Ischemic myocardial damage is an increasing cause of heart failure in the western world and has long been considered irreversible because adult cardiomyocytes are terminally differentiated and do not proliferate. Stem cells are undifferentiated cells capable of self-renewal, proliferation, and differentiation into multiple lineages permitting tissue regeneration. A number of types of stem cells are now recognised, as well as partially differentiated progenitor cells that are capable of proliferation and differentiation to multiple lineages. Reversal of heart failure would require myocardial regeneration. A number of types of stem cells are now established in 1998, aiming to cover the requirements of the human heart is safe and enhances cardiac function, when manipulated autologous bone marrow into scar tissue of the infarcted myocardium in two patients. These patients underwent coronary bypass using the PI-circuit technique and external reshaping of left ventricle in off-pump surgery. We evaluated the efficacy of this combined technique in the improvement of cardiac function. Autologous bone marrow (300ml) was obtained by bilateral posterior iliac bone aspiration at the time of surgery. Bone marrow mononuclear cells were isolated by means of a density Ficoll-Paque gradient. Then the cells were exhaustively washed and resuspended in a normal saline solution containing 5% human serum albumin. Cell count, viability and cultures were appropriately performed. Following the operation the bone marrow mononuclear cells (30ml) were injected directly to the myocardium of the left ventricle. This benefit can be seen after 24 and 29% respectively, three months following the operation. Furthermore, we observed significant reduction of the end diastolic volume of the left ventricle and improvement in the inferior-posterior wall motion, this area was not revascularized, in comparison to the previous one before the operation. These findings suggest that transplantation of un-manipulated autologous bone marrow into scar tissue of the human heart is safe and enhances cardiac function, when used in combination with myocardial revascularization and remodelling of the left ventricle. This benefit can be seen after 3 months of the bone marrow transplant and is maintained after 8 months of follow-up.

**Cellular therapy**

**R1056**

Effective combined surgical treatment and autologous bone marrow transplantation for the end stage ischaemic cardiomyopathy

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Auto-transplanted patients were 39 male and 30 female (median age 47, range 19-70 years), diagnosed as non-Hodgkin’s lymphoma (NHL=26), multiple myeloma (MM=26), Hodgkin’s lymphoma (HL=8), acute myelogenous leukemia (AML=7) chronic myelogenous leukemia (CML=1) and chronic lymphocytic leukemia (1). Nine MM patients received tandem auto-transplants, while 1 auto- and 3 allo- were second transplants. At transplantation 41 patients were in CR, 27 in PR and 11 had refractory disease. Bone marrow (BM) grafts were used in 3 cases and peripheral stem-cell grafts (PBSC) in 76. Patients were conditioned with melphalan (32), CBV (17), BEAM (14), busulfan-based regimens (12) and Thio-TEPA-based regimen (4). Engraftment was achieved in 76/79 cases. Three patients died early post-transplant. After a median follow-up of 31 months, 47 patients are alive (68%), 40 of them disease-free, and 22 have died, due either to transplant-related complications (5), or after an early (<1 year post-transplant) 9 (NHL=5, AML=3, MM=1), or a late relapse (> 2 years post-transplant) 8 (MM=6, HL=1, NHL=1). For all allo-transplants the donor was a matched sibling. Patients were 15 male and 5 female (median age 36, range 18-56 years), diagnosed with AML (9), acute lymphoblastic leukemia (ALL=4), myelodysplastic syndrome (MDS=3), aplastic anaemia (2), CML (1) and myelofibrosis (MF=1). Excluding patients with AA, disease status at transplantation was 1st CR for 11 patients, 2nd CR for 3, and active residual disease for four. Stem-cell source was BM in 5 and PBSC in 18. A standard conditioning was used in 18 cases and a reduced intensity one in 5. Engraftment was achieved in all cases. One patient died early, due to uncontrollable VOD, and 7 (ALL=4, AML=3) due to disease progression, which was early (<8 months) in 5 cases, and late (at 17 and 21 months post-transplant) in 2. Five patients developed acute GVHD grade I-II, and one grade IV. After a median follow-up of 21 months, twelve patients are alive and disease free terminally (AML=5, MDS=3, AA=2, CML=1, MF=1) but 7 of them have manifested chronic GVHD, (extensive in 4). In one patient chronic GVHD emerged after DLI, administered for smoldering relapse.

**R1058**

Automatic platelet concentrate preparation induces higher recovery of platelets

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Patients after bone marrow transplantation are among the main recipients of platelet (plt) transfusions. Aomatic preparation of buffy-coat (BC) derived platelet concentrates (PC) by the OrbiSac sytem (OS; Gambro BCT) intends to optimise production and to enable standardization of platelet concentration. We compared yield and quality parameters with conventionally produced PC (C-PC) to validate the process for PC.

Twenty PC of 4 pooled BC were produced by OS and stored for 7 days on agitation. Quality control data were compared to 20 PC prepared according to our standard operating procedures. Plt counts were analysed automatically. Residual erythrocytes and leucocytes were measured by flow cytometry. We analysed pH, pO2, pCO2, HCO3, glucose, lactate and plt activation markers (CD62p, CD63, thrombospondin [TSP]) by flow cytometry. Aggregometry with
activation of plt by collagen, measurement of hypotonic shock response (HSR) and extent of shape change (ESC) were tested in addition.

In OS-PC and C-PC plt yield was 2.72±0.37 and 2.81±0.33x10^9/unit (recovery 79.1% and 73.1%, p=0.003), residual erythrocytes were <0.16x10^9/unit, residual leukocytes <0.16x10^9/unit in all PC. On d1 values for p02 in OS-PC and C-PC were 120.9±24.5 and 107.6±30.7mmHg, pCO2 58.3±7.0 and 69.5±13.6mmHg (p<0.001), HCO3 18.7±0.9 and 19.8±1.2mmol/L (p<0.001), respectively. On d7 values for p02 in OS-PC and C-PC were 99.6±44.0 and 85.1±24.5mmHg, pCO2 23.8±2.8 and 27.9±4.4mmHg (p<0.001), HCO3 10.1±1.1 and 10.1±1.1mmol/L, respectively.

Results (mean±SD) of metabolic and activation markers and morphologic features on d1 and d7 are shown in the table. In both groups functional parameters revealed a sufficient capacity for aggregation during storage. Compared to C-PC OS-PC were significantly more efficient in recovery of plt, whereas CD62p and CD63 were significantly higher due to a higher extent of plt activation in the automatic system. A possible difference in clinical outcome of transfusion of OS-PC compared to C-PC has to be investigated in further studies.

R1059

Differentiation of bone marrow clonogenic fibroblasts in children with acute leukaemias

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Objectives: Recently the investigation of differentiation potential of bone marrow stromal cells becomes important because of their implementation in transplantation. But the structure of the stromal cells pool is still not fully known. The aim of the study - to investigate the differentiation ability of bone marrow clonogenic fibroblasts and to elaborate the standart assays for clonogenic proliferation and adipogenic and osteogenic differentiation (AD and OD) of bone marrow fibroblasts in children.

Methods: The material - bone marrow aspirates from 11 healthy donors, 15 patients with acute lymphoblastic leukemia (ALL) and 15 patients with acute myeloblastic leukemia (AML). Bone marrow stromal fibroblasts were cultured accoring A.Fridenstain in our modification. OD was induced by: b-glycerophosphat 7x10^-3M; dexametasone 1x10^-5M; ascorbic acid 2x10^-5 M. For AD were used: dexametasone 1x10^-5 M and insulin 1x10^-6 M.

Results: Optimal conditions for maximum cloning efficiency were following: isolation bone marrow mononuclear cells; density by explantation - 2x10^6/mi cells; culture medium: 199 medium with 20% human serum of any blood group. Osteogenic induction increased the proportion of fibroblasts colonies from normal bone marrow with alkaline phosphatase activity from 16.8% to 77.8%; the adipogenic inductors increased the proportion of colonies with lipid-rich vacuoles (detected by Sudan) from 31.2% to 59.8%. The cloning efficiency of stromal precursors (the number of colonies per 1x10^5 explanted cells) in the patients with AML did not differ from normal donors (47.8 and 49.7)and was much lower in ALL patients (2.7). Stromal cells from leukemia patients showed decreased (in comparison with normal donors) potency for OD in the presence of inductors (from 28.6% to 58.4% for AML; from 17.5 to 45.9 for ALL patients). The ability of stromal cells to AD did not differ in patients with acute leukemias and normal donors.

Conclusion: Standardization of stromal fibroblasts clonogenic cultivation assay is necessary for the evaluation of bone marrow stroma state. The using of osteogenic and adipogenic inductors demonstrate the possibility of in vitro differentiation of stromal progenitors and showed the differences between normal and leukemic stromal cells.

R1060

Initiation of leukapheresis for peripheral blood stem cell collection at a low level of circulating CD34+ cells

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Objectives: Most patients or donors undergoing leukapheresis (LP) for autologous or allogeneic peripheral blood stem cell (PBSC) collection require multiple LP to achieve a sufficient CD34+ cell dose (e.g., > 2.5 x 10^6/kg). LP is initiated when peripheral blood (PB) CD34+ reached a certain level (e.g., ≥ 20 per microliter). The aim of this retrospective analysis is to summarize our institutional experience of initiating LP at a lower PB CD34+ cells level of 5 per microliter and to investigate the merits of this practice.

Methods: All patients or donors underwent LP (using Cobe Spectra or Baxter Amicus) processing 3 times the blood volume. A total of 192 procedures (130 autologous and 62 allogeneic) was performed in 87 patients or donors between Jan 04 and Oct 05. Autologous patients were mobilized with chemotherapy and G-CSF (10 mcg/kg) while allogeneic donors with G-CSF (10 mcg/kg) alone. A “good” LP is defined as having ≥ 1 x 10^6 CD34+ cells/kg in the collection so that a minimum dose of 3 x 10^6/kg can be achieved in 3 sessions. CD34+ cells were measured by sequential gating (staining with antibodies against CD45 and CD34 together with forward and side-scattering).

Results: Each LP contained 6.44 x 10^6 WBC/kg (median, range: 1.3–17.5) and 2.48 x 10^6 CD34+ cells/kg (median, range: 0.14–24.9). CD34+ cells/kg in LP were correlated to PB CD34+ cell counts (r = 0.80). As shown in Table 1, initiating LP at higher levels of PB CD34+ cell (≥ 10 per microliter) increased the proportion of “Good LP”, whether “All” collections or only the first collections were considered. However, a substantial number of “Good LP” (≥ 50%) would be missed if LP was initiated at 10 or 20 CD34+ cells per microliter in PB (Table 2), but almost none at 5 CD34+ cells per microliter.

Conclusion: The result demonstrated that initiating LP at 5 PB CD34+ cells per microliter is helpful to some patients/donors. Additional criteria may need to be adopted to determine whether LP should be discontinued if mobilization is adequate to minimize resource utilization.

|  | #LP | % Good | #LP | % Good |
|---|---|---|---|---|
| PB CD34+ cells | 192 | 76.0 | 28 | 79.3 |
| ≥ 5 per microliter | 183 | 79.2 | 25 | 81.2 |
| ≥ 10 per microliter | 154 | 86.5 | 65 | 87.7 |
| ≥ 20 per microliter | 75 | 96.0 | 34 | 94.1 |
Background: With the COMTEC apheresis device (Fresenius Turku University Hospital (Turku, FIN) T.T. Pelliniemi, K. Remes cell separator with reduced product volume procedure therapy. These cells are a promising tool for future anti-tumor apoptosis, but cell count and vitality recover during 14 days culture. Due to enhanced NK proliferation cellular density became apoptotic, after three days vitality amounted 48% on the first three days of cell culture around 50% of the cells with a purity of 93% and a vitality of 97% on average. During MACS separation yielded 8.1 +/- 5.0 * 1E+06 CD56+ cells were isolated from 15 ml BC. Purity and vitality (7-AAD determination of CD56+ expression.

Results: By the use of RosetteSep 15.3 +/- 4.3 * 1E+06 NK-cells were isolated from 15 ml BC (n = 10) using the RosetteSep technique (CellSystems, St. Katharinen, Germany). RosetteSep, i.e. an antibody cocktail containing anti-CD3, -CD4, -CD19, -CD36, -CD66b, crosslinks the appropriate WBCs to glycophorin A expressed on RBCs, thereby augments the density of all unwanted cells. Centrifugation over ficoll paque separates all bonded cells from CD3-/CD56+ cells, i.e. mature NKS. In a second step NKS were purified by MACS (magnetic activated cell sorting) using anti-CD56 microbeads (Miltenyi, Bergisch Gladbach, Germany). NKS were cultured in a density of 1E+06/ml in X-Vivo 10 medium (Cambrex, Verviers, Belgium) containing 2% FCS and 1000 U/ml IL-2 at 37°C for 14 days. Cells were characterized and counted by flow-cytometrical determination of CD56+ expression. 

Results: The median CD34+ cell CE was 40% with both software versions. There were no differences between the patient groups undergoing leukapheresis with the different software versions. The median counts of CD34+ cells per harvest or mononuclear cell purity (83% vs. 81%) or platelet contamination (1.5 vs. 1.3 E12/l) were comparable. Comparing the version 02.03xx to the version 3.0xx, the median red cell contamination was significantly less in the product collected with the version 3.0xx (6% vs. 9%, p<0.001). The correlation between the pre-apheresis blood CD34+ cell count and the CD34+ cell yield per harvest was similar (r=0.88, p<0.001).

Conclusion: The PBSC collection with the COMTEC RV-PBSC procedure results in good quality apheresis products and the COMTEC device is suitable for PBSC collection in clinical practice. The software updating did not alter CD34+ cell collection efficiency.

R1061
Isolation of CD3-/CD56+ natural killer cells from whole blood derived buffy coats
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Background: Besides CD8+ T-cells natural killer cells (NKS) are an essential arm of host defense against viral infection and cancerous degeneration of cells. The aims of the present study were to isolate NKS from whole blood derived buffy coats (BCs), to enrich and stimulate them by means of cell culture and IL-2 application.

Material and methods: In a first step NKS were isolated from 15 ml BC (n = 10) using the RosetteSep technique (CellSystems, St. Katharinen, Germany). RosetteSep, i.e. an antibody cocktail containing anti-CD3, -CD4, -CD19, -CD36, -CD66b, crosslinks the appropriate WBCs to glycophorin A expressed on RBCs, thereby augments the density of all unwanted cells. Centrifugation over ficoll paque separates all bonded cells from CD3-/CD56+ cells, i.e. mature NKS. In a second step NKS were purified by MACS (magnetic activated cell sorting) using anti-CD56 microbeads (Miltenyi, Bergisch Gladbach, Germany). NKS were cultured in a density of 1E+06/ml in X-Vivo 10 medium (Cambrex, Verviers, Belgium) containing 2% FCS and 1000 U/ml IL-2 at 37°C for 14 days. Cells were characterized and counted by flow-cytometrical determination of CD56+ expression. 

Results: By the use of RosetteSep 15.3 +/- 4.3 * 1E+06 NK-cells were isolated from 15 ml BC. Purity and vitality (7-AAD staining) of the cells averaged 83 and 99%, respectively. MACS separation yielded 8.1 +/- 5.0 * 1E+06 CD56+ cells with a purity of 93% and a vitality of 97% on average. During the first three days of cell culture around 50% of the cells became apoptotic, after three days vitality amounted 48% on average. Due to enhanced NK proliferation cellular density exhibited recovery to about 2.7 * 1E+06/ml, and vitality increased to about 89% after another 11 days of cell culture. 

Conclusion: Vital natural killer cells may be isolated with high purity from BCs using RosetteSep and MACS technology. By this means initially NK-cells are affected by a high rate of apoptosis, but cell count and vitality recover during 14 day cell culture. These cells are a promising tool for future anti-tumor therapy.
PBPC graft. There was no correlation with the increase in DC and any PB parameter evaluated. In summary, G-CSF mobilisation while increasing the TNC number (up to 84 fold) does not affect NK, DC and T lymphocytes sub-populations ratio in the grafts from healthy adult donors.

Further studies are required to determine if the development, maturation and functional activity, namely cytotoxic and immune capacity of these cells are affected by the procedure.

R1064
Toxicity and efficacy of donor lymphocyte infusion after haematopoietic stem cell transplantation
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Relapse remains one of the main complications after allogeneic haematopoietic stem cell transplantation (HSCT). Donor lymphocyte infusion (DLI) is a therapeutic approach that is able to mediate antitumour effects and restore prolonged remissions. This antitumour effect has been well established in chronic myeloid leukaemia (CML) and less in other malignant diseases.

We retrospectively analysed 40 patients (14F/26M) who relapsed or were at high risk of relapse after HSCT between December 1994 and September 2005. Patients median age was 32 years (6-55). They had the following diagnosis: CML 17, acute myeloid leukaemia (AML) 3, acute lymphoid leukaemia (ALL) 6, multiple myeloma (MM) 6, Hodgkin disease (HD) 4, aplastic anemia (AA) 2, non-Hodgkin lymphoma (NHL) 1, myelodisplastic syndrome (MDS) 1.

Graft source was G-CSF mobilized peripheral blood in 35 patients and bone marrow in 5. Conditioning was myeloablative in 27 patients (in 19 of them graft was T cell depleted in vitro) and of reduced intensity (RIC) in the remaining 13 patients. Eight patients underwent a 2nd allogeneic transplantation and 4 of them later received DLI. The indications for DLI were relapse/disease progression in 35 patients and pre-emptive in 5. Conditioning was myeloablative in 27 patients (in 19 of them graft was T cell depleted in vitro) and of reduced intensity (RIC) in the remaining 13 patients. Eight patients underwent a 2nd allogeneic transplantation and 4 of them later received DLI. The indications for DLI were relapse/disease progression in 35 patients and pre-emptive in 5. The median interval between transplantation and the first DLI was 8 months (1-61). The median dose of CD3+ lymphocytes infused was 1x10^7/kg (0.05-10 x 10^7/Kg). A complete response was observed in 33% of patients (4/9 acute leukaemia, 1/6 MM, 8/17 LMC) and a partial response in 2 (1 MM and 1 SMD). There was no remission in 45% of patients (4/4 HD, 2/2 AA, 8/17 CML, 2/9 LA, 2/6 MM).

In two patients there was no available information. All the five patients relapsed after prophylactic DLI. Eight patients developed graft versus host disease (GVHD) “de novo” after DLI.

Seven patients with CML did not achieve molecular remission after DLI but did it when imatinib mesylate was associated. At the last follow up, 25 patients remained alive and 20 of them established full donor chimerism.

In summary, complete remissions induced by DLI were inferior to reported in literature. GVHD developed just in an acceptable number of cases and was controlled. Further studies need to be performed to evaluate which patients really benefit from DLI.
Cytokines

R1066
Palifermin for oral mucositis prophylaxis in autologous transplantation
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Objectives: Oral mucositis is a near universal complication of intensive chemotherapy. The patient discomfort often require i.v. narcotics and is the main cause for total parenteral nutrition. Some authors have associated the duration and the severity of mucositis with transplant outcomes. Until very recently no effective treatment or prophylaxis was available. Palifermin is the recombinant human keratinocyte growth factor and a previous study have shown its ability to decrease the length and severity of mucositis in conditioning regimens including total body irradiation (TBI). From August to November 2005 we have used Palifermin for the prophylaxis of mucositis in adult patients (pts) under autologous BMT with conditioning regimens without TBI.

Methods: Nine patients (4 females and 5 males) with a median age of 46.8 (range 27-56) have been included in an open label study included in an extended access program. The predominant diagnosis was Non Hodgkin’s Lymphoma (7). The conditioning regimen was BEAM in 7, VP16 + Melphalan in 1 and Carboplatinum + VP16 + Thiotepa in 1. Palifermin was given according to the manufacturer instructions 60 micrograms/kg body weight on 3 days before chemotherapy and on 3 days starting on day 0 after stem cell infusion. We analysed severity and length of mucositis, narcotic need, haematological reconstitution and side effects of palifermin. We have compared the results with a historical cohort matched for diagnosis, age, conditioning regimen and number of CD34+ cells transplanted.

Results: There was only one case of discontinuation of treatment due to toxicity (generalized skin oedema with severe hypotension). Severe pruritus was present in 4 pts and generalized rash in all but one patient. A statistical significant increase in weight was observed in pts treated with palifermin on day +3 (median + 4.4 kg versuss -2 kg without palifermin). The number of days under iv narcotics was lower on palifermin group (3.4 vs 4.6) and more patients did not require narcotics at all (4 out of nine versus 1 in 9). The mean of the number of days without any oral nutrition was lower on palifermin group (2.66 versus 5.22). All these differences did not reach statistical significance. The haematological recovery, antibiotic need, bacteriological isolates and length of stay on hospital were equivalent in both groups.

Conclusion: In our series palifermin decrease the severity and duration of mucositis and was associated with significant skin toxicity.

R1067
Clinical experiences with the new keratinocyte growth factor palifermin in 7 patients treated with high-dose chemotherapy followed by autologous stem cell transplantation
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Oral mucositis is a common side effect of chemotherapeutic and/or radiotherapeutic treatment in malignant diseases resulting in pain, diarrhoea, malnutrition, gastrointestinal bleeding complications, local and systemic infection leading to prolongation of hospitalization duration and increased medication and treatment costs. This side-effect of cancer treatment has to be expected in up to 50% of all treated cancer patients and the percentage is even higher in the hematopoietic stem cell transplantation setting. As the functional role of different cytokines such as tumor necrosis factor-alpha and different interleukines and probable protective factors like secretory IgA in the pathological pathway leading to mucositis is not yet well understood, neither a widely accepted prophylaxis nor a therapy is available.

We report our data of prophylactic application of the recombinant human keratinocyte growth factor palifermin in 7 autologous stem cell transplantation recipients (female 3, male 4). In 3 cases diagnosis was relaps non-hodgkin’s lymphoma, 4 patients were suffering from multiple myeloma. The patients age ranged from 31 to 65 years, karnofsky status was 100% in all. Palifermin was given as intravenous bolus injection on 3 consecutive days , third dose at least 30 hours before administration of high dose chemotherapy. Second cycle consisted of another 3 doses that were given on 3 consecutive days starting with the day of reinfusion of the stem cell harvest product. The single injection doses ranged from 3.9 to 5.4mg according to the producer’s 60 mcg/kg/d recommendation. We experienced only one case of WHO-Grade 3-mucositis which could not be distinguished from mucosal hyperproliferation due to the medication, all other patients showed no sign of oral mucositis. Diarrhoea was maximum WHO-Grade 1. Treatment related side effects consisted of mucosal oedema (vagina, tongue, palate, lids), erythema of the face and upper body, dysphagia and disturbances of taste and sensibility. In 3 cases oral mucosa lead to detachment from palate and tongue, in another patient mild temporary dyspnoea occurred. Altogether those findings were well manageable and resolved without additional measure within 3 days after last injection. We observed no septic event. The hospitalization duration was slightly reduced to former comparable patients even though the first cycle of palifermin led to a 4 days prolongation of the pre-transplant phase.

