 (68%) and 28 (88%) pts respectively. Twenty-seven pts (84%) achieved CR after R-CHOP immunotherapy plus RT. Five pts (16%) were refractory to first line treatment and received HDT followed by PBSCA. Refractory disease has been found after 4 courses of R-CHOP in 1 case, after the completion of immunotherapym program in 1 case, and at the end of RT in 3 cases. After salvage treatment 4 pts obtained CR and 1 died of progressive lymphoma.
Conclusion: HDT with autologous stem cell transplantation (ASCT) is the standard of care for eligible pts affected by refractory or relapsed Diffuse Large B Cell Lymphoma (DLBCL). However, the effectiveness of salvage chemotherapy and ASCT for refractory or relapsed disease has not been well established in PMBCL. In this regard, the small literature available provides controversial data. In particular, some little experiences show an inferior outcome in pts with PMBCL compared to pts with DLBCL. Our results show that HDT and PBSCA is an efficacious salvage treatment for pts with refractory PMBCL and this approach should be applied immediately at the first signs of unresponsive disease.

Multiple myeloma

R1361
In vivo purging with bortezomib and cyrophosphamide, followed by autologous stem cell transplantation and thalidomide consolidation in elderly patients with multiple myeloma
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Bortezomib (BOR) and cyrophosphamide (CY) have synergistic effects and are highly active in the treatment of MM. BOR is not thrombogenic and does not affect PBSC collection. It has already been used in combination with High Dose Melphalan (HD-MEL) before ASCT. Thalidomide (Thal) is active and well tolerated when used as low dose consolidation after chemotherapy or ASCT. Basing on this background we started a pilot study in high risk MM elderly patients, fit for ASCT, combining BOR, CY and dexamethasone (DEX) as induction and mobilizing therapy (CY-BOR), followed by ASCT with supplemented BOR-HD-MEL, to determine: 1-percentage of complete remission (CR); 2-clearance of Minimal Residual Disease (MRD) in PBSC harvest and in bone marrow (assessed by flow cytomtery). Pts receive four 3 weeks cycles of BOR 1.3 mg/m² and DEX 40mg/day i.v. (days 1,4,8,11) and CY i.v. 300 mg/m² (days 1,8,15). Pts achieving at least PR, are mobilized after BOR and DEX standard dose (days 1,4,8,11) with CY 3 gr/m² (day 8); GCS-F is started at day 9. Pts who mobilize an adequate PBSC amount undergo ASCT with HD-MEL (day -1) and BOR (1.3 mg/m²) on days -6, -3, + 1, +4. Three months after ASCT, pts achieving <CR start Thal 100 mg/day until relapse/progresion or unacceptable toxicity. 25 pts (median age: 66, range 52–77) were enrolled: 21 are evaluable for response before PBSC harvest: 18 responded to induction therapy (85%), 16 achieving at least VGPR (+2PR); 3 had resistant disease. 15/18 pts successfully mobilized PBSC (median 5.5; range 2.5–11.2 × 10⁶ CD34 + cells/kg) and 14 evaluable for response after ASCT: 4 VGPR, 2 CR, 4 nCR. With a median follow up of 390 days (range: 56–811) all 25 pts are alive. We did not observe thromboembolic events; 3 pts experienced grade 3 neurotoxicity, requiring reduction of dose of BOR. Flow cytometry analysis on 12 PBSC harvests shows complete clearance of clonal plasmacells in 9. Complete data concerning the MRD status will be further presented. This schedule is well tolerated and very effective also in elderly patients, allowing to collect a clonal plasmacells free harvest. Whether this will translate in an “in vivo” MRD clearance after ASCT, it should be confirmed by a longer follow up and by a PCR monitoring with patients’ specific probes (which is still ongoing).

R1362
The utility of the follow-up with serum-free light chain after autologous stem cell transplantation in multiple myeloma patients with measurable M-protein
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Introduction: We investigated the preceding changes in serial serum FLC assay until progressive disease (PD) was confirmed by the international uniform response criteria in post-ASCT patients with measurable disease.
Patients and method: We included patients, who (1) undertook ASCT for measurable disease (2) showed, at least, two serial response assessment of stable disease or complete response before PD or relapse by serum/urine M-protein, (3) had periodic serum FLC assay until progressive disease (4) showed, at least, two serial response assessment of stable disease or complete response before PD or relapse by serum/urine M-protein, (3) had periodic serum FLC assay until progressive disease (PD) was confirmed by the international uniform response criteria in post-ASCT patients with measurable disease.
Patients and method: We included patients, who (1) undertook ASCT for measurable disease (2) showed, at least, two serial response assessment of stable disease or complete response before PD or relapse by serum/urine M-protein, (3) had periodic serum FLC assay until progressive disease (PD) was confirmed by the international uniform response criteria in post-ASCT patients with measurable disease.
in 23 patients (79%) and dFLC in 21 patients (72%) preceded or accompanied with the time of PD or relapse. The preceding time was median 2 months (range -11–0) and 1 month (range, -11–0), respectively. Twenty-eight dFLC values were observed as negative values out of a total 123 data from 29 patients. Of these values, 12 were below normal iFLC concentration, 14 within normal range of iFLC (kappa 8.5–23.7 mg/L, lambda 9.5–23.5 mg/L), and 2 above normal iFLC concentration. Conclusion: About 70% of patients showed significant increase of iFLC that preceded or accompanied with the time of PD or relapse observed by M-protein by median 2 months at the cutoff of 20mg/L. In addition, interpretations of dFLC may be difficult as it is frequently observed as negative value in post-ASCT MM patients. Therefore, serial measurement of iFLC seems to have a utility in follow-up after ASCT in measurable disease. However, there should be more validation with large patients’ population.