R1068
Prevention of oral mucositis in patients undergoing radiochemotherapy and allogeneic stem cell transplantation with recombinant human keratinocyte growth factor (palifermin): a single-centre experience
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Objectives: Oral mucositis is one of several common adverse effects of myeloablative therapy, it is particularly frequent in patients receiving high-dose chemotherapy with or without total-body irradiation for hematopoietic stem cell transplantation (HSCT). Palifermin is the first agent to be approved for the prevention of oral mucositis induced by myeloablative therapy. So far, data on ist use in allogeneic HSCT are rare.

Patients and Methods: We analyzed the efficacy of palifermin on 4 consecutive patients (median age: 42, range 33-48 years) undergoing allogeneic peripheral blood stem cell transplantation (sibling donor; n=2; unrelated donor, n=2) at our institution. All patients received a conditioning therapy with cyclophosphamide and total body irradiation. Palifermin was administered intravenously in a dosage of 60µg/kg/day 3 days before myeloablative therapy and 3 days after stem cell infusion. Mucositis was assessed daily and graded according to the WHO-scale.

Results: One patient died on day +10 after transplantation due to gram-negative sepsis and multi-organ failure. The other 3 patients were eligible for assessment of mucositis. The 3 eligible patients experienced only WHO grade 2 mucositis. The drug was generally well tolerated without severe side effects. One patient developed erythema on the face, which resolved 24-hours after palifermin administration without the need of medication. In the other 2 patients no side effects were observed. Only 1 patient experience acute Graft-versus-host disease (GVHD) of the skin grade II. Currently, 3 of 4 patients are alive, 1, 3 and 4 months after HSCT.
Conclusion: Palifermin represents the first drug approved by the US FDA for reduction of incidence and duration of oral mucositis in a specific patient cohort. In our cohort of patients we observed no serious side effects and only low grade mucositis. The effect of palifermin on gut epithelia or GVHD incidence and severity has to be assessed in larger patient cohorts.

R1069
Pegfilgrastim in comparison with filgrastim after allogeneic stem cell transplantation
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Purpose: After autologous and allogeneic peripheral blood stem cell transplantation (PBSCT) Filgrastim (G-CSF) is given daily to enhance neutrophil recovery and prevent risk of infection. Peg-Filgrastim (Polyethylen-Glykol-G-CSF) has been approved in 2002. Since the clearance of Peg-Filgrastim is mediated by neutrophils, in neutropenia activity and serum concentration is prolonged and elevated. The aim of this study was to evaluate for the first time the efficacy of a single fixed dose (6 mg) in patients after allogeneic PBSCT from matched unrelated donors.

Methods: On day +5 after allogeneic PBSCT five patients (2 AML, 2 ALL, 1 CML; median age 29y) received 6mg Peg-Filgrastim s.c. The neutrophil and platelet recovery was compared to patients (n=21; 11 ALL, 6 AML, 2 MDS, 1 CML, 1 MM; median age 41,5 y) treated with daily Filgrastim (5µg/kg i.v. over 4h). Definition of neutrophil recovery was ≥ 0,5/ml, platelet recovery ≥ 20/nl on three consecutive days and without transfusions respectively. G-CSF serum levels were measured by ELISA (R&D Systems).

Results: Peg-Filgrastim was well tolerated. In patients treated with Peg-Filgrastim the neutrophil engraftment was 18 days versus 19 days in the Filgrastim group. The platelet recovery in the Peg-Filgrastim group was 22 days and 20 days in the Filgrastim group. These differences were not significant. G-CSF levels were measured after the first administration of Filgrastim or Peg-Filgrastim respectively. The results showed a neutrophil mediated clearance of Peg-Filgrastim with a peak serum level on day +2 that dropped in parallel to neutrophil recovery. The peak level of Peg-Filgrastim was about ten fold higher than the peak level of Filgrastim (111 ng/ml vs 10 ng/ml).

Conclusions: Our data suggest that a fixed dose of 6mg Peg-Filgrastim used after allogeneic PBSCT is effective and gives comparable results to daily administration of Filgrastim. Larger prospective studies are needed.

R1070
Use of pegfilgrastim after high-dose melphalan and autologous peripheral blood stem cell transplant in multiple myeloma patients
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Single dose of Pegfilgrastim is equivalent to daily filgrastim after standard dose chemotherapy in decreasing the duration of neutropenia. Daily filgrastim started within 1-4 days after autologous peripheral blood stem cell transplant (APBSCT) leads to decrease in time to neutrophil engraftment. We undertook a study of pegfilgrastim after high-dose Melphalan (HDM) and APBSCT. In all, 13 patients with multiple myeloma (Stage III Durie-Salmon Classification), eligible to undergo HDM and APBSCT, were enrolled. Patients were conditioned with HDM at a dose of 200 mg/m² intravenously on day -2. The day of APBSCT was termed day 0. The median stem cell dose infused was 4 x 10^6/Kg (range 3-7). Patients received a single dose of 6 mg pegfilgrastim subcutaneously 24 h after APBSCT. There were no adverse events secondary to pegfilgrastim. Neutrophil engraftment was defined as first of 3 consecutive days of an ANC equal or greater than 0.5 x10⁹/l after a previous nadir. Platelet engraftment was defined as platelet count of equal or greater than 20 x 10⁹/l. All patients engrafted neutrophils and platelets with a median of 10 days (range, 9-17) and 13 days (range, 10-26), respectively. The incidence of febrile neutropenia was 53.8% (7/13). The median duration of febrile neutropenia was 2 days (range, 0-7). The mean number of platelet and packed red blood cell transfusions were 3 ± 6.2 U and 0.9 ± 1.7 U. It’s interesting to note that 9/13 patients didn’t required packed red blood cell transfusion. One patient died for cerebral bleeding after engraftment, on day +21; 2 patients experienced persistent reversible neutropenia. Neutrophil and platelet engraftment were compared with a cohort of 38 patients (same diagnoses, method of stem cell collection, conditioning regimen and stem cell dose) treated at our institute, who received filgrastim at 5 mg/kg subcutaneously daily, starting at day +3 or day +5 and no statistical difference were shown (p= ns). In conclusion, Pegfilgrastim given as a single fixed dose of 6 mg appears to be safe after HDM and APBSCT. Pegfilgrastim may be convenient to use in outpatient transplant units.

Stem cell research

R1071
A set-up of an apheresis centre (CIC 327) for autologous peripheral stem cell transplantation for haematology in Southern Israel
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The concept of treating chemosensitive leukemia, lymphoma and myeloma patients with high dose chemotherapy followed by reinfusion of peripheral stem cell has been implemented in the set-up of bone marrow transplantation, and often considered as transplantation procedure. With the use of growth factors to stimulate bone marrow and allow the harvest of peripheral stem cells with an apheresis machine Haemonetics® MCS33 and cryopreservation, the procedure of autologous peripheral stem cell transplant (APST) became feasible in hematology departments, and not necessarily in bone marrow transplant units. For this reason, we decided eight years ago to set up such a system in our institute. Our team included three MD hematologists, one nurse and one apheresis technician. We equipped our institute with the necessary separator machine and after a period of ten months trial of investigation of the harvest quality, we started to treat our patients in our institute, instead of referring them to a transplant unit. Our institute is a part of a 600-bed University hospital and covers a population of 500 000 inhabitants.

In this abstract we would like to present our results. Using two Haemonetics, approximately 300 harvests have been performed with an achievement of an excellent yield of 2CD34 stem cells (median, 4.8x10^6/kg). The harvest was performed at the day care unit and then cryopreserved in liquid nitrogen. In most patients myeloablative chemotherapy was also given ambulatory. Following the reinfusion of the harvest, patients were hospitalized in isolation rooms. The median time of recovery (neutrophils >0.5x10⁹/l) was 11 days. At the end of the year 2005 (eight years activity), 120 auto-transplantations were performed, including 12 tandem. The diagnoses were: multiple myeloma in 49, non-Hodgkin’s lymphoma 33, Hodgkin’s disease 14, AML 4, ALL 2, amyloidosis 4 and CLL 14 cases. Treatment related mortality was 1.7%. We believe that such endeavor should be encouraged and advised for more hematology centers. Hematologists in training and senior hematologists have the benefit of keeping their patients
under close supervision with the challenge of further therapies, increasing the clinical level, the motivation and the interest in the field of hematooncology.

R1072
Characteristics of bone marrow mesenchymal stem cells in malignant and non-malignant haematological disorders of childhood
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Mesenchymal Stem Cells (MSCs) are multipotent progenitor cells within the bone marrow (BM) capable of differentiating into various tissue specific cells. MSCs form an integral part of the BM stroma, have immunomodulatory functions and play an important role in the support of hematopoiesis. Their multipotentiality and ease of ex vivo expansion has raised great interest in the clinical use of MSCs for tissue repair and gene therapy. In order to evaluate if malignant and non-malignant hematological diseases quantitatively and qualitatively affect BM derived MSCs, bone marrow from children with acute lymphoblastic leukemia (ALL diagnosis n=9, different phases of treatment n=29, end of therapy n=10), idiopathic thrombocytopenic purpura (n=16), autoimmune neutropenia (n=12) and control patients (n=30) was harvested and the mononuclear cell (MNC) fraction isolated. MSCs were expanded in αMEM supplemented with 10% selected FCS, characterized and compared in terms of their phenotypic characteristics, clonogenicity and ability to differentiate into adipogenic (A), osteogenic (O) and chondrogenic (C). MNCs at day 0 expressed high levels of CD34, CD45, CD29, CD90 and very low levels of CD14, CD105 and CD90. Expression of hematopoietic markers on cells at passage 1 (P1) and thereafter progressively diminished while expression of CD29, CD44, CD90 and CD105 increased approaching 100%. Cell doubling time ranged from 3 to 4 days at all passages. High clonogenicity was observed in all samples at all passages as shown by the presence of CFU-F colonies (>50 cells) with the exception of ALL samples at diagnosis which showed impaired proliferation and clonogenicity that returned to normal since remission was achieved at the following phases of treatment till the end of therapy. At P2 or P3, MSCs were differentiated towards the A, O, and C lineages by using specific induction media. Differentiation was assessed by histochemistry and RT-PCR (LPL and ap2 for A, osteocalcin, osteoprotegerin and ALP for O, aggrecan and Col II for C). P2 or P3 MSCs from all groups exhibited bi- or tri-lineage differentiation. Preliminary cloning experiments showed that MSC population is composed of cells with differing proliferation potential and clonogenicity. These results indicate that blood diseases of childhood do not affect the characteristics of MSCs which could have clinical applications particularly in hematopoetic reconstitution following transplantation.

R1073
Pegfilgrastim after autologous stem cell transplantation
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Recombinant hemopoietic growth factors are not added routinely after autologous stem cell transplantation (ASCT) in our institution. In myeloma patients, a multicentric protocol to which we participate indicates granulocyte growth factor after re-infusion, at the dose of 5μg/Kg, from day +1 to hematological recovery. Because of equivalence between a single Peg-Filgrastim (PEG) dose and daily filgrastim doses in decreasing the duration of neutropenia after standard dose chemotherapy, we used PEG after ASCT in patients affected by myeloma or lymphoma. From February 2004 to November 2005, we enrolled 53 patients, 30 suffering from myeloma (13 undergoing single-ASCT and 11 tandem-ASCT) and 23 suffering from lymphoma (13 non-Hodgkin, 10 Hodgkin), conditioned by MEL200 and BEAM, respectively. All patients received a single dose of 6 mg pegfilgrastim subcutaneously 24 h after autologous stem cells infusion. All patients engrafted neutrophils and platelets with a median time of 10 and 13 days, respectively, (regardless of the underlying disease and type of conditioning). The incidence of febrile neutropenia was 30% (16/53) with a median duration of 12 hours. We observed no adverse events secondary to PEG injection. No patient had clinically significant mucositis. We compared this cohort of myeloma patients with an historical group of autotransplanted myeloma patients treated by standard daily doses of filgrastim and of lymphoma patients transplanted without G-CSF administration. Time to neutrophil and platelet recovery was identical in both groups of myeloma patients, and appeared sensibly reduced in lymphoma patients treated with PEG as compared to no-G-CSF patients (days 21 and 19 vs. 22 and 19, respectively). We conclude that a single dose of pegfilgrastim after ASCT is safe, well tolerated and accelerates neutrophil recovery, thus decreasing time of hospitalization it seems equivalent to daily dose of filgrastim. We also documented no differences between our cohort of patients and historical groups in order of febrile neutropenia and proved infections. Since PEG disappearance from the circulation is not due to renal or hepatic clearance, but only to uptake on granulocytic cells and their precursors, this drug can be given even early after stem cells infusion, and will be utilized as soon as the engraftment occurs.

R1074
Mesenchymal stem cells are able to stimulate alloreactive immune cells
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Bone marrow-derived mesenchymal stem cells (MSC) have been suggested to be “immune-privileged” while exerting a strong immune-modulatory function as “third-party” cells in an HLA-independent manner. Therefore MSC are interesting candidates for cell and gene therapeutic applications. However, a better understanding of the mechanisms underlying their immune-modulatory potential would be very important for MSC application in clinical settings. We investigated the interaction of MSC with allogeneic immune cells conditioned by co-culturing them with activated PBMC and using them as third party in mixed lymphocyte cultures. We present data demonstrating that the immune-privileged state of MSC results from an interplay of stimulating and suppressing factors. The directly stimulating activity leads to both active lymphocyte proliferation and secretion of pro-inflammatory cytokines by allo-reactive lymphocytes. Stimulation is, however, dominant only at low MSC:effector cell (<0.1 under our experimental conditions), but outweighed with higher MSC numbers by the suppressing activity. Allogeneic mixed lymphocyte cultures as well as MSC-mediated stimulatory effects are efficiently suppressed by the addition of MSC-conditioned medium underlining the important role soluble factors play in MSC-mediated immune modulation. In conclusion, based on our data we suggest that the “immune-privileged status” of MSC reflects a sensitive balance of MHC-mediated immune activation and the suppression of immunological reactivity largely conferred by soluble factors.
Bone marrow-derived endothelial progenitor cells (EPCs) circulate in the peripheral blood (PB) of healthy subjects (HS). EPCs seem to play an important role in maintaining vessel wall homeostasis, in the neo-angiogenetic processes and in the re-endothelialization of the wall of injured vessels. The aim of the study is to assess the number and origin of circulating EPCs in children with non-malignant diseases who received allogeneic BMT from an HLA-identical sibling or a matched unrelated donor. We studied patients with Thalassemia major (n=9), Fanconi anemia (n=2), sickle cell disease (n=3), mucopolysaccharidosis (n=1), diskertatosis congenita (n=1), acquired aplastic anemia (n=3), or Chediaik Higashi syndrome (n=1). We evaluated PB samples at 21, 45, 60, 90, and 120 days after transplant. The number of EPCs was evaluated as CD34+VEGFR-2+ or CD34+CD133+VEGFR-2+ cells by cytfluorimetric analysis, and by in vitro culture. The analysis of PB samples from 10 age matched donors (HS) was included in the study. Donor or recipient origin of EPCs was assessed on at least 10 individually picked endothelial colonies by micro-satellite analysis. In patients tested 21 days after transplant the percentage of circulating CD34+VEGFR-2+ cells (median 0.1%, 0-0.5) and the percentage of CD34+ co-expressing the CD133 and VEGFR-2 antigens, representing a restricted subset of immature EPCs (median 0.06%, 0-1.3), were comparable to those found in HS (median 0.06%, 0-0.67; median 0.6%, 0-0.15, respectively). The number of EPC derived colonies was also comparable in patients tested at 21 days after transplantation (median 26/10⁶ mononuclear cells, 0-91) and in HS (median 22.5/10⁶ mononuclear cells, 11-43). Neither the percentage of circulating cell subsets, nor the number of EPC derived colonies, showed significant modifications during 120 day follow up by ANOVA test. Microsatellite analysis was performed on the EPC derived colonies of 3 patients, tested at time points ranging from 1 to 2 months. In 2 patients, all the analysed colonies were of donor origin; in the third patient all the analysed colonies were of patient origin (hematopoietic engraftment donor/recipient 95%/5%). Circulating EPCs are detectable in patients given allogeneic BMT from 21 days up to 4 months after transplantation. Further studies are needed to definitively conclude their origin and to assess whether their recovery can be correlated to the clinical outcome of the transplanted patients.

**R1075**

Circulating endothelial progenitor cells in children with non-malignant diseases following allogeneic bone marrow transplantation

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Bone marrow and peripheral blood of adults contain a subset of progenitor cells, which are able to differentiate into mature endothelial cells, thus contributing to re-endothelialization and neo-vascularization. The number of these cells in healthy subjects is rather low; almost 0.002% of total mononuclear cells and a variety of factors may further influence their number. To investigate how allogeneic stem cell transplantation (SCT) influence circulating endothelial progenitor cells (CEPCs), we obtained peripheral blood samples from 13 transplant recipient at different time points (ranging from 8 months to 5 years after transplantation). Peripheral blood mononuclear cells were separated by Ficoll density-gradient centrifugation and were seeded to fibronecint-coated well dishes containing Endocult Medium (Stem Cell Technologies). In order to remove monocytes and mature endothelial cells, non-adherent cells, at day two of culture, were harvested and further cultured for an additional three days to allow formation of endothelial colonies. The phenotype of the cells that emerged in culture was characterized by immunohistochemistry, and their origin was determined using a polymerase chain reaction (PCR)-based assay for polymorphic short tandem repeats (STRs). All samples gave rise to EPCs colonies in 5 days. The mean number of EPCs colonies/10⁶ cells was 58 ± 25.3 (range: 46-96) and it didn’t seem to correlate with the post-transplant time. The cultured cells expressed typical endothelial markers such as CD31 and von Willebrand factor (vWF). For each patient and at all time points, STR-PCR analysis showed that cultured cells came exclusively from the donor. These results demonstrate that CEPCs are detectable after SCT and that their number is independent of post-transplant time.

**R1076**

Circulating endothelial progenitor cells in patients undergoing allogeneic stem cell transplantation

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Bone marrow and peripheral blood of adults contain a subset of progenitor cells, which are able to differentiate into mature endothelial cells, thus contributing to re-endothelialization and neo-vascularization. The number of these cells in healthy subjects is rather low; almost 0.002% of total mononuclear cells and a variety of factors may further influence their number. To investigate how allogeneic stem cell transplantation (SCT) influence circulating endothelial progenitor cells (CEPCs), we obtained peripheral blood samples from 13 transplant recipient at different time points (ranging from 8 months to 5 years after transplantation). Peripheral blood mononuclear cells were separated by Ficoll density-gradient centrifugation and were seeded to fibronecint-coated well dishes containing Endocult Medium (Stem Cell Technologies). In order to remove monocytes and mature endothelial cells, non-adherent cells, at day two of culture, were harvested and further cultured for an additional three days to allow formation of endothelial colonies. The phenotype of the cells that emerged in culture was characterized by immunohistochemistry, and their origin was determined using a polymerase chain reaction (PCR)-based assay for polymorphic short tandem repeats (STRs). All samples gave rise to EPCs colonies in 5 days. The mean number of EPCs colonies/10⁶ cells was 58 ± 25.3 (range: 46-96) and it didn’t seem to correlate with the post-transplant time. The cultured cells expressed typical endothelial markers such as CD31 and von Willebrand factor (vWF). For each patient and at all time points, STR-PCR analysis showed that cultured cells came exclusively from the donor. These results demonstrate that CEPCs are detectable after SCT and that their number is independent of post-transplant time.

**R1077**

Early CD34+ cells recirculate after autologous peripheral blood stem cell transplant and peg-filgrastim administration for haematological malignancies

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CD34 protein is widely accepted as reliable marker for identifying hematopoietic stem or progenitor cells in bone marrow and in peripheral blood. CD34+ cells represent a heterogeneous cell population consisting of primitive uncommitted and pluripotent progenitors as well as committed stem cell. Previous studies showed that these progenitors, mainly myeloid-committed subsets, are detectable in the early phase following infusion of autologous/allogeneic stem cell at day +9 (Albo et al, Haematologica 2004).

Based on these findings, we investigated the kinetics of appearance of CD34+ cells after autologous peripheral blood stem cell transplant (aPBSCT) and administration of Peg-filgrastim 6mg at day +1, and its correlation with haematological engraftment.

We studied in 16 consecutive patients (pts), affected by haematological malignancy and treated with aPBSCT (Table 1), the percentage of CD34+ cell in peripheral blood every other day from day + 2 until patient discharge. These cells were detectable starting from day +10 after transplantation. The peak of the CD34+ cells was at day +12 (range 10-16), at this day WBC were 6015/mmC (range 1060-13940/mmC) and the number of CD34+/mmC were 7.85 (range 0.8-116). There was no correlation between total number of CD 34+/kg infused and day, absolute number and percentage of CD34+ peak.

Statistical analysis demonstrated a significant negative correlation (r=-0.53, p=0.035) between age of pts and peak of CD34+, while a significant positive correlation (r=0.58, p=0.019) between age of pts and day of CD34+ peak. When haematological reconstitution after aPBSCT we observed a significant positive correlation between day of CD34+ peak and time to absolute lymphocyte>0.5 x10⁹/ALC (r=0.59, p=0.016), PMN>5x10⁹/l (r=0.53, p=0.035), Plt>100x10⁹/mmC (r=0.71, p=0.002), length of hospitalization (r=0.51, p=0.044).

This data seems to link up the appearance of CD34+ cells with the bone marrow reserve; younger pts release higher number of CD34+ in peripheral blood after aPBSCT and these cells are detectable sooner than in the older pts. Furthermore, time to ALC>0.5 x10⁹/l and PMN>0.5x10⁹/l recovery, Plt>100x10⁹/mmC and length of hospitalization are longer in pts that release later CD34+ cells, i.e. the older pts. Early appearance of CD34+ cells after aPBSCT and peg-filgrastim might be considered as surrogate marker of bone marrow reserve. Further analysis of CD34+ subset are ongoing to confirm these data and to clarify their significance.
Table 1. Characteristics of patients and engraftment

| Sex (F/M) | 5 F / 11 M |
|-----------|------------|
| Age       | 46.5 y (range 20-62) |
| Malignancy| 4 Myeloma Multiple, 4 Hodgkin disease, 8 Non-Hodgkin Lymphoma |
| CD34+ / kg infused | 6.375 x 10^6 cells (range 2.29-14.16) |
| Time to PMN=0.5 x 10^9/l | 9 days (range 7-14) |
| Time to Plts>50 x 10^9/l | 14 days (range 11-68) |
| Time to Plts>100 x 10^9/l | 17 days (range 14-49) |
| Length of hospitalization | 21 days (range 10-30) |
| Time to ALC=0.5 x 10^9/l | 13.5 days (range 8-40) |

R1078
Evaluation of donor/recipient origin of mesenchymal stem cells after allogeneic hematopoietic stem cell transplantation in paediatric patients
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Mesenchymal stem cells (MSCs) are endowed with multilineage potential and immunomodulatory ability, these properties rendering them attractive for tissue engineering and immunotherapy. However, it is still a matter of debate whether donor MSCs have a sustained engraftment in the host bone marrow (BM) after allogeneic hematopoietic stem cell transplantation (HSCT). In particular, studies on the fate of MSCs transplanted with cord blood (CB) are lacking. The aim of this study was to analyse the donor/recipient origin of MSCs in pediatric patients receiving an allogeneic HSCT. Thirty-six patients undergoing allogeneic HSCT for either malignant (24 cases) or non-malignant disorders (12 cases) were enrolled in the study; 19 patients received CB transplantation (CBT, 13 from a related and 6 from an unrelated donor) and 17 patients BM transplantation (BMT, 7 from a related and 10 from unrelated donor). Results were also compared with those obtained in 11 adults given HSCT for either malignant (7 cases) or non-malignant (4 cases) disorders. MSCs were grown from BM aspirates taken 2-17 months after HSCT. MSC samples at the third-fourth passage were phenotypically characterized and resulted to be positive for CD73, CD105, CD14 (<2%). Donor/recipient origin of MSCs was assessed by amelogenin assay (in case of male recipient/female donor) and microsatellite analysis. MSCs were grown from 29 pediatric patients; in 8 samples (4 after BMT and 4 after CBT) a confluent layer of cells did not grow, leading to an insufficient quantity of MSCs for chimerism analysis. Molecular analysis on MSCs demonstrated a full recipient chimerism in 10/13 and in 10/15 of the assessable pediatric patients given BMT and CBT, respectively. A mixed MSC chimerism with donor cells was observed in 3 patients transplanted with BM cells and in 5 children given CBT. Chimerism analysis performed on peripheral blood mononuclear cells (PBMCs) of the same patients, showed a full donor chimerism in all children given BMT but one, while a mixed chimerism was detected in 6 out of 19 children given CBT. A full recipient MSC chimerism was observed in all adult patients, who also displayed a full donor PBMC chimerism. These data suggest that BM soil of pediatric patients might be more favourable than that of adults for the engraftment of transplanted MSCs and that MSCs able to engraft in the host can also be transferred with CB.
**Graft engineering**

**R1080**

Cryopreservation of peripheral blood progenitors for autologous transplantation in haematological malignancies with different concentration of cryoprotectant - five-year single-centre experience

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In this study we present our five year center experience with cryopreservation of PBSC and autologous transplantation in 42 patients with hematological malignancies treated in a period 2000-2005 at Department of Hematology, Skopje. Material and methods: diagnosis of patients were (9 AML, 11 NHL, 10 MM, 12 HD) and median age at transplant was 34 years (7-63). Mobilization of PBSC was provided with Etoposide (VP-16) + G-CSF 10mcg/kg in AML patients, and high dose Cyclophosphamide 4-5gr/m2+G-CSF 10mcg/kg or alone G-CSF 10 mcg/kg in patients with lymphoproliferative diseases. Collected PBSC were cryopreserved in solutions with 5% DMSO in 20 patients and 10% DMSO in 22 patients, computer programmed until -80C, and stored different period in lique refrigerate nitrogen on -196C. Autologous transplant was preformed with conditioning consisting of myeloablative high-dose chemotherapy, BuCy in AML patients, high dose Mel in MM patients, BEAM or hd ICE in NHL patients and BEAM in HD patients. Cell viability was assessed by fluorescence microscopy using acridine orange dye exclusion.

Results: A total of 103 PBSC cryopreservation procedures were preformed in our group of patients with median 3 (2-5) apheresis procedures. Median period from storage of cryopreserved PBSC grafts until thawing was 46 days (32-60). Total number of infused CD34+cells was between 2.0-15x10^6/kg and median number of mononuclear cells was 4.2x10^6/kg(1.7-7.2). The amount of infused DMSO solution ranged between 210-650ml (median 430ml) with DMSO concentration ranging 23ml- 50ml (median 35ml) in a group preserved with 10%DMSO and 13-23ml (median 19ml) in 5% DMSO cryopreserved grafts. The viability of the fresh harvests before storage was median 97% (range 68, 5-99, 9%). The poorer viability was associated with harvest cell count. Bellow 300x10^6/L the median viability was 98% and only 0/24 cases had <85% viable cells. Harvests count above 300x10^6/L the median viability was 78% (67.8%-99%). In a group of patients that received PBSC grafts preserved with 10% DMSO, also revealed signs of mild DMSO infusion related toxicity (22%-vs14%). Hematopoietic recovery was similar in both groups, sor Ne>0,5x10^11/L on day +9 (6-10), Plt >20x10^11/L on day +12 (11-14).

Our results confirm that the infusion of cryopreserved autologous PBSC in hematological malignancies revealed successful engraftment in all patients and good cell viability. We did not registered “hard to mobilize” patients and graft failure.

**R1081**

ThermoGenesis AXP™ and BioArchive™ systems for automated cord blood banking

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Background: Good tissue practices (cGTP) in cord blood banking require product uniformity and reproducible mononuclear cell recovery and viability, suggesting that automation could be critical to facilitating cGTP-compliance for cord blood banks. Processes that lend themselves to automation are cord blood volume reduction, controlled rate freezing, storage and retrieval that avoids unnecessary transient warming events. We have evaluated the AutoXpressSystem (AXP), that allows for automated volume reduction in a closed system. The AutoXpress consists of a microprocessor-controlled device and a disposable closed blood bag set that provides for the separation of CB into a freezing bag, an erythrocyte bag and an excess plasma bag. The mononuclear cell product is concentrated into a uniform volume in the freezing bag, ready to be cryoprotected and fully compatible with the BioArchive System, a system that allows the controlled-rate freezing, liquid nitrogen storage and retrieval of 3,600 CBUs.

Study Design: The efficiency with which cord blood hematopoietic progenitor cells can be concentrated into the freezing bag of the AutoXpress bag-set was determined using the CD34 cell marker and colony-forming unit (CFU) counts as principal indices. The product was cryoprotected with 10% DMSO, frozen in the BioArchive system, stored for 2-4 weeks under the liquid N2 level and then retrieved and thawed using the standard clinical protocol. Twenty-three consecutive cord blood units were evaluated for cell recovery by measuring the collection and product volumes, the hematocrit and the counts of total nucleated cells (TNC), mononuclear cells (MNC), CD34+ cells and colony-forming units (CFU) before and after AXP processing. We also determined these indices after thawing, storage in the BioArchive System and thawing. Results: Results are presented as the mean S.D. (N=23) for all values. The AXP process achieved MNC fraction volumes of 19.7 0.3 ml with a final average hematocrit of 29.8 2.6%. The post-processing recovery of CD34+ cells was 98.2 8.0% and those of CFU 94.6 7.0%, of MNC 97.9 4.9% and of TNC 84.8 9.2%. Less than 1% of TNC were lost into the excess plasma bags. Granulocytes, accounting for 15% of TNC and less than 0.5% of the CD34+ cells were lost into the red cell bags. Post-thaw the recoveries of CFU and viable CD34+ cells were 96 4.8% and 94 2.1%, respectively.

Conclusions: The AXP efficiently and reproducibly separates cord blood mononuclear and CD34+ cells into a consistent, uniform volume. These cells retained their viability post BioArchive freezing, storage and retrieval (>94%). Thus, AXP coupled with the BioArchive System supports a very high quality standard for automated cord blood processing.

**R1082**

The influence of cryopreservation and the duration of frozen storage

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Among the factors which enable successful transplantation, the ability to store and subsequently recover sufficient viable hematopoietic stem cells to reestablish hematopoiesis is critical. The goal of this study was to evaluate the impact of freezing procedures and cryopreservation in liquid nitrogen into -176°C on cell viability, WBC, CD34+ cells recovery and clonogenic capacity. In the retrospective study 74 samples derived from patients with hematological malignancies (n=66) and healthy donors (n=8) were analysed. Median duration of the product storage was 3.6 years (1 day – 15.6 years). The viability (%), WBC (G/L), and CD34+ (%) all decreased significantly after cryopreservation (p=0.000001) with median relative changes of -15.3%, -13.2%, and -10.7%, respectively. After thawing the viability equaled 79.5% (36-96%), WBC 46.3G/L (1.9-190.8G/L), and CD34+ cells 0.47% (0.01-7.0%).

In multivariate analysis the following factors were associated with poor recovery: 1) viability: presence of malignant disease (p=0.000002), use of cyclophosphamide (Ctx) for mobilisation (p=0.00008), 2) WBC: presence of malignant disease (p=0.003), older age (p=0.003), 3) CD34+ cells: presence of malignant disease (p=0.006), storage duration (p=0.000003).
After thawing the median number of clones/10⁴ cells was as follows: 1) CFU-GM on day 7: 11 (0 – 70.2), 2) CFU-GM on day 14: 276 (14 – 95.5), 3) BFU-E: 11.3 (4.7 – 48), 4) CFU-GEMM: 1.9 (0 – 7.2). The clonogenicity was negatively influenced by: 1) CFU-GM on day 7: presence of malignant disease (p=0.00004), chemotherapy-containing mobilisation regimen (p=0.01), Ctx for mobilisation (p=0.00004), 2) CFU-GM on day 14: older age (p=0.048), presence of malignant disease (p=0.000002), chemotherapy-containing mobilisation regimen (p<0.000001), 3) BFU-E on day 14: presence of malignant disease (p=0.00002), chemotherapy-containing mobilisation regimen (p<0.000001), 4) CFU-GEMM on day 14: diagnosis other than CML (p=0.00009).

We conclude that both diagnosis and mobilisation regimen have impact on recovery and clonogenicity of cryopreserved hematopoietic stem cells. On the other hand, even long-term storage enables preservation of vial cells with high clonogenic potential, which may be used for transplantation.

R1083 Immune reconstitution after HLA-haploidentical transplantation using unmodified marrow and CD6-depleted blood stem cell: not different from HLA-identical transplantation

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Immune reconstitution (IR) is a key role of allogeneic hematopoietic stem cell transplantation (HSCT) not only because persistent immune defects is related to post-transplant infectious morbidity, but also because it may influence the risk of relapse and the development of secondary malignancies after HSCT. Many factors have an impact on IR, especially the degree of genetic differences between donor and recipient. Several studies have shown extreme slow IR in patients receiving T-cell depleted graft from human leukocyte antigen (HLA)-haploidentical (HAP) donor. Differently, we have used unmodified marrow on day 0 and CD6-depleted mobilized blood cells (MBC) on day 6 for haploidentical transplantation. CD6-depleted MBC are devoid of CD4-positive cells, they contain CD34-positive cells, NK-cells and a minority of CD8-positive cells. To compare the IR during the first year post transplant between HAP and HLA-identical (ID) recipients, we have carried out a prospective, longitudinal analysis of IR in 15 HSCT patients in each group. We assessed reconstitution of naive CD4 T cells, B cells, natural killer cells, dendritic cells and monitored thymic output by using TCR rearrangement excision circles (TRECcs). Surprisingly, reconstitution did not differ between the groups. This study reveals that IR in HAP transplantation using unmodified marrow and CD6-depleted MBC is not worse than ID group.

Graft-versus-host disease

R1084 Autologous graft-versus-host disease – rare complication of haematopoietic cell transplantation

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Background: Autologous graft versus host disease (GVHD) is a novel self-limited autoaggression syndrome, encountered in the autologous PBSCT (peripheral blood stem cell transplantation) setting with spontaneous occurrence in patients receiving CD34+ enriched autografts.

Aim: To present the case report, concerning the occurrence of a rather rare form of GVHD in a patient with CD34+ enriched auto-PBSCT for Ewing sarcoma.

Case report: We present the case of a 14 years old female patient with Ewing sarcoma of the scull and right hip who underwent autologous PBSCT with a CD34+ enriched graft. The preparative regimen consisted of busulfan and melphalan. Engraftment occurred on day +11 for neutrophils and on day +12 for platelets. At day +22 signs of acute GVHD involving only the skin occurred initially in the axillae, flexion sites of the elbows and popliteal region and shortly afterwards involving the face, neck, trunk and abdomen. Beside maculopapular exanthema, bulla formation and large epidermal desabasion were observed, an image corresponding to stage IV of acute GVHD. The patient remained feverless, without pruritus or other signs or symptoms. At the time of onset the investigation of immune reconstitution presented marked lymphopenia (absolute values per microliter): CD4+ = 44, CD8+ = 34, CD16+/CD56+ = 173. No other modified biological parameters could be found. Skin biopsy revealed lymphoid, predominantly CD8+ infiltration. In 50 days the lesions progressively disappeared, with the maintenance of residual hyperpigmentation and nail keratoses.

Conclusion: Autologous GVHD, confined to the skin without any clinical-biological evidence of internal organ disease is possible self-limited condition. The aim of the study was to show the case of autologous GVHD, that is not a diagnosis. This study reveals that IR in HAP transplantation using unmodified marrow and CD6-depleted MBC is not worse than ID group.

R1085 Why will an amotosalen-based protocol for extracorporeal photopheresis be valuable?

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Bone marrow transplantation has the potential of providing a complete cure of the disease symptoms of hematologic malignancies and, ultimately, with an appropriate safety profile, the symptoms of hemoglobinopathies as well. Even in the case of related fully matched bone marrow transplantation, there is morbidity and mortality associated with Graft-versus-Host-Disease (GVHD). Successful application of mismatched (related-haploidentical and unrelated) bone marrow transplantation (BMT) in patients with leukemia or lymphoma requires that improved outcomes of BMT be obtained, coupled with the avoidance or successful treatment for the GVHD now experienced in existing mismatched BMT protocols. These two goals may require use of reduced-intensity conditioning (RIC) in support of the transplantation. Extracorporeal Photopheresis (ECP) has proven to be effective as a treatment modality for GVHD following transplantation and is gaining popularity as a treatment protocol. Yet, there remain significant technical challenges in the application of ECP. As a solution to many of these problems, we propose the development of a more simple and less expensive process for the psoralen photochemical treatment of the transplant patient's autologous leukocytes. This process will define the therapeutic dose of photochemically treated cells required for clinical responses equivalent to those which have been observed to be most effective using presently approved ECP instrumentation and protocols. However, this new process uses Amotosalen, a more photochemically efficient psoralen, and it uses lymphocytes collected by the conventional methods of blood banks. The greater photo-sensitivty of the psoralen compound results in far shorter exposures of the target leukocytes to UVA light, leading to less non-specific damage of the cells, leaving them metabolically active, although unable to divide. It is our premise that these are the ideal properties that photochemically treated lymphocytes must have in order to
maximize the positive immunological responses associated with a successful photopheresis treatment.

R1086

UVA1 phototherapy as a treatment for sclerodermic chronic graft-versus-host disease refractory to immuno-suppressive therapy

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Chronic graft versus host disease (cGVHD) is the most common late complication affecting long-term survivors of allogeneic HSCT. Several organs are targets of cGVHD but the skin is the tissue mainly affected. Two subtypes of involvement, cutaneous lichenoid and scleroderma, have been described, based on clinical and histopathological examinations. Chronic cGVHD is usually treated with immuno-suppressive drugs which, however, are not effective in about 20-40 % of patients. In this setting of refractory/resistant patients, extracorporeal photochemotherapy and ultraviolet A (UVA) radiation often show a positive clinical outcome, but the results are limited by the low number of treated cases. In our institute, UVA1 phototherapy has been used for treating 3 patients with advanced cutaneous cGVHD (generalized sclerodermoid skin involvement) resistant to conventional immuno-suppressive therapy. Patients enrolled gave fully informed consent and they underwent skin biopsies before and after UVA1 phototherapy. Moreover, the cutaneous elasticity has been evaluated by means of an elastometer. UVA1 radiation (340-400 nm) was emitted by a GP-24H irradiation unit with a dose of 60 J/cm². Irradiance was measured with a spectroradiometer and found to be 95mJ/cm² at skin level. Patients were treated 5 times per week for a total of 6 weeks (total 1800 J/cm²) and the cutaneous lesions were carefully inspected and palpated before starting every treatment. Patients were conditioned with a reduced intensity regimen and received unmodified G-CSF mobilized PBSC from matched related donors. GVHD prophylaxis consisted of CSA and MTX. All patients had failed to respond to at least three lines of immuno-suppressive therapy. At the end of sessions the clinical response was assessed subjectively and objectively, and it was graded as good (obvious softening), moderate (mild softening) and poor (no change). Two patients had a good response and one a moderate response reporting remarkable softening of skin lesions after UVA1 phototherapy without side effects. Clinical responses were associated to an improvement of histopathological findings. The evaluation of skin elasticity showed a significant increase in resilience, hysteresis, and distensibility. Our results demonstrate the efficacy of UVA1 phototherapy. It appeared, in the setting of cutaneous cGVHD, as a procedure well tolerated and effective particularly for patients who do not respond to standard immuno-suppressive treatments.

R1087

Rituximab in chronic graft-versus-host disease of the lung

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We report about a 24 year old male patient in whom severe obstructive chronic graft-versus-host-disease (cGVHD) of the lung improved with Rituximab, an anti-CD20 antibody. The patient had undergone HLA-matched unrelated allogeneic peripheral stem cell transplant for his acute myeloid leukaemia in August 2003. With discontinuation of cyclosporine after day +100 mild cGVHD of the skin and liver occurred, but improved spontaneously within a few weeks. In January 2005 the patient presented with severe dyspnoea associated with an afibrile upper respiratory tract infection. The work-up revealed severe obstruction on lung function tests, nodular infiltrates in computed tomography scan (CT), acute bronchitis in bronchoscopy; but no infectious agent in bronchial lavage. Initial and long term treatment with various antibiotics, antifungal agents and several immuno-suppressants (prednison, rapamycin, mycophenolatmofetil, cyclosporine A, cyclophosphamide) was unsuccessful. After a single dose of Rituximab (375mg/m²) dyspnoea improved for a few days, but symptoms reappeared. We started a regular weekly Rituximab application in July 2005 resulting in marked clinical improvement, slight improvement on lung function (peak expiratory flow, PEF) and regression of the nodular infiltrates in CT scan despite tapering off other immuno-suppression and intermittent respiratory infections. The patient was able to participate in household thresholds again. Rituximab therapy was discontinued in November 2005 because of patient’s admission with spontaneous pneumothorax. The patient is now being evaluated for lung transplantation. Like in our case, monoclonal anti-CD20 antibody has recently shown efficacy in cGvHD and several autoimmune diseases, probably due to elimination of B cells which may act as antigen presenting cells for T cells and as a source of autoantibody production.

Conclusion: We propose regular weekly Rituximab application as a treatment option in cGVHD of the lung.

R1088

Aim: peak level measurements of cyclosporin A useful in allogeneic stem cell transplantation?

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Peak level plasma levels of Cyclosporin A (CsA) 2 or 4 hours after drug administration (C2 or C4) correlate with the degree of calcineurin inhibition and with the AUC much better than trough levels (C0). In solid organ transplantation dosing according to C2 levels rather than C0 led to reduced rejection rates. In allogeneic stem cell transplantation CsA doses are still adjusted according to C0 levels. To investigate whether C2 and C4 levels would be useful in CsA dosing after stem cell transplantation these measurements were performed in consecutive patients in addition to C0 levels. If intravenous CsA was given, peak levels were assessed after the end of a 4 hour infusion. So far 16 serial measurements were undertaken in 10 transplant patients (6 AML, 1 ALL, 1 SAA, 1 Myeloma, 1 CLL). There was only a weak correlation between trough and peak levels of CsA and between dose and plasma levels irrespective whether the drug was administered orally or intravenously. Although all trough levels were within the target range of 250 – 450 ng/ml peak levels were all below 1000 ng/ml. There was no evidence of so-called “late absorbers” as all C4 levels were lower than the C2 levels. Two patients showed evidence of microangiopathic hemolytic anaemia, however peak and trough levels of CsA were not different from other patients. All but two patients had signs of acute GvHD. In view of the CsA doses given and the trough levels, the peak levels were considerably lower than expected from
results in liver or renal transplantation. This was even more surprising as most patients received itraconazole, a drug known to increase CsA levels by interfering with CsA metabolism in the liver.

In summary C2 monitoring is feasible and demonstrates lower peak levels than expected in solid organ transplantation. These levels should lead to a lesser degree of calcineurin inhibition. Whether this translates in increased rates of GVHD remains to be seen with a larger patient number. If this holds true CsA dosing with higher C2 target levels might decrease acute GVHD after stem cell transplantation as previously shown for rejection rates after liver transplantation.

R1089
Clinical and histopathological features of oral chronic graft-versus-host disease following allogeneic reduced-intensity conditioning haematopoietic stem cell transplantation: a single-centre blind study

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Introduction: Oral involvement of chronic graft-versus-host disease (GVHD) occurs in 80% of patients suffering from cGVHD and the oral cavity may be the primary or even the only site of cGVHD involvement.

Lichen planus-like lesions are the most distinctive oral changes of cGVHD. The histopathologic changes of oral cGVHD include epithelial atrophy, apoptotic bodies, hydropic degeneration of the basal cells, and a mononuclear sub-epithelial cell infiltrate with lymphocyte invasion.

Aim: The aim of this blind study was to compare clinical and histological features of oral mucosa in patients who underwent allogeneic reduced intensity conditioning hematopoietic stem cell transplantation (RIC HSCT).

Patients and Methods: This study enrolled 10 adult patients who consecutively underwent allogeneic RIC HSCT for hematological malignancies between October 2004 and October 2005. All patients were assessed for the presence of oral cGVHD by oral examination performed on the day +100 at the Unit of Oral Pathology and Medicine, University of Milan. Clinical lichenoid changes of the oral mucosa were regarded as positive for cGVHD. Following informed consent, an incisional biopsy was taken from oral mucosa of all patients, either with or without oral cGVHD lesions. Biopsies were examined by a pathologist who was unaware of clinical aspect of the mucosa (blind).

Results: Biopsies were taken from 4 patients with clinical evidence of oral cGVHD and 6 patients with apparently normal oral mucosa. Biopsies were performed at a time point ranging from 96 to 165 days (median 113.5 days) after transplantation.

Sites of biopsies were buccal mucosa and gingiva. Histological cGVHD changes were detected in all the 4 patients having also clinical evidence of oral cGVHD, and in 4 out of 6 patients with apparently healthy mucosa.

Conclusions: While histological changes of oral mucosa without corresponding clinical changes are not sufficient to make a definite diagnosis of oral cGVHD, their detection might be of considerable help to predict the onset of the disease following RIC HSCT. A longer follow up of patients showing histological changes with no clinical counterpart, will possibly elucidate whether such changes are indeed predictive of the occurrence of clinically evident lesions.

R1090
Evaluation of safety and tolerability of extracorporeal photochemotherapy in paediatric patients affected with graft-versus-host disease

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Objectives: Extracorporeal photochemotherapy (ECP) is a therapeutic option for treatment of acute and chronic graft versus host disease (aGvHD and cGvHD) resistant to standard drug therapy. In the pediatric context, ECP procedure has some technical limitations when compared to adult subset. Low body weight, muscular access, hypersensitivity to hypocalcemia, fluid balance and transfusion demand may represent a limitation for ECP treatment in children. We report our experience in 15 very low body weight children (<15 Kg) affected with aGvHD and cGvHD in terms of safety and tolerability of ECP.