R1363
Reduced-toxicity conditioning with fludarabine and busulfan prior to autologous stem cell transplantation in multiple myeloma patients. A single-centre experience
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Background and objectives: In Multiple Myeloma, reduced intensity conditioning (RIC) regimens consisting either of melphalan/fludarabine or total body irradiation (TBI; 2 Gy) and fludarabine are usually preferred to myeloablative conditioning regimens before stem cell autologous transplantation (alloSCT). Although the transplant related mortality may be lowered, these latter programs may also be associated to a higher incidence of relapse. We performed a pilot study to test the safety and efficacy of a reduced toxicitiy program based on i.v. Busulfan (3.2 mg/kg/day × 4 days) combined with Fludarabine (40 mg/ kg/day × 4 days) and antithymocyte globuline (ATG) before allo-SCT in multiple myeloma.

Patients and methods: Between April 2007–July 2009 10 patients with a median age of 51 years entered this study. The donor was an HLA identical sibling in 6 cases and a compatible unrelated in 4. All patients but one had relapsed after a previous double autologous transplant program; after salvage therapy, at time of autologeneic transplant, 3 patients were in complete and 7 in partial remission.

Results: In all patients the transplant was followed by stable engraftment and showed a complete hematopoietic chimerism at day +100. The median number of CD4 lymphocytes at day 100 was 190/mcl (range 34–517). Grade III-IV acute graft-versus-host disease (GVHD) was observed only in one patient while 3 patients developed limited extension chronic GVHD. With a median follow-up of 450 days (130–903) the overall survival (OS) at 2 years is 71% (95% CI 26–92%) with no transplant-related mortality (TRM). Two patients progressed and died while 8 patients are alive, seven of whom in complete or very good partial remission. One patient achieved a further remission after being treated with lenalidomide and cellular therapy.

Conclusions: Allogeneic transplantation after a myeloablative dose of Busulfan combined to Fludarabine and ATG is feasible in intensively pretreated multiple myeloma patients leading to stable engraftment, complete hematopoietic chimerism and good immune reconstitution without relevant toxicities. This program may be considered particularly for patients relapsing after high doses of Melphalan and autologous transplant.

Solid tumours

R1364
Brescia International Working Group: a proposal for a European cooperative protocol on haploidentical stem cell transplantation in paediatric solid tumours
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The First International Workshop in Haploidentical Stem Cell Transplantation in high risk paediatric solid tumours was celebrated on July 2009 in Brescia, Italy. The Associazione Bambino Emopatico Onlus and Ospedale Bambini leads by Dr. F. Porta made the Workshop possible. Recognizing the little progress made in the survival of children with metastatic and refractory solid tumours and the graft versus tumour effect mediated by allogeneic stem cell transplantation (ASCT) with haploidentical donor reported by different groups, we propose to explore haploidentical ASCT as alternative treatment in an International cooperative European protocol. A well-designed protocol should be developed in centres of excellence in ASCT. Research should be based on immune reconstitution and immune cytotoxicity, especially Natural Killer cells (NK) alloreactivity. The main parameters of the study will be safety and efficacy. At the workshop we proposed a multicenter pilot study consisted on haploidentical stem cell transplantation with T depletion graft. In our protocol we include patients with a partial responsive or relapsed or metastatic Alveolar Rhabdomyosacoma stage IV, Soft-Part Sarcoma stage IV, Ewing’s Sarcoma (without pulmonary metastasis), multi site Osteosarcoma and Neuroblastoma not responsive to chemotherapy, autologous transplant and therapeutic MIBGS. Two are the protocols for stem cell manipulation and conditioning (CR). The first consists on CD3/CD19 cells depletion, associated with a CR based on Melphalan, Fludarabine and Thiohepa, the second on CD34+ cells selection with a CR based on Thiopeta, Fludarabine and Treosulfan. The protocol includes possible use of expanded NK cells delayed donor lymphocyte infusion (DLI) as a strategy to improve survival. We will compare the results obtained in the different European centres in order to have a statistically significant date, necessary to include ASCT in standard therapy for high risk paediatric solid tumours.