Methods: ECP consists of 3 distinct steps: 1) collection of mononuclear cells by Spectra Cobe (version 6.0) cell separator device processing 2 blood volumes; 2) ex-vivo dilution with saline and addition of B-MOP to the bag, transfer of the buffy coat in a UV-A permeable bag and irradiation at 22°C; 3) reinfusion of the cells to the patient after 2 hours to avoid hypotermia. 15 patients, median age 4.2 years (range:2-5) median weight 11.5 kg (range:7-15) were treated. A central venous catheter was positioned in all patients. Our treatment protocol consisted in 3 procedures per week in aGvHD and 2 procedures per week in cGvHD, both followed by 2 procedures every 2 weeks for 2-3 weeks then by 2 procedures per month. 8 patients were affected with aGvHD (grade II-IV with skin, liver and gut involvement); 7 patients had extensive cGvHD (skin, mucosae, liver, lung). The cell separator device was primed every time with filtered and irradiated RBC. Calcium gluconate was continuously administered by pump during the procedure. The ACD/whole blood ratio was always settled at 1:20. All patients were monitored for blood pressure and heart rate during the entire procedure.

Results: The total number of ECP procedures performed was 375, with a median of 25 (range:8-65) procedures/patient. 5/15 (33.3%) patients experienced mild hypotension, 6/15 (40%) moderate hypotermia and 2/15 (13.3%) hypocalcemia (nausea and vomiting); no procedure was discontinued. The transfusion demand did not augment during all the course of treatment; no life-threatening infections were recorded.

Conclusion: We demonstrated that ECP is feasible and safe even in very low body weight patients on condition that ECP is performed adopting some simple precautions. Our experience broadens the cohort of patients who can benefit from this therapeutic option.

R1091
Analysis of the outcome and adverse factors influencing the related matched donor HSCT performed in a Lower Silesian centre for cellular transplantation, Poland

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89 haematological patients - malignancies (CML: n=29; AML:n=23; ALL:n=20) and SAA (n=17); children (n=33) and adults (n=56) were transplanted with BM (n=72) and PBPC (n=17) from HLA-identical sibling donors in years 1990 to 2000. All leukemia patients received myeloablative conditioning (BuCy or BuCyVp); SAA patients were conditioned with Cy or CyATG. Chimerism was tested and proved to be complete in all but 5 cases (death before day +30). During 13 years of follow up 31 patients died of transplant related causes (Regimen Related Toxicity:n=2, RRT/aGvHD:n=2, Infection: n=7, aGvHD/Multi Organ Failure:Inf:n=8, cGvHD/MOF/Inf:n=12) or relapse (n=8). aGvHD was seen in 44,2% cases (grade1=19,7% of patients with hematological recovery, grade2=14%, grade>2= 10,5%).
Severe aGvHD (grade>2) was seen only in leukemia patients, who received toxic conditioning, but not in SAA conditioned only with Cy+ATG. aGvHD was more frequent and severe in pts with a high grade of toxicity (among patients with low RRT there were 63% cases of aGvHD=0 and no cases of aGvHD=3, among patients with high RRT there were 38% aGvHD=0 and 22% aGvHD=2). In addition to toxicity infections and inflammation seemed to aggravate aGvHD as shown by the presence of an elevation of serum CRP at the advent of severe aGvHD. Apparently the presence of toxicity and aGvHD had an additive negative effect on survival. The incidence of cGvHD among all patients living>day+100 was 49% and increased with i) diagnosis: SAA(11,8% of SAA cases),AML (47,4%),ALL(53,9%),CML(73,9%).ii)previous aGvHD: all surviving patients with aGvHD>1 developed cGvHD. Cumulative survival is better in following :i)SAA vs ALL/LALL/CML (p=0,003),ii)ALL, CML early stage vs advanced (p=0,02),iii) conditioning BuCy vs BuCyVp (p=0,02),iv) gender: female advanced (p=0,02),v) conditioning BuCy vs BuCyVp (p=0,02),vi) ALL, CML early stage vs Cumulative survival is better in following:i)SAA vs ALL/LALL/CML (p=0,003),ii)ALL, CML early stage vs advanced (p=0,02),iii) conditioning BuCy vs BuCyVp (p=0,02),iv) gender: female advanced (p=0,02),v) conditioning BuCy vs BuCyVp (p=0,02),vi) ALL, CML early stage vs advanced (p=0,02),vii) age: children vs adults (p=0,26),viii) gender: female advanced (p=0,02),vii) conditioning BuCy vs BuCyVp (p=0,02)

Objectives: Distinct eosinophilia rarely occurs in chronic graft-versus-host disease (GvHD) after allogeneic hematopoietic stem cell transplantation (HSCT), but is often seen in hematological malignancies, including lymphoproliferative diseases. A hypothesis exists that marked eosinophilia in chronic GvHD is mainly of non-colonial origin and, in general, is associated with favorable prognosis. The aim of this study was to investigate the serum levels of secretable eosinophil and mast cells proteins in the patients with chronic GvHD and other hematological malignancies.

Patients and Methods: Six post-transplant patients with chronic GvHD with eosinophilia were followed up after allo-HSCT. Thirty hematological malignancies in patients with marked eosinophilia (>0.4 x 10^9/L) were also observed, including cases of lymphoproliferative (n=20) and myeloproliferative (n=10) diseases. Eighteen patients with bronchial asthma (BA) comprised a reference group for polyclonal eosinophilia. High content of peripheral blood eosinophils served as the major criterion of patients' selection and was confirmed by flow cytometry. Eosinophil cationic protein (ECP) and tryptase were measured in serum by fluorimunoenzyme assay (Pharmacia, Sweden).

Results: The percentages of circulating eosinophils in patients with lymphoproliferative (median: 11.7%; range, 2-78%) and myeloproliferative diseases (median 16.8%; range, 1-68%), and in cases of chronic GvHD after allo-HSCT (median 16.3%; range, 3-70%) were significantly higher than in BA patients (median 10.2%; range, 5-23%); D=0.01. The levels of total ECP were markedly increased in the patients with lymphoproliferative diseases (median: 18.8 ng/mL; range, 2-197 ng/mL) compared to BA (median: 10.5 ng/mL; range, 2.8-80 ng/mL); P=0.001. However, the serum levels of tryptase and ECP in total group of hematological patients (median: 6.5 ng/mL; range, 2 to 24.1 ng/mL) were not increased, as compared with asthma cases (median: 5.4 ng/mL; range, 1.9 to 24.4 ng/mL); P=0.05. Likewise, in chronic GvHD with eosinophilia, the serum contents of both ECP and tryptase did not differ from those in BA.

Conclusion: The significant differences in ECP levels in blood serum in the patients with lymphoproliferative diseases and myeloproliferative disorders (median 16.8%; range, 1-68%), in chronic GvHD after allo-HSCT (median 16.3%; range, 3-70%), and in cases of chronic GvHD after allo-HSCT (median 16.3%; range, 3-70%) were significantly higher than in BA patients (median 10.2%; range, 5-23%); D=0.01. The levels of total ECP were markedly increased in the patients with lymphoproliferative diseases (median: 18.8 ng/mL; range, 2-197 ng/mL) compared to BA (median: 10.5 ng/mL; range, 2.8-80 ng/mL); P=0.001. However, the serum levels of tryptase and ECP in total group of hematological patients (median: 6.5 ng/mL; range, 2 to 24.1 ng/mL) were not increased, as compared with asthma cases (median: 5.4 ng/mL; range, 1.9 to 24.4 ng/mL); P=0.05. Likewise, in chronic GvHD with eosinophilia, the serum contents of both ECP and tryptase did not differ from those in BA.

R1093 Intracellular markers of eosinophils and mast cells in patients with lymphoproliferative and myeloproliferative disorders associated with high-grade eosinophilia

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Graft versus malignancy

R1094 Natural killer cell activity in the early phase after allogeneic stem cell transplantation: impact of conditioning strategies

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Background: Research into the role of Natural killer (NK) cells in allogeneic stem cell transplantation (SCT) has been greatly expanded recently. NK cells may contribute to the GVHD reaction possibly without exerting a GVHD effect. Using a novel flow cytometric assay, which detects the lytic granule membrane protein CD107a as a marker for NK cell degranulation, we investigated the effect of in vivo T cell depletion and the type of conditioning on NK cell function in the early phase after allogeneic SCT.
Methods: Peripheral blood mononuclear cells were collected at day +30 and +90 after allogeneic SCT and incubated with the NK sensitive cell line HL60 (3 hours at 37°C, E:T ratio 1:1). PE-Cy5 conjugated anti-CD107a antibody was added prior to incubation, monensin (0.05mM) was added after 1 hour of incubation. Finally, cells were further stained against CD56, CD3 and CD16 and the proportion of CD107a positive NK cells was measured by flow cytometry and the absolute number of degranulating NK cells was calculated. Results were compared to values from 15 healthy controls.

Results: Twenty two patients were investigated of whom 14 had been treated with a conventional dose conditioning regimen and 8 had received a reduced dose regimen. At day +30, the proportion of NK cells with cytotoxic activity (CD107a+/CD56+) was significantly reduced as compared to normal donors (2.6% vs. 5.6%, p<0.001). At day +90 the percentage of degranulating NK cells in five evaluated patients was within normal range (mean 5.7%). The predominant proportion of degranulating cells was in the CD56dim/CD16- subpopulation (mean 9.8%).

At day +30 the percentage of CD107a+ cells averaged 1.9% after conventional conditioning compared to 4.0% after reduced intensity conditioning (p=0.21). The absolute number of degranulating NK cells was significantly reduced after conventional conditioning (4.1/µl vs. 19.8/µl, p=0.011). Neither the percentage (2.5% vs. 2.9%, p=0.77) nor the absolute number (9.4/µl vs. 13.5/µl, p=0.59) of CD107a+ NK cells differed significantly in patients with and without ATG induced T cell depletion at day +30.

Conclusion: According to our data cytotoxic activity of NK cells is reduced after allogeneic SCT. The absolute number of NK cells with cytotoxic activity is significantly higher after reduced intensity conditioning which may impact on the outcome of this strategy. We saw no significant influence of antibody mediated in vivo T cell depletion by ATG on NK cell activity during the first two months post SCT.

R1095 Role of NK alloreactivity in matched unrelated donor bone marrow transplantation in lymphoid neoplasia: single-centre experience
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It is known that NK alloreactivity is involved in the control of neoplastic cells in the setting of apioleid bone marrow transplantation (BMT) in patients with acute myeloid leukemia (AML). The role of NK alloreactivity in the setting of marrow unrelated transplant (MUD) in lymphoid neoplasia is still controversial. In a series of 15 patients (9 Acute Lymphoid Leukemia, 4 Non-Hodgkin’s Lymphoma L, 2 Severe Aplastic Anemia), we investigated whether NK alloreactivity is involved in the control of lymphoid neoplastic cells. In addition, using monoclonal antibodies (MoAbs), we evaluated the expression of Killer Immunoglobulin-like Receptors (KIRs), Killer Lectine-like Receptors (KLRs), and Natural Cytotoxicity Receprors (NCRs) in patients under study.

According to HLA-Cw alloreactivity, patients were separated into two groups: a group of 6 patients with potential NK alloreactivity (group A: 3 ALL, 1 NHL, 2 SAA), a second group of 9 patients lacking NK alloreactivity (group B: 6 ALL, 3 NHL). The mean follow up was 24 months ± 6 months. 100% of group A patients were alive, whereas 66% of group B were still in remission, indicating a role for alloreactivity in lymphoid neoplasia. Concerning expression of NK receptors, group A was characterized by the high expression of KIRs, potentially involved in alloreactivity in 2 out of 6 patients. KLR was represented by CD94/NKG2A in 5 out of 6 and in one case by CD94/NKG2C. This last case showed a recovery of CD94/NKG2A phenotype after 18 months. NCRs and NKG2D were usually expressed, with exception of NKP44. Group B was characterized by an heterogeneous pattern of expression of KIR, whereas CD94/NKG2A was expressed in 7 out of 9 cases and 2 cases expressed NKG2C. Interestingly, one of these two cases relapsed during follow-up. NCRs were usually expressed, with the only exception of NKP44.

Our data indicate that NK alloreactivity might be an advantage for survival in patients affected by acute lymphoid neoplasia. No correlation could be demonstrated with expression of Natural Killer Receptors (NKRs) and clinical behaviour, suggesting that analysis at clonal level is mandatory to get insights into the mechanism involved.

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Infectious complications

R1096 A rare complication after allogeneic stem cell transplantation: acute axonal neuropathy
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Despite considerable progress in the management of allo-HSCT, infection remains an important cause of morbidity and mortality after transplant. CMV is frequently involved in the infectious pathology after HSC transplantation. We present a 18-year old women with high-risk acute lymphoblastic leukemia who underwent matched sibling PBSC transplantation. She received standard conditioning regimen (Endoxan 120 mg/kgc and TBI 12 Gy). GvHD prophylaxis received was with CsA and methotrexate. She developed grade II GvHD (cutaneous and intestinal) on day +16 with resolution under corticotherapy. Starting with day +50 she had weakness, osteoarticulary and muscular pain with functional impairment. Neurological examen and the electrophysiological study showed an acute axonal neuropathy. She had more than 16 000 CMV copies in the blood PCR. She received 250 mg bid i.v Ganciclovir for 21 days as induction therapy and than 300 mg/d for more 4 weeks with complete remission of the neurological symptoms

R1097 Successful treatment of very early reactivation of cerebral toxoplasmosis after stem cell transplantation
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A 15 year old female patient was diagnosed with CML in chronic phase and stem cell transplantation (SCT) was planned from her one antigen mismatched sister. Pre-transplant routine investigations revealed a high antibody titer against Toxoplasma gondii (IFT 1:5120, KBR 1:40, IgM negative) indicating the history of a previous infection. Cerebral magnetic resonance imaging (MRI) ten days before transplantation showed no abnormalities.

The patient was conditioned with busulfan (16 mg/kg), cyclophosphamide (120 mg/kg). Because of the HL-A mismatch rabbit ATG (Fresenius, 60 mg/kg) was added. Graft-versus-host disease (GvHD) prophylaxis consisted of cyclosporine A and MTX. PBSCs containing 7.2 *10^6/kg CD34pos stem cells were transfused. Engraftment occured rapidly on day +12.

On day +11 the patient developed severe headache and hours later generalized seizures. Computered tomography showed multiple patchy hypointense lesions in the subcortical white areas with corresponding nodular signal enhancements in T2 weighed MRI analysis suggesting an infectious process. EEG revealed pronounced focal abnormalities in the left hemisphere. Previous cerebral magnetic resonance imaging (MRI) obtained 10 days before transplantation showed no abnormalities. A cerebrospinal fluid (CSF) analysis revealed a high titer of IgM against Toxoplasma gondii. The patient was treated with 250 mg/2 days i.v Ganciclovir and showed a complete remission of seizures.
Transient oral cavity and skin complications after mucositis preventing therapy (palifermin) in a patient after allogeneic PBSCT. Case history

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Mucositis is a common side effect of chemotherapy and radiotherapy with no effective treatment. It occurs when cancer treatment destroys the rapidly dividing epithelial cells, particularly in the oral cavity, leaving the mucosal tissue open to ulceration and infection. The aim of this study was to assess the state of oral mucosa in a patient after allo-PBSCT who has received palifermin, a recombinant human keratinocyte growth factor.

Materials and methods: A 19-year-old male was treated in the Department of Haematology of the Medical University in Warsaw due to the AML. Conditional chemotherapy was applied, according to the BuCy 4 + ATG regimen and allogeneic haematopoietic cells transplantation from an unrelated donor. He was receiving palifermin (60 microg/kg/d) intravenously for 3 consecutive days immediately before the initiation of conditioning therapy (on days -13, -12, -11) and after transplantation (on days +3, +4). On day +5 mucous membrane was pale and swollen, with linea alba visible on cheeks. Superficial glosissitis and viral pharyngitis were noted. Beginning with day +5/+6 proliferative gingivitis was observed. On day +9 gingival contour was altered and the gingiva covered nearly completely tooth crowns of all teeth. The gingiva were whitened, as if covered by thick epithelium. Slight gingival hyperplasia was still observed on day +24. During the forming of gingival hyperplasia the patient had a subjective “membrane growing” sensation with tingling and itching. He reported an oral cavity pain score of 0 in the 10-point pain scale. Since day +4/5 skin rash coexisted, spreading over hairy head skin, face, dorsum and chest. Disseminated papulopustular (acne-like) lesions were observed. Some of them were related to the hair follicles. Skin changes were present till day +15. Neutropenic fever was noted on day +6 (absolute leucocytosis 0.1 G/L). Concomitant medications: Orungal 2 x 200 mg p.o., Heviran (aciclovir) 5 x 400 mg p.o., Tazocin 4 x 4.5 g i.v. on days +2 and +3, Maxipime 3 x 2.0 g i.v. on days +3 till +7, Vancomycin 2 x 1 g on days +5 till +14, metronidazol 3 x 500 mg i.v. on days +4 till +11, Neoral 2 x 100 mg i.v. since day +3 till +11, Neoral 2 x 150 mg since day +5 and 2 x 150 mg by mouth till +14, 1 tablet/d since day +6.

R1098

Progressive fatal respiratory failure due to pseudomembranous aspergillosis tracheobronchitis following allogeneic stem cell transplantation

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Invasive fungal infections are frequent and often fatal in patients following allogeneic hematopoietic stem cell transplant (HSCT). Long-term immunosuppressive therapy for graft versus host disease (GVHD) seems to be the most predisposing factor. We present a 16 year old girl with Paroxysmal Nocturnal Hemoglobinuria (PNH). Due to unfavourable course (severe attacks of hemolysis requiring hemodialysis, Budd-Chiari syndrome) she was indicated for HSCT. She consequently failed to engraft two unmanipulated grafts from HLA mismatched (B, Cw alleles) unrelated donor. First time bone marrow was infused with CD34 7x10/6/kg, conditioning consisted of treosulfan, cyclophosphamide and ATG. Second time peripheral blood stem cells (PBSC) were used with CD34 6x10/6/kg after fludarabine, cyclophosphamide and ATG. After 3 months lasting aplasia, third allogeneic graft (PBSC – CD34 4,5x10/6/kg) was given with no conditioning surprisingly followed by rapid and full trilnieage engraftment. She than developed severe acute and chronic extensive GVHD, treated with cyclosporine A, steroids, tacrolimus, mycophenolate, sirolimus and anthymocyte globuline. During post-transplant course she suffered from BKV hemorrhagic cystitis, repeated CMV reactivations and colitis, drug induced nephropathy and steroid diabetes. She had a long-term and lasting preemptive voriconazole prophylaxis. For acute hemodynamic instability due to severe gastrointestinal bleeding 7 months after HSCT she was transferred to ICU and electively intubated. At that time she was also heparinized for acute vein thrombosis. Early on she started to desaturate, chest X-ray showed unilateral atelectasis. Bronchoscopy revealed whitish membranes, plugs and casts causing extensive obstruction of both lungs. Cultures grew Aspergillus fumigatus. Therapy including caspofungin, nebulized amphotericin B and repeated mechanical removal of obturating membranes failed to stop progression and patient died due to isolated respiratory failure 10 days upon arrival to ICU. Autopsy confirmed fungal involvement (lungs, liver, lymph nodes, skin, kidneys, and brain). Pseudomembranous myotic tracheobronchitis may be a rapidly progressing complication in heavily immunocompromised patients. Complex therapy including even combination of potent antifungals may fail to overal fatal course. Even in patients on antifungals this complication must be actively looked for with early use of bronchoscopy and exact identification of pathogen.

R1100

Triple antifungal therapy for severe systemic candidiasis allowed performance of allogeneic stem cell transplantation

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Systemic candidiasis is a rare but life threatening complication in immunosuppressed patients undergoing allogeneic SCT. Combination of new antifungal agents might improve outcome in these patients.

Here, triple anti-myocytic therapy is described in an ALL patient in urgent need of allogeneic bone marrow transplantation. The patient with T-cell acute lymphoblastic leukemia presented with thrombocytopenia due to disseminated infection with candida species. Despite the early initiation of antifungal therapy and subsequent engraftment the patient died due to fungal sepsis. The patient received a third allogeneic graft from HLA matched sibling donor with CD34 2.5x10/6/kg. Treatment consisted of posaconazole, voriconazole and amphotericin B. The patient recovered a complete haematological remission and obtained a complete molecular remission as indicated by the resolution of infections. The patient is now alive and well with no signs of disease relapse.
containing Fludarabin, Cytarabin, Etosopide and Amsacrine occurred. Even after CLAEG (Cladribine, Etosopide, Cytarabin and Campath-1H) the patient still had 35% leukemic bone marrow blasts requiring high dose chemotherapy with allogeneic stem cell transplantation. One day after start of the conditioning regimen the patient showed myeloid skin manifestations and blood cultures became positive for candida cruzei despite fluconazol prophylaxis. Because of the limited sensibility of Fluconazol resistant candida species to liposomal Amphotericon B and the high mortality rate in patients with systemic candidiasis Voriconazol was added immediately to liposomal Amphotericon B. Subsequently, the patients body temperature increased and Caspofungin was added. Since the myelotic skin manifestation responded to this triple anti-mycotic combination allogeneic peripheral blood stem cell transplantation from an unrelated donor could be performed. Although the fever resolved later the patient showed signs of a septic shock requiring intravenous administration of dopamine. With the unchanged triple antifungal therapy the patient became afebrile, skin manifestations showed complete resolution and cultures became negative. Three months after the onset of systemic candidiasis the patient was fully active with no signs of fungal infection and in haematological and molecular remission. This case shows, that the entire intensive conditioning chemotherapy could be administered and allogeneic stem cell transplantation is feasible in patients with systemic candidiasis when such combined antifungal treatment is given.

R1101
Successful treatment of invasive aspergillus with a combination of antifungal and growth factor therapies and donor granulocyte infusions
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Neutropenic patients, especially following aggressive chemotherapy, are at high risk for infectious complications. These are an important contributory factor to the treatment related morbidity and mortality (TRM). Because neutrophils represent the first line of host defense, granulocyte transfusion therapy is a tempting therapeutic approach. Although such therapy has been employed sporadically for several decades, clinical trials have been compromised by technical problems and low granulocyte yields resulting from inadequate donor stimulation. The discovery of granulocyte colony-stimulating factor (G-CSF) as a means to elevate blood neutrophil counts in healthy donors has rekindled interest in granulocyte transfusion therapy. We describe a 55-year old female patient with an acute myeloid leukaemia (AML) who underwent remission induction chemotherapy. In absence of repopulation of the peripheral blood the bone marrow showed persistent AML. Despite antifungal prophylaxis the patient developed an invasive pulmonary aspergilliosis. Voriconazol, G-CSF and caspofungin were added, and granulocyte infusions were started because of the continuing neutropenia. The granulocytes were collected from donors with a compatible blood group through leucapheresis after stimulation with G-CSF and dexamethasone (8mg), and then irradiated. The transfusions were given three times a week. Conditioning for a reduced intensity (RIC) allogeneic peripheral blood stem cell transplant (PBSCST) with a HLA-identical brother was started. In total 15 granulocyte infusions were given; of which 5 after transplantation, the last on day +10 after PBSCST. They were discontinued when the ANC count reached 1.0x10^9/l. All granulocyte infusions were tolerated very well. Four months after transplantation she is in a complete hematologic remission without signs of graft-versus-host-disease (GVHD), and without signs of active pulmonary infection. The precise role of donor granulocyte infusions remains to be delineated, partly because of the lack of defined clinical trials. We conclude that granulocyte transfusion therapy may be useful for neutropenia-related fungal infections in patients with hematologic malignancies. The use of granulocyte infusions during a RIC-PBSCST procedure does not seem to lead to an increased risk of GVHD or hamper engraftment.

R1102
Allogeneic haematopoietic stem cell transplant in patients with haematological diseases and previous invasive fungal infection
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Introduction: Patients with haematological diseases previously diagnosed with invasive fungal infection (IFI) are considered to be at high risk of suffering reactivation of the infection during subsequent intensive chemotherapy. Methods: In the last 2 years 3 patients with haematological diseases (2 AML and 1 acquired aplastic anaemia) and previous invasive aspergillosis have undergone allogeneic haematopoietic stem cell transplant in our centre. All patients received as primary antifungal therapy combination of liposomal amphotericon B (Ambisome) and caspofungin displaying complete or good partial radiological resolution of the infection. Itraconazole and voriconazol was continued as secondary prophylaxis. Conditioning regimen consisted of busulfan and cyclophosphamide in patients with AML and ATG and cyclophosphamide in the aplastic anaemia patient. Cyclosporine in combination or not with metothrexate was the regimen administered to prevent GHVD. Results: In a patient with AML no clinical or radiological signs of reactivation of fungal infection were observed through the transplant procedure. In the other patient with AML itraconazole was changed by liposomal amphotericon because of unresponsive fever. In the patient with aplastic anaemia combined therapy with amphotericon and caspofungin was initiated because of radiological worsening, but galactomannan antigen was negative in all analysis performed. No patient has died because of infectious complication during or after transplant. Conclusion: Availability of new antifungal agents does allow pre-transplant therapy of previous IFI, aiming to achieve a clinically undetectable state of infection, and an adequate antifungal treatment during transplant to diminish risk of reactivation of fungal infection in allografted patients. However, the optimal use of antifungal agents or their combinations remains to be determined and more studies are necessary to confirm our experience.

R1103
Successful treatment of primary invasive aspergillus of the bowel after autologous stem cell transplantation
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The frequency of invasive fungal infections, in particular infections due to Aspergillus and other moulds, has increased over the past two decades. Whereas invasive aspergillosis mainly involves the respiratory tract, lung or sinus, the lower gastrointestinal tract is rarely affected, most often in the frame of secondary dissemination. Primary invasive aspergillosis of the gut is a rare event associated with high mortality and has not been reported to date in patients after autologous stem cell transplantation (SCT). We report on a 10-year-old boy who developed isolated intestinal aspergillosis soon after autologous stem cell transplantation for PNET of the central nervous system. The boy received broad-spectrum antibiotics because of...
neutropenic fever, and antimicrobial prophylaxis included trimethoprim-sulfamethoxazole, metronidazole, acyclovir, fluconazole and topical amphotericin B. Antimycotic therapy was started because of persistent fever and abdominal pain, and rising serum levels of galactomannan and the isolation of Aspergillus fumigatus from the stool suggested invasive aspergillosis. The boy underwent enterostomy on day +19, and diagnosis of intestinal aspergillosis was pathohistologically confirmed. No other site of invasive aspergillosis was evident. The patient was treated with antimycotic combination therapy consisting of liposomal amphotericin B, voriconazole and caspofungin. The clinical condition slowly improved over the next months. Enterostomy was removed on day +120 and antimycotic treatment has been stopped soon after. Currently, on day +140, the boy is at home without major gastrointestinal complaints. We conclude from this case that primary intestinal invasive aspergillosis can occur in patients undergoing autologous stem cell transplantation, and therefore, this diagnosis has to be considered in this setting in patients suffering from fever and abdominal pain. In case of positive galactomannan antigenemia, an extensive search for invasive aspergillosis should be performed and, at the same time, early antifungal therapy should be started.

**R1104**

**Tuberculous arachnoiditis after a second allogeneic haematopoietic stem cell transplantation for acute lymphoblastic leukaemia in CR2**

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Mycobacterial disease is a rare and difficult diagnosis in hematopoietic transplant recipients. We report the case of a twenty-year-old woman who underwent a second Matched Unrelated Donor (MURD) HSCT for a null ALL in second complete remission. Conditioning regimen consisted on Cyclophosphamide 120 mg/msq; Busulfan 16 mg/Kg and Thiotepa 750mg/msq. On day +44, while receiving acyclovir prophylaxis, and a previous Cotrimoxazole prophylaxis, she developed an acute ascending paraparesis with anesthesia and reflex loss and loss of sphincter control. MRI imaging showed Gadolinium contrast enhancement on cauda equina roots. CSF analysis showed elevated cell count (116/mm³), Normal Range NR: 0-5/mm³), elevated CSF protein concentration (261mg/dL; NR: 1-40mg/dL), and no hygrocorticorcraquia (54mg/dL with simultaneous plasma concentration of 84mg/dL). Flow cytometry analysis showed 96% T lymphocytes, 0.09% non clonal B lymphocytes, 3.5% monocytes and 0.4% neutrophils. Bacterial, fungal and mycobacterium presence was ruled out and empirical antiviral treatment consisting on Foscarnet (180 mg /kg /day), immunoglobulin (0.5g/kg/48hours) was started. Methylprednisolone 1g per day for tree days flowed by 30mg per day for three consecutive weeks. No clinical response was observed and neurotrophic viral infection was excluded by PCR technique. A second lumbar puncture was performed 17 days later showing a lower number of lymphoid cells (10/mm³) but with an increase in protein concentration (321mg/dL) without hypogluccorraquia; moreover auramine positive rods were present. Antituberculous treatment with Rifampicin (600 mg/d), Isoniazide (300 mg/d), Ethambutol (2.5g/d) and Pyrazinamide (2g/d) were started, with no clinical improve but with an increase in protein concentration (321mg/dL). Flow cytometry analysis showed 96% T lymphocytes, 0.09% non clonal B lymphocytes, 3.5% monocytes and 0.4% neutrophils. We conclude from this case that primary intestinal invasive aspergillosis can occur in patients undergoing autologous stem cell transplantation, and therefore, this diagnosis has to be considered in this setting in patients suffering from fever and abdominal pain. In case of positive galactomannan antigenemia, an extensive search for invasive aspergillosis should be performed and, at the same time, early antifungal therapy should be started.

**R1105**

**Pentastomiasis of the liver in a patient following unrelated stem cell transplantation with estimated hepatic acute graft-versus-host disease**

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We report a case of human hepatic pentastomiasis (Armillifer armillatus) in a 22-year old man with AML who immigrated to Germany from Togo in 2002 and died as a result of relapse, 37 days post HLA-matched unrelated stem cell transplantation. At day +30 post transplantation, diarrhoea occurred and gastroscopy with excisional biopsy revealed an acute graft versus host disease of the stomach and upper small bowel. For treatment we started with a tapering schedule of methylprednisolone (5mg/kg body weight/day). Three days later we observed a hyperbilirubinemia and slightly increased liver enzymes. The abdominal ultrasound showed an increased size of the liver and an increased portal perfusion. The findings were interpreted as a hepatic involvement of the acute Graft versus Host disease (aGvHD) and a salavage treatment with mycophenolate motefil was initiated. The patient died as a consequence of intracerebral bleeding in a cerebral infiltrate of the leukaemia. At autopsy, in addition to the cerebral findings, we found multiple pentastomides (documented by photographs) up to 3cm length (spec. Armillifer armillatus) subcapsular, in the parenchymatous tissue and in the Portal veins. No aGvHD-like inflammatory infiltrate in the liver was observed.

**Late effects and quality of life**

**R1106**

**Trilineage hypoplasia after initial neutrophil engraftment in patients with acute myeloid leukaemia undergoing autologous transplantation with Bu/Cy conditioning:**

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Myeloablative conditioning with autologous stem cell transplantation (ASCT) is a treatment option for AML patients with lack of sibling donor. Hematopoietic engraftment may be prolonged depending on the type of myeloablative regimen, mobilization regimen, dose of MNC and patients age. In a 5 year period (2000-2005) we realized a total of 9 autologous transplantsations with cryopreserved peripheral blood cells (PBSC) and Busulfan and Cyclophosphamide conditioning. We present two cases (22%) of AML (standard
Combination of rabbit antithymocyte globulin (ATG Fresenius) and cyclosporine A (CsA) with short term methotrexate were used. Due to renal toxicity CsA was early switched to tacrolimus and corticosteroids were started at day+22. Week after she developed steroid resistant acute GVHD grade IV with skin and gut involvement. For that she was successfully treated with combination of steroids, mycophenolate mofetil and sirolimus (day+27 through day+71). During period of acute GVHD severe keratoconjunctivitis sicca with deep corneal defects has been developing and visual acuity had deteriorated. There were increasing pannus and calcified deposits (calcareous degeneration). Serum calcium and phosphate levels were normal at several times points. Long-term immunosuppression improved symptoms of GVHD but healing of corneal defects was not reached despite local therapy of antibiotics and corticosteroids. At day+133 patient underwent removal of calcium deposits and both eyes were covered by amniotic membrane. At day+303 keratoplasty in the left eye was performed with transient improvement of visual acuity. Two weeks after keratoplasty calcified plaques have recurred in the transplanted tissue and fast visual loss reappeared. We suggest that ectopic corneal calcifications are probably associated with persistent epithelial and stromal defects and keratoconjunctivitis sicca as a symptom of persistent active chronic GVHD. Combined immunosuppressive therapy should therefore continue. We plan to repeat surgery after that period under better control of GVHD. Patient is now one year after SCT with no signs of leukaemia relapse with full donor hematopoiesis, but prognosis of vision still remains at this moment uncertain. Supported by VZ FNM MZO 00064203

R1107
Neurological long-term follow-up after allogeneic bone marrow transplantation
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To improve our knowledge about the neurological outcome after allogeneic bone marrow transplantation (bmt), we have started a prospective study already 6 years ago. So far we could show that within the first year after transplantation a significant proportion of patients (65%) had developed neurological sequelae. Besides well-defined neurological complications more than half of the study population suffered from new neurological abnormalities of unknown origin predominantly affecting the peripheral nervous system. In a small subgroup of patients already central nervous signs, cognitive deficits and white matter lesions could be detected and this was in relation to chronic graft-versus-host disease (GVHD)/immunosuppression. To determine whether central nervous system (CNS) involvement during GVHD might manifest at a later time in more bone marrow recipients, the following patient - a 47 year old female patient - was included. The patient received a neurological examination, underwent a neuropsychological test battery and standard MRI sequences. Long-term follow-up results will increase the insight onto the spectrum, incidence and etiology of neurological sequelae after allogeneic bmt. They will be discussed in relation to retrospective and experimental data, which suggest involvement of the CNS during GVHD.

R1108
Keratoconjunctivitis sicca with recurrent calcium deposition in the cornea and severe visual loss due to GVHD
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We present severe keratoconjunctivitis sicca with recurrent calcium deposition in the cornea after keratoplasty in a patient with extensive chronic graft versus host disease (GVHD). A 17-years-old girl with myelodysplastic syndrome (RAEB) underwent stem cell transplantation (SCT) using peripheral blood stem cells of two alleles mismatched unrelated donor (B, Cw). Conditioning regimen consisted of busulfan, cyclophosphamide and melphalan, for GVHD prophylaxis

risk) patients (two females 48 and 40 years at transplant) that underwent autologous transplantation at Department of hematology, Skojo. Mobilisation of PBSC was preformed with VP-16 2000 mg/msq + G-CSF 10µgr/kg in one patient and HD-ARAC 3gr/mscq3, Idarubicine 10mg/msq+G-CSF 10µgr/kg in other two patients. A minimum of 2.0x10^9/kg MNC and 3.0x10^9/kgMNC respectively were collected and preserved in 5% DMSO solution. We registered engraftment for Ne>0,5x10^9/L on day +11 and +13 and for Plt>20x10^9/L on day +13 and +16. Patient were followed up in outpatients and pancytopenia was registered one month after transplant with Plt<20 x10^9/L with mild haemorrhagic complications Plt transfusions dependence, Hb < 80g/L at day +11 and +13 due to transfusion dependence (on two week interval), WBC < 3.0 x10^9/L with cytokine treatment (twice a week G-CSF 375 µgr/kg). Bone marrow biopsy revealed trilineage hypoplasia, no signs of organomegaly, no microbiological and viral findings. One patient has recovered completely 15 months after transplantation and the other is still in good physical condition but present pancytopenia 12 months posttransplant. We conclude that prolonged pancytopenia is due to Bu/Cy conditioning, the age could be a significant factor for starting myeloablative conditioning prior autologous transplant as well as minimum MNC dose could prolong immune reconstitution.

R1109
Hashimoto encephalitis after allogeneic stem cell transplantation
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Thyroid dysfunction is an important problem in patients receiving bone marrow transplantation. However there was no case of Hashimoto encephalitis in a patient after stem cell transplantation. We describe the case of 47 year old female patient who underwent allogeneic bone marrow transplantation and who developed Hashimoto encephalitis. AML patient was subjected to allogeneic stem cell transplant as reduced intensity conditioning (FLAG-Ida) in October 2002. Early posttransplant period was complicated by reactivation of CMV infection and prolonged peripheral cytopenia. 24 months after transplant the patient chimerism analysis revealed graft rejection without AML relapse. 32 months after transplantation the patient developed fatigue, loss of appetite, vomiting but also psychiatric symptoms: hallucinations and paranoid ideations. CT scans does not revealed anything specific. Typical psychiatric treatment was not efficient. Later patient experienced episodes of epilepsy and developed cerebellar ataxia and progressive unilateral paresis with impaired consciousness. In accessory investigations – in CSF elevated level of proteins and EEG abnormalities were noted, blood and CSF cultures were negative, thyroid hormones level were slightly decreased, TSH and ATPO antibodies titers were elevated. Results of MRI confirmed disseminated pathologic changes in white matter. Hashimoto disease with encephalitis was diagnosed and the patient was treated with high dose Methylprednisolone i.v. for 5 days with rapid improvement noticed within first 48 h of the treatment. The neurological state normalize within one week. Maintenance treatment with decreasing dose of oral Prednisone is carried on. Occurrence of Hashimoto encephalitis in described patients seems to be connected with chronic graft rejection process and impaired prolonged regeneration of immunity after transplant. The role of different infections mainly viral should also be investigated.
Purpose: BMT improvement must take in consideration ethical issues regarding donors. Based on donors refusal cases report, our analysis underlines the significance of identifying «unwilling donors». Moreover, we are concerned about the process of obtaining informed consent of family members to undergo HLA histocompatibility tests in order to prevent psychological consequences of peripheral blood and bone marrow donation.

Background: Studies about psychological issues of bone marrow donation show that donors may be worried about their health status (Switzer et al., 1997; Molassiotis et Holroyd, 1999). Switzer et al. correlate donor difficulties to their hesitation during donation decision process and consider that screening donors motivations is useful to a psychoprophylaxis approach.

Other authors note that families donors are even more exposed to psychological problems (Chang et al, 1998). Directly concerned by recipient reactions to BMT, family donors (Wolcott et al, 1986) may feel unconscious guilt in cases of unsuccessful or complicated BMT (Futterman and Wallisch, 1990). Deeper psychodynamics of BMT process indicates that donor can confuse biological (HLA) and psychological identity and be anxious about being ill. (Alby, 1988, 1990; Ascher, 1994, 2004). Topall-Rabanes et al. (2000) notice that about 20% of 202 studied family donors are classed as «reluctant»: for these subjects, donation decision is not considered as a real choice. They suffer from health problems and feelings of regret even long time after donation.

Methods and Results: We reviewed the records of donor refusal cases representing 2% of 400 allografts performed in our twenty years BMT practice. In-depth analysis of patients psychosocial situation, quality of social support, family members relationships, patient-family-staff communications modalities, in particular regarding to donation information process.

We will present these cases and retrospective analysis to introduce ethical questions on HLA histocompatibility testing. Conclusion: Regarding to our experience and to our data, it seems that information process of family members about bone marrow donation may lead to an impossible choice. Appointed by HLA testing as donor, family member is moved to suffer of biological determination and to become «unwilling donor». Protecting these persons is a medical responsibility. More research is necessary on this subject.

R1111

Paranoiaca reactions in patients with haematological malignancies after bone marrow transplantation

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Background: Paranoiaca reactions (PR) are the reactive states with domination of the overvalued ideas or delusion disorders (sensitive, litigious, invention and so on). PR occurred not so often in the course of BMT but there are the most disturbing for hematologists among the all patients with personality disorders.

Aims: To specify the typology of paranoiaca reactions and development of its treatment at the patients with hematological malignancies after BMT.

Methods: It was surveyed by the clinical method 104 patients received BMT in 2001-2004 in National Research Center for Hematology, Moscow, for the following hematological malignances: AML (n = 21), ALL (n = 8), CML (n = 21), HD (n = 9), MDS (n = 10), MM (n = 17), NHL (n = 15), ST (n = 3). There are 14 patients with paranoiaca reactions (persecution, sensitive, litigious, invention) were possible to allocate among the mental disorders at our patients.

Results: Paranoiaca reactions with ideas of persecution (n = 7) are the most often in our sample. In such cases patients suspect those around them, especially medical personnel, in preconceived attitude to them and, possibly, to conspire for damnification of patients. Such ideas have a systematic character and are resistant for treatment.

Sensitive paranoiaca reactions (n = 2) are joined with perception of physical handicap and characterized by sensation of slighting attitude and mockery those around patient and by confidence in dissemination of detractive rumours.

Ligitive paranoiaca reactions (n = 4) are characterized by low-systematized and insufficiently well-founded requests and multiple joined with hematological malignancies such as requests to compensate the prejudice applied by disease or laying claims to medical personnel are at fault, in opinion of patient, in the prejudices joined with disease.

Invention paranoiaca reactions (n = 1) in our sample are characterized by elaboration of self-treatment methods of his/her disease.

Treatment of examined states was significantly resistant and included antipsychotic and anxiolitic medications.

Conclusions: PR at the patients with hemato-oncological disorders after bone marrow transplantation are the separate problem demanding the special attention both the hematologist and the psychiatrist, especially relative to its treatment.

R1112

Quality of life and symptoms in long-term survivors of childhood blood cancer post bone marrow transplantation/ stem cell transplantation

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Background: Quality of life (QoL) is increasingly used as a treatment outcome along with traditional clinical outcomes in children with cancer undergoing bone marrow transplantation (BMT)/stem cell transplantation (SCT). The aim of our study was to estimate QoL parameters and symptoms in survivors of childhood blood cancer after BMT/SCT.

Patients and Methods: Fifteen survivors were evaluated 1-13 years (median, 3 years) after allogeneic BMT/SCT for acute leukemia (12), chronic leukemia (2) and myelodysplastic syndrome (1). Median age at transplantation – 13 yrs (range 1.5–21.0), girls/boys–10/5. PedsQL\textsuperscript{TM} Generic Core Scales and SF-36 questionnaires were used for QoL assessment in the group younger than 18 yrs at the time of the survey and in the group 18 yrs and older, respectively. NJ Children Cancer Symptom Inventory and MD Anderson Symptom Inventory were used for symptom assessment in the younger and older groups, respectively. For comparison 56 healthy controls (20 – for younger group; 36 – for older group) matched to survivors by age and gender were included in the study.

Results: No significant differences in QoL parameters (physical, psychological and social functioning) between survivors and control group were revealed. Only School Functioning for children younger than 18 yrs was lower in the group of survivors (61 vs 77, p<0.05). Seven survivors experienced moderate or severe symptoms (5 to 10 scores on 0-10 scale). Four of them had pronounced psychological symptoms. Other pronounced symptoms were chronic pain, fatigue, lack of appetite, shortness of breath, drowsiness and nausea. Six survivors had at least two moderate or severe symptoms.

Conclusions: QoL parameters, namely, physical, psychological and social functioning in long-term survivors of childhood blood cancer post allogeneic BMT/SCT is comparable to healthy people. However, nearly half of
survivors experience different symptoms in long-term period after transplantation. This confirms the importance to monitor and control late related symptoms in order to preserve QoL of long-term survivors of childhood blood cancer.

R1113 Differential diagnosis of T-cell lymphoproliferative disease after allogeneic haematopoietic progenitor cell transplant
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Two cases are presented of a rapidly developing cervical lymphadenopathy 19 days after allogeneic peripheral blood stem cell transplant for acute myeloid leukemia. Patient #1, a 57 year-old female, developed painful bilateral cervical adenopathy (up to 2 cm) overnight, fever (39.2°C), and a fine maculopapulous rash. No further enlarged lymph nodes were found by computed tomography, and the right cervical mass was removed. Histology showed infiltration of the lymph node by atypical lymphoid cells that expressed CD3 and CD4. Molecular analysis revealed monoclonal rearranged TCR gamma chains. Histology of the skin showed a leukocytoclastic vasculitis without any signs of acute GVHD. EBV PCR was consistently negative in peripheral blood and lymph node. The diagnosis of T-PTLD was made, and the patient received a 10-day course of methylprednisolone. With this regimen, the patient’s condition rapidly improved, and the fever, lymphadenopathy, and rash resolved completely within a few days. Patient #2, a 46 year-old female, developed painful acral bullous erythematosus lesions on her mouth on day 17 post transplant which progressed to generalized maculopapulous rash. Histology showed acute graft versus host disease (GVHD) of the skin. On day +19, the patient presented with painful bilateral cervical adenopathy (up to 1.8 cm), and abdominal ultrasound and MRI showed no other regions of lymphadenopathy. Histology of one removed lymph node demonstrated a highly proliferating, diffuse infiltrate of CD3 positive T cells with 60% of T cells expressing CD4 and 40% expressing CD8, respectively, as well as scattered CD20 positive cells. Molecular analysis detected a polyclonal pattern of TCR rearrangement and an oligoclonal immunoglobulin receptor rearrangement. EBV PCR was negative in peripheral blood and lymph node. The clinical scenario was interpreted as concomitant lymphadenopathy associated with acute GVHD, and the patient received methylprednisolone for 10 days and completely recovered. This report highlights the necessity to remove suspiciously enlarged lymph nodes, developing after allogeneic transplant, in order to distinguish between T-PTLD and lymphadenopathy accompanying acute GVHD.

Minimal residual disease

R1114 The evaluation of T-cell receptor gamma gene expression for prognosis of relapse development after stem cell transplantation
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Background: The hematopoetic stem cells transplantation (HSCT) in patients with hematological disorders is the most radical method of the therapy. The early diagnostics of relapse in post-HSCT period could improve significantly the therapy effectiveness. The purpose of this study was to develop the minimal residual tumor cells detection method by studying the mediated mechanisms of organism’s reaction to tumor clone. Materials and methods: Nineteen patients with different hematological malignancies, who were undergone the HSCT, have been included in the research. Total mRNA was extracted from peripheral blood leukocytes, sampling in the time of conditioning regime completion just prior to stem cells transfusion. We performed the RT-PCR with primers specific to V-J-gamma junctions (TCR-gamma gene). Specific signal was detected in 2% agarose gel.

Results: Absence of TCR-gamma gene expression at Day-0 were significantly more often in group of the patients who were staying in remission after HSCT (p=0.013). In group of the patients with myeloablative pre-transplant conditioning the significantly differences in TCR-gamma gene expression depending on the fact of relapse has not been revealed. In group of the patients with reduce intensity conditioning regime the positive correlation between the presence of TCR-gamma gene expression at Day-0 and relapse in post-HSCT period was observed (p=0.047).

Conclusion: The definition of TCR-gamma gene expression before hematopoetic stem cells infusions is the method of a tumor process activity estimation during the therapy, especially in case of using the reduce intensity conditioning regime. We suggest this criterion as the early prognostic factor for the relapse developing in post-HSCT.

R1115 MRD-directed adoptive immunotherapy following allogeneic stem cell transplantation fails to permanently revert disease progression in childhood acute lymphoblastic leukaemia
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Between VIII/2000 and VII/2004 we performed allogeneic haematopoietic stem cell transplantation (HSCT) in consecutively 36 children with acute lymphoblastic leukaemia (ALL). We analysed pre- and post-transplant minimal residual disease (MRD) levels using quantitative RQ-PCR targeted to immunoglobulin and/or T-cell receptor rearrangements in 25 of them with available targets (with adequate sensitivity and specificity according to ESG-MRD-ALL criteria). Seven of patients with detectable MRD prior HSCT (n=8) relapsed after transplant and one died in CCR before day +100 due to transplant related complications. In the group of pre-HSCT MRD negative patients (n=17), only one relapse appeared. In a total of 4 patients, there was a time-frame for an attempt to avert the disease progression detected by RQ-PCR. In one BCR/ABL+ patient, imatinib mesylate dose was increased and 3 doses of DLI (5.7x10^7/kg CD3+ cell) were administered. Despite this, patient relapsed +690 days after HSCT. Second BCR/ABL+ patient developed molecular relapse despite chronic GVHD. Therefore, immunosuppression was quickly discontinued and imatinib mesylate was administered. He achieved temporary molecular remission but 6 months later died of CNS disease progression. Third patient developed MRD positivity +60 days after HSCT and therefore immunosuppression was quickly tapered. GVHD reactivation required steroid and CsA treatment. After GVHD resolution immunosuppression was ceased and three escalating doses of DLI (1x10^6, 1x10^7, 1x10^8 CD3+ kg) were given. Nevertheless, this did not avert haematological relapse. This patient after high-dose chemotherapy achieved second complete remission (CR) with MRD negativity prior to second HSCT and now is 11 months after HSCT in continuous CR and MRD-negative. The fourth patient (BCR/ABL+) developed relapse despite three DLIs and imatinib mesylate treatment given for positive MRD +90 after HSCT. Adoptive immunotherapy (rapid cessation of immunosuppression, infusion of DLI in 4 to 6 weeks interval and/or use of imatinib mesylate in BCR/ABL+ ALL) after the
transplantation was not successful in our cohort in relapse prevention and might only postpone the manifestation of relapse and facilitate further efficacious chemotherapy and re-transplantation. We are confident that all effort should be aimed to a better control of the pre-transplant MRD levels.

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R1116
The dynamics of chimerism evolution determines the differential outcome of various transplant settings
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Background: Several factors such as the intensity of the conditioning regimen, the T-cell content of the graft or the GVHD prophylaxis, influence the degree of chimerism after SCT. Objective: To evaluate the dynamics of chimerism after different SCT settings (ablative, reduced intensity conditioning -RIC- and T-cell depleted -TCD-) and its influence in the success of the procedure.

Patients and Methods: 68 SCT: 32 ablative (including 7 from MUD), 19 RIC and 17 TCD (including 8 from haploidentical donors and 2 from MUD with 1 HLA disparity). Chimerism analysis was performed by FISH or STR-PCR (sensitivity 1%). Samples: bone marrow (BM) and peripheral blood (PB) on days +30, +100, +180, +365 and once a year thereafter, as well as PB and leukocyte lineages (T lymphocytes CD3+, B lymphocytes CD19+ and myeloid cells CD15+ isolated (purity >95%) by immunomagnetic means. AutoMACS, Miltenyi Biotec), every 2 weeks, starting on day +15 and until complete chimerism (CC) was achieved. Results of chimerism follow-up were censored once the diagnosis of relapse or rejection was established.

Results: Incidence of mixed chimerism (MC) on day +30 (ablative: BM 27%, PB 15%; RIC: BM 40%, PB 41%, CD3 40%; TCD: BM 31%, PB 33%, CD3 54%), as well as its dynamics (MC on day +100: ablative BM 8%, PB 4%; RIC BM 8%, PB 15%, CD3 22%; TCD BM 20%, PB 40%, CD3 37%) were different in the three SCT settings. Moreover, the percentage of recipient cells (%R) was significantly higher after RIC and TCD than after ablative SCT, as well as in T lymphocytes than in BM or PB (7/8 cases with simultaneous studies showed MC in T lymphocytes and CC in PB). All RIC SCT evolved to CC by day +180 while TCD SCT showed persistent MC (2 patients with stable MC after one year). The incidence of rejection was greater after RIC (2/19) and TCD (4/17) than after ablative SCT (2/32). All these patients showed MC, mainly in T lymphocytes, which allowed early diagnosis and successful treatment with immunosuppression withdrawal and donor leukocyte infusion. Patients with CC in PB/T lymphocytes on day +30 had a higher incidence of GVHD≥II than those with MC. In the present series, however, a relationship between chimerism and relapse, disease free survival or overall survival, was not observed.

Conclusions: SCT with greater incidence of MC (RIC and TCD) favor immune tolerance between donor and recipient which reduces the risk/severity of GVHD at the expense of a higher incidence of graft rejection.

R1117
Reliable quantification of haematopoietic chimerism after allogeneic stem cell transplantation by real-time quantitative PCR analysis
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Introduction: Increasing mixed chimerism (MC) represents a poor proptic factor. after allogeneic stem cell transplantation (SCT). Moreover, to define the best timing of immune-suppression withdrawal and donor lymphocytes infusion, a strict monitoring of donor hemopoiesis is needed. Methods: We evaluated 18 donor/recipient pairs using a quantitative real-time PCR (qrt-PCR) with the aims 1) to evaluate the informativeness of this chimerism assay and 2) to compare qrt-PCR analysis with standard methods such as fluorescence in situ hybridization (FISH) for mismatched sex pairs or variable nucleotide tandem repeats (VNTR) for matched sex pairs. Qrt-PCR (LightCycler 2.0, Roche) was performed on bone marrow and peripheral blood samples collected monthly, using eleven biallelic DNA genetic system located on chromosomes 1, 6, 9, 11, 17, 18, 20, X and Y. Glyceraldehyde phosphate dehydrogenase (GAPDH) gene was used as active reference. Before quantification, donor and recipient DNAs were genotyped using primers and probes specific for all genetic markers. Patients had a median age of 43.5 years (range 26-70) and were affected by acute leukemia (n=12), or lymphoproliferative disorders (n=6). Standard regimen was used in 10 cases, reduced intensity conditioning in 4, while 4 patients underwent an unrelated SCT. Median follow-up of the 18 patients was 16.5 months (range 4.2-34.4). Results: Both qrt-PCR and FISH detected donor/recipient differences in 100% of pairs, while VNTR was not informative in 25% of sex matched pairs. Mixed chimerism was observed in 8/18 patients (44.4%) using qrt-PCR and in 3 of the 16 patients (18.7%) evaluable with standard methods. Overall, 5/18 patients (27.8%) relapsed; before relapse, mixed chimerism was observed in all patients by qrt-PCR and in 3/5 by FISH/VNTR. Qrt-PCR detected mixed chimerism 45 days (range 0-315) earlier than standard methods. In 2 cases in which VNTR was either not informative or not predictive for relapse, the interval between detection of mixed chimerism by qrt-PCR and relapse was 30 and 315 days, respectively.

Conclusions: chimerism determination using qrt-PCR is more informative than standard methods and may represent an useful tool for the follow up of allogeneic SCT.

Reduced-intensity transplants

R1118
Reduced intensity transplant programme. A single-centre experience
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From 2001 we introduced a reduced-intensity conditioning regimen for allogeneic transplants(alloc). This regimen is tailored for old patients (pts) or for those who have previously received an allo or autologous BMT. At now we have transplanted 15 pts; 2 of them received previously an autologous and 1 an allo BMT. They were 10 males and 5 females and the donors were 9 males and 6 females. The mean age of the pts was 50 yrs (range 22-68 yrs).The diagnoses were: 2 HD,7 NHL, 1 MDS, 2 Renal Cancer, 1 Sarcoma, 1 Myeloma and 1 CML; the 3 solid tumors were all metastatic, 4 pts were in PR, 5 in II CR, 1 in III CR and the one with MDS at the onset. The ABO compatibility
was:complete for 8, minor for 2 and major for 5. The CMV status was: donor/pt positive for 13 and positive donor/negative pt for 2. The conditioning regimen consisted of Thiopeta, Fludarabine and Cyclophosphamide except for the pt with CML who received Busulphan and Fludarabine. The source of stem cells was peripheral blood in 14 and bone marrow in 1 and the stem cells were cryopreserved after the harvest. The GVHD prophylaxis consisted of CsA and short course MTX. The 14 pts who received peripheral stem cells the mean cell dose was 4x10^6/Kg CD34+ cells, for the one who received bone marrow was 2.9x10^6/Kg MNC. The mean time to reach PMNs >5x10^9/L and PLTs >20x10^9/L was respectively 15 days (range 13-20 days) and 24 days (range 15-55 days). The mean number of transfused RBC and PLT units was respectively 4 (range 0-8) and 4 (range 1-7). The mean grade of mucositis (according to the WHO classification) was 2. The major complications during neutropenia were 5 FUO, 5 gram + bacteremias and 2 cerebral aspergillosis. No cases of VOD of the liver were observed. 8 pts had aGVHD (grade 1), 7 grade 2 and 1 grade 3. Two pts developed CMV reactivation after allo; the complication was cured with Ganciclovir. Nine over 15 pts died: 4 for disease (the 3 solid tumors but for the 2 with renal cancer a response even if transient was observed and 1 NHL), 1 for acute GVHD and 2 for cerebral aspergillosis. The chimerism was complete for all the evaluable pts at 30 days. None needed DLI. Six patients are in CR with a median follow-up of 2 year (2 weeks in patient 1 and 11 chronic phase and 1 in ii chronic phase) and 1 relapsed. This pilot study demonstrated an acceptable regimen-related toxicity (according to the age and previous chemotherapy including transplants), the possibility to reach a very early full-donor chimerism and to cure high risk pts.

R1119
Reduced-intensity allogeneic stem cell transplantation in relapsed and refractory Hodgkin’s disease
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Introduction: Autologous stem cell transplantation (autoSCT) is usually preferred to alloSCT, due to its widespread availability, lack of the immunological problems intrinsic to the development of graft-versus-host disease (GVHD), and the infrequent bone marrow involvement present in Hodgkin’s disease (HD), for patients undergoing high-dose chemotherapy/radiotherapy. Allogeneic SCT has been associated with a high transplant-related mortality (TRM) in patients with HD due to a high incidence of GVHD and of fatal infectious events after transplantation. The poor outcome of these patients after alloSCT may reflect in part the advanced status of the disease at transplantation and the poor performance status of the patient population allografted. Furthermore, the high TRM present in the conventional alloSCT setting has never allowed a proper evaluation of a possible graft-versus-Hodgkin’s effect. In an effort to reduce the TRM associated with alloSCT, low-intensity regimens have been developed; the curative potential of these protocols would rely on the graft-versus-leukemia effect of the allogeneic infusion more than in the conditioning regimen per se. Case: In our hospital a total of 3 patients with relapsed Hodgkin’s disease underwent reduced-intensity conditioning (RIC) allogeneic stem cell transplantation (alloSCT) from an HLA-identical sibling. We explored reduced-intensity allografts using Fludarabine-Melphalan conditioning and early withdrawal of immunosuppression as an alternative to palliative chemotherapy. Graft-versus-host disease (GVHD) prophylaxis was mini-methotrexate and ciclosporine with the curative potential of these protocols would rely on the graft-versus-leukemia effect of the allogeneic infusion more than in the conditioning regimen per se. Conclusion: Although the number of HD patients allografted with reduced-intensity protocols is low and the follow-up still short, it seems that the reduced-intensity allogeneic stem cell transplantation is effective in relapsed and refractory Hodgkin’s disease where autografts have failed.

R1120
Remarkable reduction of acute GVHD and infectious complications after reduced-intensity conditioning and low-dose (30 mg) campath-1H
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During the four month-period from May, 2005 to September, 2005 we performed reduced intensity (RIC) allogeneic transplantation for five patients with their HLA indentical sibling donors. The median age of the 3 female and 2 male patients was 40.7 years (32.8-57.1). Two patients had chronic lymphocytic leukemia (CLL), one Hodgkin’s disease (HD), one follicular NHL grade I (FL) and one myelodysplasic syndrome (MDS). The HD patient allografted after previous autologous stem cell transplant. The FL patient was in complete remission, the others were in partial remission before transplant with low tumour burden. The conditioning regimen consisted of 1x30mg Campath-1H and Fludarabine 5x30 mg/m² for all patients, adding Melphalan (140 mg/m²) for the lymphoid malignancies, and Busulphan (8mg/kg) for the MDS patient. For GVHD prophylaxis 3mg/kg cyclosporin A (continuously) +8mg/kg methyltrexate was given on days 1, 3, 6. All patients engrafted. One patient developed grade I acute GVHD. Two patients had febrile neutropenia, one developed central venous line infection. No one had CMV reactivation or disease. After a median of 173 day-follow up (100-180 days) all patients developed full donor chimera tested by VNTR PCR. The two CLL patients are in remission proven by flow cytometry (less than 1% CD19/CD5+ cells). The FL patient is in CR and received four courses of Mabthera (375mg/m²) as maintenance therapy. The MDS patient is in CR according to BM histology, but still thrombocytopenic (60 G/L). The Hodgkin’s patient has active disease with minimal tumor burden with mixed chimerism at day 100, now waiting for DLI. Conclusion: low dose (30 mg) Campath-1H + CSA/MTX GVHD prophylaxis is a well balanced regimen regarding the incidence and severity of acute GVHD, infectious complications and GVL effect after RIC conditioning. These preliminary results - especially concerning the late infections complications - compare much better to those we observed in our previous series of RIC transplants with higher doses (90 or 100 mg) of Campath-1H, or that we read in the literature. With the publication of these preliminary results we would like to underline the message that less is better regarding Campath-1H in reduced intensity conditioning.

R1121
Fludarabine-based reduced-intensity conditioning for allogeneic transplantation in children with malignant and non-malignant diseases
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Fludarabine-based reduced-intensity conditioning (RIC) was analyzed in 40 children with malignant (n=31) and non-malignant diseases (n=9) between May 2001 and June 2005. Conditioning regimens consisted of fludarabine 30 mg/m²/day x 5 days plus melphalan 140 mg/m²/day x 1 day (n=25) or plus oral busulphan 4 mg/Kg/day x 2 days (n=11) or plus
cyclophosphamide 60 mg/kg/day x 2 days and globuline antithymocyte 2.5 mg/kg/day x 2 days (n=4). The patients
were grafted with bone marrow (n=7), cord blood (n=4) or PBSC either unmanipulated (n=6) or CD34+ selection (n=20)
or CD3/CD19 depletion (n=3). GVHD prophylaxis was performed with CsA + Mtx (n=29), CsA only (n=7) and CsA +
steroids (n=4). Donors were either related (n=25) or unrelated (n=15). The median number of CD34+ cells infused was
5.75x10^6/kg recipient bw (range 0.25-47.7).

Results: There were a rapid recovery of neutrophils (median 13 days; range 5-29) and platelets (median 15 days; range 5-
56). The median length of hospital stay was 17 days ( range 9-77). With a median follow-up of 10 months (range 3-40) the
incidence of aGVHD and cGVHD were 16±6% and 24±8% respectively. The probability of TRM was 10±5%. Patients
grafted with manipulated PBSC had the lowest TRM (5±3%). The relapse incidence was 35±11%. High number of infused
CD34+ cells (p=0.066) and cGVHD (p=0.01) was associated respectively. The probability of TRM was 10±5%. Patients
died of relapse or progressive disease (n=5), aGVHD with a lesser RI. The event-free survival was 57±10%.

Objectives: Neutropenia is a major risk factor for early transplant related mortality in children undergoing
haematopoietic stem cell transplantation (HSCT). In case of infection, refractory to antibiotic therapy the combination with
allogeneic granulocyte transfusions is a logical approach to manage this problem. These patients are chimeras of at least
declared three different cell populations (recipient – stem cell donor – granulocyte donor). Two patients where followed closely by
single nucleotide polymorphism (SNP) analysis to reveal the duration and percentage of leukocytes derived from
allogeneic donors.

Patients and methods: One patient suffering from Evans syndrome and was transplanted with cord blood of a MMUD
and adequate dosing of cytoreductive and immune suppressive treatment appeared critical for rapid trilineage engraftment.

Conclusion: Fludarabine-based RIC provide a good alternative to myeloablative conditioning for allogeneic transplantation
either malignant or non-malignant disease in children.

R1122 Reduction of treatment-related mortality in non-
myeloablative allogeneic transplantation
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Introduction: Treatment related mortality is the trade off of allogeneic transplants. Although the probability of TRM has been reduced to 15-25% following non-myeloablative (reduced intensity conditioning) allogeneic transplant it remains a barrier to administer these transplants. We desired to identify mechanisms that compromise safety or would enhance safety.

Methods: The different outcome parameters in transplantation were defined as safety or efficacy parameter and per safety and efficacy parameter the literature was searched for investigational method, prophylactic, supportive or therapeutic measurement. Furthermore the toxicity scales were reviewed and known side effects were listed and include in the search.

Results: Outcome parameters defined as safety parameter included engraftment, disease recurrence; side effects of major concern, acute and chronic graft versus host disease, infection, death as well as life threatening side effects defined by the safety scales. More that first complete remission was considered and adverse safety parameter.

1) engraftment failure was noted after inadequate cytoreductive treatment such as 2 cGy TBI and cyclophosphamide or underdosing of fludarabine (<90 mg/m²); adequate dosing of cytoreductive and immune suppressive treatment appeared critical for rapid trilineage engraftment.

2) TBI appeared associated with higher risk of graft versus host disease and conditioning with drugs only.

3) In vivo T-cell depletion by f.e anti-thymocyte globulin was associated with high risk of cytomegalvi reactivation.

4) T-cell depletion of the graft is associated with increases risk of relapse

5) B-cell depletion of the graft seems to reduce the risk of chronic graft versus host disease

6) CMV positivity is not a prerequisite for CMV reactivation

7) A combination of immune suppressive agent reduces the risk of acute graft versus host disease

Discussion: Based on these and less stringent criteria we defined a practice guideline and make recommendations for reduction of side effects and treatment related mortality. The method developed model will be presented.

R1123 Monitoring of mixed chimerism by single nucleotide polymorphism analysis in two children supported with granulocyte transfusions after allogeneic stem cell transplantation
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Introduction: Allogeneic stem cell transplantation (Allo-SCT) is the only curative treatment for most patients with high risk acute myeloid leukaemia (AML)/myelodysplastic syndrome (MDS) through a graft vs. leukemia effect. The development of RICs allow the use of this strategy in older patients who were not candidates due to high toxicity with conventional regimens.

Patients and Methods: In this study we included all patients with AMD/MDS who received a Reduced-intensity allogeneic stem cell transplantation (RIC ALLO-SCT) fromApril/1999 to April/2005. There were 29 patients (12 women), median age 51 years (55-71). Sixteen patients had AML (5 advanced phase) and 13 MDS (8 advanced phase) Conditioning regimen consisted in fludarabine 150 mg/m² and busulfan 8-
10 mg/kg in 27 patients and other in 2 patients. Graft versus host disease prophylaxis consisted in cyclosporin in all
patients with methotrexate in 17 patients or mycophenolate mofetil in 12 patients.

Results: After a median follow up of 455 days (46-1540) 15 patients are alive and without disease. One year overall survival and disease free survival (DFS) is 60% (50-70%) and 50% (40-60%), respectively. Transplant related mortality (TRM) was the cause of death in 7 patients, 3 months and one year cumulative incidence (CI) of TRM were 11 % (4-31%) and 22 % (10-49%), respectively. Eight patients relapsed (median time: 184 days (75-593); one year CI 23% (12-47%).

Twelve patients developed acute graft versus host disease (aGVHD); CI 39% (24-64%). Fourteen of 24 evaluable patients developed chronic GVHD; CI 67% (50-90). The development of cGVHD was associated with better DFS: 74% (63-88%) vs. 25% (5-46%) P=0.04.

Conclusion: RIC ALLO-SCT is a curative option in patients with advanced age diagnosed of AMD/MDS with an acceptable TRM. Age should not be an exclusion criteria in patients with AML/MDS for ALLO-SCT. cGVHD is associated with better DFS.

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R1125
Treosulfan/fludarabine conditioning for allogeneic haematopoietic stem cell transplant in patients with haematologic malignancies

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Allogeneic stem cell transplant (allo HSCT) is a curative approach for patients with hematologic malignancies. However, it is associated with high treatment related morbidity and mortality. Because transplant related mortality increases with advanced age, advanced disease and unrelated donors, patients older than 50-55 years may be excluded from this procedure. Reduced intensity conditioning and new preparative regimens are therefore explored to allow HSCT to a wider patient population. The aim of this study was to evaluate efficacy and toxicity of the combination Treosulfan (water soluble alkylating agent, Busulphan derivative) and Fludarabine as preparative regimen for allo-HSCT in patients receiving match sibling or unrelated donors for advanced heavily pretreated hematologic malignancies. Since July 2005 to November 2005 8 patients (3 ALL, 3 AML, 1 CML, 1 MM ) entered this study. Mean age was 41 years (range 22-61). Conditioning consisted of Treosulfan 12 gr/m² for 3 days, Fludarabine 30 mg/m² for 5 days, Cyclosporine plus short MTX and anti-Thymocyte globulin (Thymoglobulin) at a total dose of 6 mg /kg. All patients engrafted; mean time to neutrophil recovery ≥500 x10^9/L was 14 (range 11-18) days , to platelets ≥20000x10^9/L was 16 ( range 12-25) days. No conditioning regimen related deaths was observed. Three (3) patients experienced GI toxicity (2 grade 1, 1 grade 4), 2 patients had grade 2 liver toxicity. No acute GVHD was observed. All patients are alive with a follow-up ranging from 20 to 120 days. Despite the short follow-up, in this preliminary report we underline that Treosulfan-Fludarabine –ATG conditioning is characterized by reduced toxicity; long term follow-up is necessary to evaluate OS, DFS and relapse in these patients.

R1126
Successful salvage of acute myeloid leukaemia relapsing early post-allogeneic stem cell transplant with reduced-intensity conditioning and second allografting

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Background: Prognosis of patients with AML relapsing within a year of allografting is poor. Management options are limited and response to donor lymphocyte infusion(DLI) is poor due to disease kinetics. Second allografting using conventional conditioning is unpopular due to high transplant related mortality(TRM). There is paucity of data on second allografting with reduced intensity conditioning(RIC) as a salvage for relapses post-allografting. Herein, we report 2 such patients who were successfully salvaged and achieved a durable complete remission(CR) with RIC and second allografting.

Case 1: A 16 year old male with normo-cytogenetics AML received an allograft from a HLA identical sibling after myeloablative conditioning. He relapsed 5 months after transplant with loss of donor chimerism. He was salvaged with FLAG regime followed by serial DLIs and achieved CR2 which lasted 3 months before relapsing again. He was reinduced with FLAG regime followed by PBSC infusion from the original donor. He received no post-transplant immunosuppression with the development of grade 1 graft versus host disease(GVHD). He has since achieved 100% donor chimerism and remains in CR3 for more than 12 months.

Case 2: A 55 year old male with myelodysplasia transforming into AML received DLI(20q) and his first allograft from a HLA identical sibling after non-myeloablative conditioning. Donor chimerism declined after 3 months and blast counts continued to rise despite serial DLIs. He was reinduced with idarubicin and cytarabine, followed by a second allografting from the original donor with RIC (fludarabine 25 mg/m² 3-7 to -4, melphalan 100 mg/m² 3-3 days). Donor chimerism ensued and he remains in CR for more than 12 months. Immune suppression was tapered with development of grade II GVHD.

Conclusion: Remission durations after the second allografting exceeded those after the first. A RIC regime with second allografting is a more effective modality than DLI in the case of relapse. A RIC regime is less toxic associated with second transplants, while allowing for engraftment of infused PBSCs with execution of GVL effect. Immune modulation by aggressive manipulation of post-transplant immune suppression also appears to be a key element in successful second allografting. This salvage approach offers a practical, well-tolerated and potentially curative treatment for patients who in most circumstances, would have been precluded from further active management.
transfused beyond day +100 after allo-SCT. When comparing these 55 patients, to the group of 35 patients who were never transfused, platelets count prior to RIC allo-SCT (median count 130.000/L vs. 161.000/L; P=0.07) and the occurrence of severe acute GVHD (P=0.0001; 100% of patients with grade 3-4 acute GVHD were transfused) were the parameters significantly associated with platelets transfusion needs. In this cohort, 69 pts could be assessed for platelets recovery at day +100: among them, 58 (84%) had a platelet count >50.000/L. At day +100 after allo-SCT, a diagnosis of myeloid malignancy (AML, CML or MDS) was associated with a delayed platelet recovery (P=0.03).

Overall, these observations show a significantly lower rate of platelets transfusions and a quicker kinetics of platelets recovery after RIC allo-SCT. In addition, the low level of myeloablation observed after RIC, may offer a window of opportunity for testing of megakaryocytic growth factors, towards further improving the safety and outcome of RIC or nonmyeloablative allo-SCT.

Paediatric issues

R1128
Successful unmanipulated allogeneic PBSCT from matched unrelated donor in an 8-year-old boy with previously diagnosed pulmonary aspergillosis: case report
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Fungal infections have become the major cause of infectious morbidity and mortality in patients undergoing bone marrow transplantation (BMT). In many cases invasive aspergillosis infections create a major therapeutic dilemma and contraindication to marrow transplantation. We report on a 8 years old boy with secondary acute myeloid leukemia, who underwent unrelated donor peripheral blood stem cell transplantation (PBSC) with previously diagnosed pulmonary aspergillosis and successfully recovered from the infection. Probable invasive pulmonary aspergillosis (IPA) was diagnosed in the patient acc. to EORTC-criteria. A large diffuse wedge-shaped infiltration was observed in thorax CT scans two months before PBSCT and throughout the early posttransplant period. Liposomal amphotericin B (L AmB) 5mg/kg/d i.v. and voriconazole p.o. 2 x 4-6 mg/kg/d were administered for the whole peritransplant period. After conditioning regimen incl. Treosulfan 3x14mg/m², Melphalan 100mg/m², ATG Fresenius 4x5mg/kg the patient received PBSC (14.4 x 106 cells CD34+/kg recipient bw) from the unrelated donor, who was mismatched at 1 A*- allel. CsA, MTX and ATG were used as GvHD prophylaxis. A rapid and sustained allogeneic engraftment (neutrophils > 0.5 G/L on day +11, thrombocytes > 50 G/L on day +19) was observed. The posttransplant period was uneventful except for 2 weeks long lasting exhausting morning cough and subacute breathing difficulties requiring passive oxygen therapy. Three weeks after PBSCT, bronchoscopy with broncho-alveolar lavage revealed no pathogens. Nevertheless it was decided to continue treatment with L AmB 5mg/kg every second day. In control thorax CT the infiltration was considerably smaller and no indication was found to perform an open lung biopsy. The patient received further treatment with voriconazole p.o. for months. Regularly performed thorax CTs revealed a continuous regression of pulmonary changes. The patient remains alive and well in CR 11 months from transplant without any pulmonary abnormalities, except for a slight thickening of the interlobar groove and is given itraconazole p.o. This report demonstrates that administration of full-dose antifungal therapy and shortening the neutropenia period due to peripheral blood stem cell transplantation allow the successful outcome, even in high-risk patients with previous aspergillosis.

R1129
Effective treatment of combined pulmonary complications in a girl with high-risk acute lymphoblastic leukaemia after allogenic haematopoietic stem cell transplantation
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Post-transplant pulmonary complications are not rare and their mortality is still very high. We present 11-old year girl with high-risk acute lymphoblastic leukemia after allogenic hematopoietic cell transplantation (HCT) from matched related sibling donor. Conditioning regimen consisted of fractionated total body irradiation (12Gy) and high-dose etoposid (60mg/kg). As graft versus host disease (GVHD) prophylaxis Cyclosporine A was used. Graft contained 3.26x10^6 CD34+ cells. On day +5 persistent fever occurred, antibacterial and antifungal treatment were administrated. On day +7 5ug/kg G-CSF was added. We observed symptoms of respiratory failure. On day +10 WBC was 400/ul, cutaneous GVHD grade III appeared and steroids were administrated. On day +12 she had all symptoms of pulmonary oedema, was intubated and mechanically ventilated. Simultaneously rapid hematopoiesis reconstitution was observed: leucocytes>1.0 G/l and granulocytes>0.5 G/l on day +11, and platelets>50 G/l on day +14. After few days of the gradual improvement, her status suddenly deteriorated. Chest X-ray showed fluid in the alveolar space in both lungs. Pentamidine was added to treatment. Severe but stable status (opportunity to controlled ventilation, FiO2 above 80%) lasted about 3 months. She was treated with wide-spectrum antibiotics and antifungal drugs (liposomal amphotericin, voriconazole) although her blood cultures were still negative. In this time we also treated her with anti-TNF-alfa antibodies. Because of probable invasive aspergillosis and candidiasis we introduced treatment with caspofungin. Gradual improvement was observed: significant decrease of the requirement for oxygen, possibility to reduction of controlled pressures and respiratory rates and simultaneous improvement of radiologic changes. After few days of Continuous Positive Airway Pressure ventilation right pneumothorax appeared and continuous suction drainage had been used for three weeks. Simultaneously she was detoxicated from thiopental and morfine using fenobarbital and methadone. On day +128 mechanical ventilation was discontinued. Requirement for passive oxygen therapy gradually decreased. Nowadays chest tomography shows mild pulmonary fibrosis and bronchiectasia. The patients is alive, in good general status, under intensive physical and
pulmonary rehabilitation with stable full donor chimerism without immunosuppression. Nevertheless regular long-term pulmonary follow-up is still required.

R1130
Severe CNS damage in patient with mixed chimerism and reactivation of multiple herpetic infections after haploidentical SCT with T-cell depletion for familial haemophagocytic lymphohistiocytosis

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CNS symptoms and their severity strongly correlate with outcome of patients with familial haemophagocytic lymphohistiocytosis (FHL). Here we describe a case of a patient without primary CNS involvement related to FHL who developed progressive CNS damage of unknown aetiology after allogeneic stem cell transplantation (SCT). The patient was diagnosed at the age of 2.5 years with FHL, mutation in perforin gene was not proved, no signs of reduced perforin expression were observed. He was treated according to protocol HLH94 and then transplanted in clinical and expression were observed. He was treated according to protocol HLH94 and then transplanted in clinical and haematological remission of FHL from his haploidentical father (peripheral blood stem cell – CD34 positive selection; CliniMACS) because the matched donor was available. Myeloablative conditioning consisted of busulfan, cyclophosphamide and rATG. Soon after engraftment he suffered from CMV reactivation and he consequently experienced acute graft rejection. Following OKT3 and steroids further T cell depleted graft SCT was infused 26 days after 1st SCT. Early after second engraftment he developed severe acute encephalopathy and syndrome of inadquate ADH secretion (hyponatraemia, neurologic seizures,...), HHV6 variant B was at that time detected in cerebrospinal fluid (CSF) and blood. This was early followed by marked EBV and B cell proliferation treated with rituximab. After engraftment, complete donor haematopoesis was confirmed with exception of almost full autologous recovery of T lymphocytes (split chimaerism). At day+80 all T lymphocytes (3.8x10^9/L) were activated (expressing HLA DR) and were of host origin. Eleven months after SCT HHV7 was repeatedly detected in peripheral blood and later frequent pharmacologically almost uncontrolled epileptic seizures started initiating progressive mental retardation. Throughout this whole period repeated MRI scans and examinations of CSF, blood and marrow failed to document involvement of CNS due to uncontrolled FHL. One year after SCT cytotoxic assays showed significantly decreased activity of T cells. Despite the number of donor origin T cells started to predominate the host ones only 2 years after SCT, there were no clear clinical signs of FHL. Unfortunately the patient developed irreversible CNS damage. We speculate that multiple herpetic infections were responsible for proliferation of activated autologous T lymphocytes that contributed to severe CNS damage in this patient.

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R1131
Double haploidentical stem cell transplantation in children with severe combined immunodeficiency

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Consecutive twenty seven patients with inborn errors: four inborn metabolism: three metachromatic leukodystrophy, and one mucopolysacaridosis type 1: Hurler’s syndrome; also one osteopetrosis, two mayor thalassemia, two Fanconi anemia, and eighteen immunodeficiencies: twelve severe-combined immunodeficiency (SCID), two Wiskott-Aldrich syndrome and four hemophagocytic lymphohistiosiysis, were transplanted in our centre during the last nine years.

We report two cases of SCID: SCID type JAK3 deficiency (T-, NK-, B+) and major histocompatibility complex (MHC) II deficiency which were treated with double haploidentical parental donor positively selected HLA-mismatched. CD34+ progenitor cells were isolated from peripheral stem cells, after mobilization with granulocyte colony-stimulating factor (G-CSF) at standard dose, by the Isolex 300 (Baxter) and Clinimac(Milteny) systems selection devices respectively. The first case was a male, 3.5 months years old with JAK3 deficiency (T-, NK-, B+) who was undergone to transplant without conditioning regimen; value of peripheral CD34+ cells infused was 20.25 x10^6/kg and the mean CD3+ cells number was 1.6x10^5/kg, and 3.63 log T-cell depletion; she had graft rejection at day +71 and therefore was undergone to second haploidentical from the same family donor: father, this time with no-myeloablative conditioning with fludarabine (F) + melphalan + anti-thymocyte globuline (ATG) and prophylaxis for graft-versus-host disease (GVHD) with cyclosporine (CyA), he died ten days after the second transplant by heart insufficiency and metabolic failure.

The second patient was a female, 36 months years old with MHC class II deficiency, she was undergone to haploidentical transplant with myeloablative conditioning based F+ busulfan and ciclophosphamide and ATG. The value of peripheral CD34+ cells infused was 20.25 x10^6/kg and the mean CD3+ cells number was 1.6x10^5/kg, and 3.63 log T cell depletion; she had graft rejection at day +180 and mixed chimerism therefore was underwent to second haploidentical transplant from the same parental donor: mother, the conditioning regimen was based OKT-3 and dexametason, she died five days after the second transplant by lung bleeding.

Despite poor prognosis at diagnosis of these cases, and the engraftment failure regardless mega-doses of CD34+, this approach could be a feasible therapeutic option for patients lacking a suitable donor.

R1132
Pegfilgrastim for mobilisation of peripheral blood stem cells in children

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Introduction: Mobilized peripheral blood stem cells (PBSC) represent the most important source for autologous stem cell transplantation, even in children with malignancy. The current practice is administration of G-CSF alone or in combination with chemotherapy. Recently, a polyethylene glycol (PEG)- conjugated form of G-CSF (pegfilgrastim) has been licensed. Preliminary data indicate it has the same effects of filgrastim in terms of elevation of absolute neutrophil count, mobilization of PBSC, and reduction of duration of chemotherapy-induced neutropenia, with the obvious advantage that these effects could be sustained for several days from a single injection without added toxicity.

In a recent experience the efficacy of a single dose (6 microgr) of pegfilgrastim, in combination with salvage chemotherapy, was tested in an open-label phase II study of 25 pretreated patients. The authors concluded that pegfilgrastim as an adjunct to chemotherapy is a predictable and highly effective mobilization regimen in pretreated lymphoma patients (Isidorins 2005).

Very limited experience is available on the use of Pegfilgrastim in children. In the only two available report it was used to shorten the duration of severe neutropenia after cytotoxic chemotherapy in five children with Ewing sarcoma (Te Poole EM 2005) and in seven pediatric cancer patients (Wendelin G 2005). We are not aware of any report on the use of pegfilgrastim for mobilization in children.
In conclusion, CD3/CD19 depleted peripheral stem cells from CD56+ cells was detected. With 304/µl CD3+, 67/µl CD4+, 239/µl CD8+ and 360/µl complete donor chimerism. At day 60, good immune recovery was fast with more than 100/µl CD3 and CD4 positive cells although T-cells were severely depleted, T-cell regeneration was observed, which responded to prednisolone. Noteworthy, immunosuppression was performed using MMF. GvHD Grad I/+13. Acute toxicity was mild (grade I). Post transplant program underwent, according to the treatment protocol, autologous transplant repeated three times and engrafted (PMN > 500/microL) on day +11, +11, and +15.

Conclusions: Mobilization with peg-filgrastim was safe and efficient in our patients; failure to mobilize was observed only in an heavily pre-treated patient with Ewing sarcoma.

R1133
CD3/CD19 depleted peripheral stem cells for matched unrelated recipient in combination with a reduced intensity regimen – a new option in children lacking a matched sibling donor?
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Stem cell transplantation (SCT) is the treatment of choice for patients suffering from severe aplastic anaemia (SAA) who do not respond to immunosuppressive treatment (IST). Patients transplanted from an HLA-identical sibling have an excellent prognosis compared to patients who were grafted from alternative donors. T-cell depletion by CD34 positive selection of peripheral stem cells reduces the risk of GvHD considerably. This, however is followed by an increased risk of graft rejection. Most recently, it could be shown, that changing the graft processing from CD34 positive enrichment to depletion of CD3 and CD19 positive peripheral cells does facilitate and improve engraftment in children transplanted from HLA-haploidentical parents. Consequently, also patients with SAA who are at highest risk for graft rejection might benefit from such a new graft processing technique.

A 7-year-old girl who failed to respond to IST received an allograft from MUD. The conditioning regimen consisted of Fludarabine (5x40mg/m²), Thiopeta (2x5 mg/kg), Melphalan (2x70mg/m²) and OKT 3 (17x 0.1 mg/kg). G-CSF mobilized peripheral blood stem cells (PBSC) were purified using anti-CD3/CD19 microbeads (Miltenyi). Recovery of CD34+ progenitor, CD56+ NK cells and CD14+ monocytes was more than 70% each. Residual T- and B-cells were < 0.008 and 0.02 %, respectively.

In total, 12.05x10^6 CD34+ cells; 30.3x10^6 CD56+ cells; 33.6x10^6 CD3+ cells and 8.8x10^6 CD20+ cells per kg were administered. Without G-CSF, engraftment occurred on day +12 for leucocytes and both neutrophils and platelets on day +13. Acute toxicity was mild (grade I). Post transplant immunosuppression was performed using MMF. GvHD Grad I was observed, which responded to prednisolone. Noteworthy, although T-cells were severely depleted, T-cell regeneration was fast with more than 100/µl CD3 and CD4 positive cells detectable already on day +25. Chimerism analysis showed complete donor chimerism. At day 60, good immune recovery with 30x/µl CD3+, 67x/µl CD4+, 239x/µl CD8+ and 360/µl CD56+ cells was detected.

In conclusion, CD3/CD19 depleted peripheral stem cells from a MUD in combination with a reduced intensity conditioning regimen could be a promising option in the treatment of patients with aplastic anaemia not responding to immunosuppressive treatment and lacking a matched sibling donor.

R1134
Allogeneic stem cell transplantation in children with idiopathic myelofibrosis
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Idiopathic myelofibrosis (IMF) comprises myelofibrosis, extramedullary haematopoesis, hepatosplenomegaly and pancytopenia. In adults IMF represents a poor prognosis, progressive fibrosis and leukaemic transformation are frequent. Allogeneic hematopoietic stem cell transplantation (HSCT) is a treatment option but is connected with high risk of graft failure and toxicity. In children the disease is rare and variable, stable course or spontaneous remission has been reported. We describe two cases of IMF in children. In a girl mild anaemia and thrombocytopenia were first documented at the age of 3 years. At 8 years pancytopenia was found, trephine bone marrow (BM) biopsy revealed normocellular haematopoesis with myelofibrosis. She remained in a good clinical state with a stable blood count but further BM biopsies showed decreased cellularity with myelofibrosis. 2.5 years after the diagnosis her blood count dropped off, hepatosplenomegaly was noted and BM biopsy revealed marked myelofibrosis. 2 months later at the age of 11 years HSCT was therefore performed (matched unrelated donor, Flu+Mel+ATG), ANC engrafted on day 20, platelets on day 25, complete donor chimaerism achieved on day 21. Corticosteroids started on day 160 for mild extensive GvHD. BM biopsy at day 180 remained hypocellular with myelofibrosis, however blood count was normal. 17-year-old male presented with pallor, quickly developed pancytopenia with blasts. BM biopsy showed myelofibrosis, increased blasts, trisomy +8. HSCT from a HLA identical sibling was performed 2 months later (Flu+Mel+ATG), ANC engrafted on day 16, platelets on day 62. Complete donor chimaerism was achieved on day 14, no GVHD. At day 180 BM biopsy did not display myelofibrosis, peripheral blood count was stable. Clinical deterioration started 10 months post-HSCT with fever, weight loss, hepatosplenomegaly, mixed chimaerism, thrombocytopenia, blasts and extramedullary infiltration of breast. Trephine and breast lump biopsy confirmed relapse of IMF in transformation to AML-M7 with trisomy +8 and trisomy +21. Second HSCT from the same donor was carried out 11 months after the first HSCT (Bu+Cy+Mel). GvHD grade II treated with steroids manifested on day 11. ANC engrafted on day 13, platelets not engrafted. Despite artificial ventilation a diffuse alveolar haemorrhage resulted in death on day 37. Reduced intensity conditioning composed of fludarabine and melphalan is preparative regimen of choice for HSCT in children with IMF.

R1135
Angiogenic factors in children after allogeneic stem cell transplantation
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There are no studies on the connection between graft versus host disease (GvHD) and angiogenesis. However, Chen et al have shown in Nat Med (2004) that the vascular endothelial growth factor-C (VEGF-C) - vascular endothelial growth factor receptor-3 (VEGFR-3) -axis has an effect on alloimmunity, and that the blockade of VEGFR-3 signaling is immunosuppressive. Both VEGF-C and Angiopoietin 2 (Ang2) are important in angiogenesis and lymphangiogenesis. In this study we measured the levels of these two factors in children after URD-SCT (unrelated allogeneic stem cell transplantation).

Patients and methods: Nine patients aged 4-16 yrs were included, six of whom developed significant acute GvHD (aGvHD, gr ≥ 2) while three did not. The diagnoses of the
aGvHD patients were ALL (acute lymphoblastic leukemia) (n=3) and SAA (severe aplastic anemia) (n=3). The non-GvHD patients also had ALL (n=2) and SAA (n=1). GVHD prophylaxis consisted of cyclosporin and short methotrexate. The serum concentrations of VEGF-C and Ang2 were analyzed by ELISA at 2-102 days posttransplant, 3-5 samples/ao/patient.

Results: The VEGF-C and Ang2 concentrations (median, range) were 1400 (545-10960) pg/ml and 2160 (875-6685) pg/ml, respectively. The presence or absence of aGvHD did not make any difference in the levels. Neither did we find correlation between hemoglobin, white blood cell count, bilirubin, CRP or sedimentation rate and these two angiogenic factors. The VEGF-C levels were significantly lower than in our previous study on ALL patients at diagnosis. The individual maximal Ang2 levels correlated with survival (≥ 7mo follow-up, p=0.014). Both absolute lymphocyte count and platelet count correlated with VEGF-C levels, probably because these cells are known to produce VEGF-C.

Conclusion: Our results did not support the hypothesis about correlation of the levels of angiogenic factors with GvHD. Instead, there was a novel finding about low concentration of Ang2 being predictive of a good outcome. Our findings pose important questions on the emerging role of angiogenic factors in the evaluation of the pathogenesis of GvHD.

R1136

Stem cell transplantation after a treosulfan-fludarabine based conditioning regimen not containing cyclophosphamide for children with Shwachman-Diamond syndrome

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Background: Hematopoetic stem cell transplantation (HCT) does appear to be a therapeutic option for children and adolescents suffering from Shwachman-Diamond syndrome with severe cytopenias and/or myelodysplastic syndrome. The paucity of experience with children undergoing HCT for SDS has been the major obstacle for recommendations regarding time point, transplant regimen, and patient subgroup benefiting most from HCT. Most but three reported patients received a preparative regimen either consisting of Bu/Cy or Bu/TBI. However, severe early toxicity with cardiomegaly, myocardial fibrosis, and cyclophosphamide associated cardiomyopathy have been described. Therefore, we tested the feasibility of a cyclophosphamide free protocol using fludarabine, treosulfan, and melphalan as a conditioning regimen.

Methods: Between 2004 and 2005 two children with SDS were enrolled. Age at transplantation was 17 and 7 years. Both patients received conditioning with fludarabine (30 mg/m²/day x 6), treosulfan (12 g/m² x 4), melphalan (140 mg/m² x 1), and campath-1H. All children received a non manipulated fresh bone marrow graft. The first patient from a HLA-identical sibling, the second from a 10/10 locus matched unrelated donor. Mean cell doses transplanted were 3.7 x 10⁸ nucleated cells/kg BW (2.9 x 10⁹/kg and 6.2 x 10⁹/kg).

Results: Both patients achieved donor derived engraftment, no GVHD exceeding grade II was observed, and both maintained donor chimerism at 100%. All patients developed grade III mucositis. On patient experienced a cerebral seizure early after transplant most likely caused by CSA toxicity. GVHD prophylaxis was switched to MMF and the patient fully recovered from this single event. Apart from this, no RRT > grade II was seen. All patients are alive after a follow up of 17 and 4 months.

Conclusion: A fludarabine based, cyclophosphamide free conditioning regimen seems to be a feasible approach for matched sibling and matched unrelated HCT in children and adolescents with SDS. Larger numbers and a longer follow-up are needed to make these results more comparable to traditionally used preparative regimens.

R1137

A prospective comparison of immune reconstitution after autologous haematopoietic stem cell transplantation in children

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Introduction: Autologous haematopoietic stem cell transplantation (auto HSCT) has become an established therapy for numerous advanced paediatric solid tumours. After haematopoietic stem cell transplantation all recipients experience a period of immunodeficiency. Regeneration of adequate T-cell numbers and repertoire diversity are key elements in the recovery of immune competence.

Patients: Immune reconstitution was studied in 28 children (34 transplants; median 5.5 years; range 13m-18y; 10 female, 18 male). Blood samples were drawn before auto HSCT, on days 14 (take), 30, 60, 100, 200 and 365 and >15months.

Methods: We analyzed lymphocyte subpopulations using flow cytometry. Intracellular cytokines (IFNgamma, IL2, TNFalpha, IL4, IL5, and IL10) were determined by FACS after in vitro stimulation with PMA, ionomycin and brefeldin for 24h. Additionally, we measured IL2, IL15 and TGFbeta in unstimulated sera by ELISA. Additional we measured TREC’s and spectatypes.

Results: As to lymphocyte subpopulations after auto HSCT, T-cells were the first to regenerate. In T-cells and CD4+ T-cells the memory phenotype (CD45RO+) predominated. As to cytokine levels in unstimulated sera, we saw high levels of IL4 shortly after transplantation, levels decreased to pre-transplant values during one year. TGFbeta levels increased during the first year and decreased thereafter. IFNgamma levels remained stable. In stimulated T-cells, IFNgamma and TNFalpha increased in the first year and went down afterwards. Interestingly, high IFNgamma levels after transplantation correlated significantly with a better survival.

R1138

Non-irradiation containing conditioning regimen for children with Fanconi's anaemia

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Fanconi Anemia is an inherited disorder that leads to progressive bone marrow failure. The only curative treatment of the severe aplastic anemia that ultimately develops in these patients is allogeneic stem cell transplantation. Patients with Fanconi Anemia have increased chromosomal fragility. As a result they are prone to both short and long term complications when conditioning regimens containing radiation are used. We report on 5 patients (3 males and 2 females) with Fanconi anemia who were transplanted from matched siblings without using radiation as a part of conditioning. Median age at time of transplant was 9 years (3-18 years). None of the patients had MDS changes or leukemia prior to transplant. Conditioning regimen consisted of fludarabine 120 mg/kg, cyclophosphamide 20 mg/kg and rabbit ATG 20 mg/kg. Peripheral blood was the source of stem cells in 3 patients, while bone marrow was the source in 2 patients. GVHD prophylaxis consisted only of cyclosporine. At a median follow up time of 368 days (48-568 days), 4 patients are alive with normal hematopoiesis. One patient failed to engraft. He was transplanted again with a different conditioning; however he had late rejection and died of sepsis 269 days after second transplant. Median CD 34+ cell count infused was 4.6 million (1.2-7.6 million). Median time to neutrophil and platelet engraftment was 9 days and 74 days, respectively. Two patients had fever with ATG; one patient had bacteremia during the neutropenic period. Two patients developed fungal infections after engraftment. Two patients
had mild VOD. Three patients received VOD prophylaxis consisting of urisdilid and spironolactone. CMV reactivation occurred in 4 patients and was treated with ganciclovir. Grade 1-2 skin and liver acute GVHD occurred in 2 patients. Limited chronic GVHD occurred in 2 patients. One patient developed extensive chronic GVHD. Conditioning without radiation is well tolerated in Fanconi anemia patients and results in prompt engraftment. Infectious complications appear to be high.

R1139
Autologous peripheral blood stem cell transplantation in low weight paediatric patients: methods and clinical outcome
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Peripheral Blood Stem Cell (PBSC) collection may be difficult in low weight bone marrow patients, with technical and clinical problems related to vascular access, low total blood volume, citrate toxicity, high extracorporeal volume, and patient's tolerance.
Methods: We present our experience with 12 consecutive children weighing ≤ 20 kg, diagnosed with acute leukaemia (3) and solid tumors (9), which were collected and transplanted between September 1994 and February 2005 at our centre. Patients mean body weight was 15 kg (range 7-20); median age was 4 years (range, 1-7 years) (Table 1). Harvesting of PBSC was started after 5 days of cytokine alone (G-CSF 10 mcg/Kg/24hs. S.C.). Procedures were performed using a Baxter CS-3000 Plus separator primed with a mixture of irradiated and white cell-depleted red cells resuspended in 5% albumin and diluted with saline to match the patient's haematocrit. Heparin and ACD-A was used for anticoagulation (heparin 5000 UI in 1000 ml ACD-A) in patients weighing < 10 kg. The median number of leukapheresis was 2 (range 1-4), processing 2.5 volemia in each session. The platelet count decreased significantly after each procedure without requirement of platelet transfusions. Special monitoring of toxicity was done. The children were not sedated and showed no serious side-effects. All PBSC were cryopreserved with DMSO 5% and stored at -80°C in mechanical freezer.
Results: The median time from cryopreservation to transplantation was 29.5 (17-130) days, and the median number of infused mononuclear cells and CD34+ cells were 5.05 (2.8-14.7) x10^8/Kg and 2.5 (0.5-4.3) x10^5/Kg, respectively. The median number of infused post-thawing CFU-GM was 23.3 (17.4-39.1) x10^3/Kg. All patients showed a safe and sustained engraftment. Median time to reach 500 and 1000 neutrophil per microL was 12 (10-17) and 12 (10-16) days. Median time to 20 and 50 platelets level per litter was 20 (10-50) and 33 (12-84) days (Table 2).

Conclusion: Our experience shows that our PBSC collection and cryopreservation method is a safe and efficient procedure in children weighing less than 20 Kg., with sustained haematopoietic reconstitution.

R1140
An approach to retrospective validation and performance monitoring of PBSC collection and other white cell procedures using the Cobe Spectra cell separator: “Cells collected” as a function of “cells processed” by 2 different methods
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Validation of cell separator machines for a specific apheresis procedure poses a number of challenges. In particular, for white cell collection procedures it is not feasible for individual centres to carry out a prospective validation process prior to introduction of the procedure, because there is no way of performing a “dummy run” of a white cell procedure without actually putting a donor on the machine. We therefore attempted retrospective validation of our Cobe Spectra cell separator machines for PBSC collection using the Mononuclear Cell (MNC) procedure by estimating efficiency of the MNC procedure for each of the individual machines, in terms of the total mononuclear cell dose achieved in the apheresis product as a function of the number of mononuclear cells processed by the machine. There should be a direct correlation between total mononuclear cells processed by the machine and the final MNC dose in the product. The difficulty is in estimating “total mononuclear cells processed by the machine”, which will only ever be an approximation. We estimated “Cells Processed” using 2 different methods: Method 1: the donor’s peripheral mononuclear cell count (lymphocytes plus monocytes) multiplied by the Run Time; Method 2: the number of Total Blood Volumes processed multiplied by the donor’s peripheral mononuclear count.
Retrospective audit was performed on 70 PBSC collections carried out on five Spectra machines over an 18-month period. Total mononuclear cell dose in the apheresis product was graphed as a function of “cells processed” as calculated by both methods above. Both methods showed a clear, statistically significant linear correlation, but there was less scatter and a lower p value using Method 1 (R=0.64, p<0.0001). It was noted that the gradient of the trend line is a measurement of the efficiency of PBSC collection. The data was therefore subdivided depending on which of the 5 Spectra machines had been used for collection, and individual data analysis was performed for each machine. This showed that the five machines all performed the MNC procedure with very similar efficiency (coefficients of trendlines 0.522 to 0.593). The same process can easily be applied to ongoing performance monitoring of the five machines, and our aim will be to do this annually from now on.

R1141
T-cell receptor molecular analysis in paediatric patients undergoing allogeneic bone marrow transplantation
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Allogeneic bone marrow transplantation (ABMT) is one of the powerful therapies for several hematological malignancies. Multiple mechanisms contribute to the graft success, such as the entity of primary disease, donor bone marrow availability, minimal residual disease (MRD) and complications including infections and acute graft-versus-host disease (GVHD). GVHD represents a major complication of ABMT and is the main cause of morbidity and mortality. The present study takes into account the recovery of the T-cell compartment before and after ABMT in 14 pediatric patients affected by
different hematological malignancies. To evaluate the pattern of accumulation of T cells, we investigated the usage of T cell receptor (TCR-beta) chain variable regions (TCRBV) and the complementarity-determining region 3 (CDR3) up to 2 year follow-up after ABMT. Increased expression of some TCRBV families were observed in patients during the months after ABMT. In the following months after ABMT, we confirmed the presence of the same T cell clones and, sometimes, we showed the appearance of a new TCRBV family subset. We observed a random distribution of overexpressed TCRBV families and we did not show a preferential expression of a peculiar TCRBV.

In order to clarify whether cells expressing a TCRBV region were clonally expanded, we performed CDR3 spectratyping and sequencing analysis. A predominant polyclonal pattern was observed in donors and patients before transplantation while at 1 and 3 months after BMT some clonal subsets were identified. In the majority of patients the presence of these subsets persists until 24 month after ABMT.

A skewed TCRBV repertoire and oligoclonal/monoclonal subsets was identified. In the majority of patients the presence of these subsets persists until 24 month after ABMT.

A skewed TCRBV repertoire and oligoclonal/monoclonal subsets we observed may explain the post-BMT immunodeficiency detectable after transplantation and may reflect responses to pathogens or alloantigens in correlation with clinical GVDH.

This study was supported by a grant from Fondazione Città della Speranza.

R1142 Measurement of FOXP3+ regulatory T-cells in allogeneic cell products by flow cytometry and staining of cytospin smears
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Introduction: Tregs are involved in the control of immune responses to foreign antigens and play an important role in the pathophysiology of GVH-reactions. They are characterized by expression of CD4+, CD25+ and the transcription factor Foxp3. Although Tregs are of emerging interest in allogeneic cell therapy, their precise enumeration in heterogeneous cell products has been extremely difficult. Using monoclonal antibodies (moAbs) against Foxp3 and immunoenzymatic labelling at the single-cell level in paraffin-embedded tissues, we have investigated different techniques to identify Tregs in cellular products and tissue biopsies.

Methods: 20µl of the anti-human PE conjugated Foxp3 antibody (clone PCH101, ebioscience) were used for intracellular labelling of 1x10^6 cells of CD19 depleted, MACS sorted CD4+/CD25+ cell fractions of peripheral blood mononuclear cells. The abcam goat polyclonal FOXP3 antibody was used for double immunoenzymatic labelling of FOXP3/CD3 and FOXP3/CD25 of PBMC cytospins, of MACS-sorted CD4+CD25+ selected cell fractions and of paraffin-embedded tissues.

Results: Nuclear staining of the FOXP3 and measurement by flow cytometry showed bright results in MACS-sorted peripheral blood cells as well as in cytospins. Gating on CD4+ cells of the CD19 depleted, CD25 enriched cell fraction, 67% of the CD4+ cells are positive for the Foxp3 marker. The frequency of FOXP3+ Treg in the peripheral blood lymphocytes of healthy individuals ranged between 2.4 % of total lymphocytes, like previously described. In double labelling cytospin analyses of FOXP3 and CD25, we confirmed co-expression of CD25 on all FOXP3 positive cells. Counting 10 high-power fields, 63% of the CD3+/CD25+ cells are FOXP3+. In healthy individuals PBMC-cytospins we found 5% CD3+/CD25+ FOXP3+ cells. In double labelling analyses of FOXP3 and CD25 in paraffin embedded tissues, we confirmed co-expression of CD25 on all FOXP3 positive cells. Only a few weakly CD25-stained cells were negative for FOXP3 staining, indicating a preferential staining of the CD25-high cell population.

Conclusion: Direct staining of cellular products with moAbs against nuclear FOXP3 and subsequent flow cytometry can give similar results as nuclear Foxp3-staining in cytospin preparations or the assessment of CD4+CD25+ cells by flow cytometry. These new techniques allow straightforward identification and quantification even of very low numbers of Treg in peripheral blood subsets or other tissues.

R1143 Patient age and granulocytic contamination of apheretic harvests are important factors for adverse events after infusion of cryopreserved HSC
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Adverse events (AE) after cryopreserved cellular infusions are frequent and seldom can be life-threatening. DMSO is considered important in their pathogenesis, however other factors could well play a role. We have prospectively collected data on AE presenting after 175 HSC infusions following high dose chemotherapy performed in our centre during a 4 y. period in patients affected with haematological neoplasm. Stem cell source was PBSC in 153 cases while Bone Marrow in 22. In all cases an endotoxin-free DMSO was used. One or more AE was registered in 51/175 infusions (28.6%). Gastrointestinal complaints were reported in 17% of all infusions, skin rashes in 5.7% of cases, shakings in 4%, cough in 5.1%, fever in 2.2%, shortness of breath in 1.2%, dizziness in 1%, headaches in 0.5%. In univariate analysis patient age over 60 was significantly associated to a higher incidence of AE, in fact incidence of AE was 25 % below 50 years of age, 26% in 50-60 decade and 47% over 60 years (P=0.04). Frequency of AE was higher after PBSC than after BM (32% versus 4.5%, chi-test: p=0.002). In univariate logistic regression factors found important for AE in PBSC group were Total Number of Cells infused /kg (P=0.01) and Volume/kg of DMSO infused (p=0.006). Adverse Events were more frequent also when Total Number of Granulocytic cells/ present in PBSC harvest was >0.5x10^7/kg in respect to infusions containing a total number of granulocytic cells below this threshold (14% versus 46%, chi test: P=0.0004). All aforementioned factors were evaluated in multivariate logistic regression and age of patient (P=0.008) and granulocytic contamination over 0.5x10^7/kg (P=0.001) were still significant while the total volume of infused DMSO loose importance (P=0.1). No cardiovascular events were recorded during infusions, however we registered a statistically decrease of blood pressure and a statistically decrease of cardiac frequency. A significant correlation existed between reduction of cardiac frequency and volume/Kg of DMSO infused (r=0.220, P= 0.005). In conclusion while non cardiovascular AE are dependent from patient age and from granulocytic contamination of apheretic harvests, cardiovascular changes are influenced only by volume/Kg of DMSO infused. As far as non cardiovascular adverse events are concerned, particular attention should be paid in infusions of HSC in patients over the age of 60 years and when the grafts have a granulocytic contamination over to 0.5 x10^7/Kg.
Stem cell source

R1144
Haematopoietic stem cell transplantation for haematological malignancies: a 5-year single-centre experience
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Hematopoietic stem cell transplantation (HSCT) is widely used therapeutic method in the treatment of patients with hematological malignancies. In this study we present our results in 5 years experience in transplantation for hematological malignancies. In a period 2000-2005 a total of 97 transplants have been realized, 30 allogeneic sibling and 67 autologous transplants. In the group of patients treated with allogeneic SCT, 60% of patients were in active disease prior transplantation, with diagnosis (19 AML; 6 CML; 1 AA; 1 NHL; 1 CLL; 2 ALL); ratio males: females = 2:1; median age 34 years (20-54). Bone marrow (BM) as source of HSC was used in 4 patients and 26 were preformed with peripheral blood stem cells (PBSC) with donor sex ratio 1:1 (15/15). Conditioning was provided with Bu/Cy (13pts). Bu/Cy+Mel (7pts). BEAM (2pts), hdICE(1pts). nonmyeloablative FLAG/Ida(4pts), Flu/Mel(1pt). The amount of infused fresh bone marrow was 1010ml(950-1100ml) with MNC 3.5x10^8/kg(2.5-4.5) and PBSC 4,3x10^8/kg (2.5-6.2). Median number of transfused blood products was for Er median 3 doses (0-6) and Pt 19 doses (5-34). Engraftment for Ne>0.5x10^9/L and Plt >20x10^9/L was recognized on +15(8-25). Median follow up for both groups was 35 years (7-63), males: females=1:1. 40% of patients with lymphoproliferative 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, Melphalan. The amount of infused fresh BM was 1010ml(950-1250ml) with MNC 4,0x10^8/kg(2.9-5.8) and PBSC 3.85x10^8/kg (1.7-6.0). Median number of transfused blood products was for Er median 3 doses (0-6) and Pt 35 doses (0-73). Engraftment for Ne>0.5x10^9/L and Plt >20x10^9/L was recognized on +15(0-25). Median follow up for both groups was 30 months (3-60). TRM in allogeneic recipients was 4 patients (13,3%) with survival of patients transplanted in CR of 84,62%, and in the autologous group TRM was 6 patients (8,9%) with survival of 62,69%.

R1145
Successful mobilisation of haematopoetic progenitor cells for autologous blood stem cell transplantation with administration of G-CSF and chemotherapy in patients with lymphoma and myeloma (single-centre experience)
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Introduction: The administration of a combination of chemotherapy and cytokines G-CSF is associated with a significantly increased efficacy of stem cell mobilization compared with either modality alone.

Method: The aim of this study was to evaluate the efficacy of G-CSF preceded by chemotherapy (cyclophosphamide 4g/m² sq for 1 dose) for hematopoietic progenitor cell mobilization for lymphoma and myeloma patients. We started G-CSF as a fixed dose 480MU SQ every day as soon as the leukocyte counts began to rise after chemotherapy induced myelosuppression. Leukapheresis was commenced at the time when leukocyte count rose up to 1000/uL, and repeated for 2-4 consecutive days until target number of CD34+ cell, at least 2x10^6/kg was collected.

Results: Thirty-five patients (male to female, 19:16, age range 21-65, lymphoma 25, myeloma 10) underwent a total of 73 courses of leukapheresis for hematopoetic progenitor cell collection prior to autologous transplantation from April 2002 through May 2005. The target amount of marrow was harvest in all patients. All the patients achieved good engraftment after autologous transplantation. The mean days required for WBC count to be over 1,000/uL was 8-16 days. Patient’s age, sex, underlying malignancy, exposure to chemotherapy before mobilization did not show any statistically significant correlation.

Conclusion: We can conclude that chemotherapy followed by G-CSF administration is an effective way for mobilization of hematopoetic progenitor cell and verified itself as a good mobilization method.

R1146
Cyclophosphamide + GCSF as mobilising schedule in multiple myeloma and malignant lymphoma patients: factors associated with mobilisation efficiency
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Background: Cyclophosphamide (CY) at dose of 1.5 grs/m² and GCSF is cortile blood to mobilize blood stem cells and to support high dose therapy in patients (pts) with multiple myeloma (MM) or malignant lymphoma (ML). However, 20-25% of pts do not achieve the minimum stem cells dose needed for a rapid hematopoetic engraftment and risk factors for poor mobilization are not well known.

Methods: 93 pts diagnosed of MM or ML and candidates to autologous stem cells transplant (ASCT) between October 96 and March 05 were treated with CY 1.5 grs/m² (d0) followed by GCSF 10 mcg/kg/d from d+7. All pts were treated as outpatients and as a whole, 20% would be considered as a failure to get an adequate stem cells collection.

Results: Thirty-five patients (male to female, 19:16, age range 21-65, lymphoma 25, myeloma 10) underwent a total of 73 courses of leukapheresis for hematopoetic progenitor cell and verified itself as a good mobilization method.

Conclusions: 1) A combination of CY + GCSF is a safe, predictable and effective for the most of MM or ML pts (80%).
2) We have not identified any premobilization factors predicting poor mobilizer pts.

**R1147**

**Report of umbilical cord blood transplantation in Iran**

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Introduction: Umbilical cord blood (UCB) may be an alternative source for allogeneic transplantation for the treatment of hematological malignancies and genetic disorders in patients without suitable donors particularly in ethnic minorities. Early experience with umbilical cord blood transplantation (CBT) demonstrated a lower incidence of graft-versus-host disease (GVHD) even though the procedure was performed with HLA-disparate grafts. The major drawbacks of CBT are slow hematopoietic recovery and a high incidence of graft failure, as a result of a lower number of progenitors infused.

Materials and Method: We collect the data of 13 patients who had undergone Cord Blood Transplantation by reviewing their records from the date of their transplantation up to the date of last contact. Analysis of the data has done via using SPSS software.

Results: 13 patients received CBT which in our center during the past 6 years, Consist of 8 Talassemia, 2 Severe Combined Immune Deficiency (SCID), 1 AML-M2, 1 MPS-1 (Hurler Syndrome). Of all the 13 patients 9 (69.23%) were male, and 4 (30.77%) were female. The median age was 4 years old; (8 months -14 ys) respectively. Donors were HLA-identical siblings in 9 (69.23%) patients and unrelated in 4 (30.76%) patients. The conditioning regimen for 78.92% of patients was Busulphan, and Cyclophosphamide. 8 patients had not GVHD, (3 of them died in first 100 days), 2 had only GI GVHD, 1 had GI and Skin GVHD, and 2 had GI, skin and also liver GVHD. The median duration of hospitalization for transplanted patients was 50 days. Transplant mortality rate in the first 100 days was 30%. The median follow-up duration was 189 days, with minimum and maximum of 7 and 1923 days respectively and during this period the overall survival (OS) is 74.07% and the disease free survival rate (DFS) was 37.50%.

Conclusion: Cord blood is a considerable alternative source for hematopoietic stem cells for allogeneic transplantation for malignant or nonmalignant hematopoietic disorders. As our cases were few we can not conclude definitely about the advantages or disadvantages, but in our study cord blood recipient from HLA-identical siblings had lower GVHD and mortality than unrelated donors.

**R1148**

**Peripheral blood stem cell collection: from outsourcing to in-service process. An opportunity to optimise the procedure. Results after one year at EIO – Milan**

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Peripheral blood stem cell (PBSC) collection by leukapheresis (LAF) represents the standard method to obtain blood stem progenitors. This procedure is usually referred to Transfusion Centers joined with Transplant Units. A possible pitfall of this kind of management could be the impossibility to perform “ad hoc” the procedure in any time. From 97 a transplant program with PBSC collection is ongoing at the EIO; until June 94 the LAF has been performed by the transfusionist team outsourcing and then governed by the medical-nurse staff of our Division. Aim of the study was to evaluate the outcome of this shift in management.

Methods: Firstly we considered what kind of LAF-related variable could be examined to optimise the service for the patients in terms of harvest quality and assistance and for the Institute in terms of reduction of time and cost of the procedure. Secondly we organized a special training with the aim to perform the LAF in-service using our know-how and resources. Successively a comparative analysis of the data referring one year of our management has been performed and compared to the data collected during one year of the previous management. In particular all the following variables of the procedure have been analyzed by a specific data-base: a)Quality of the stem cell product; b)Comfort for the patient; c)Time related to procedure; d)Costs.

Results: From July 94 to July 95 we performed 94 consecutive peripheral blood stem cell transplantations. The staff was operative 24hours every day from Monday and Sunday included. The two populations of patients were matched for age, sex, diagnosis, stage of disease and previous treatment. The collection target were >4.0x10⁶/kg and >2.0x10⁷/kg CD34+ for donors and patients respectively. The table shows the collection results obtained. The best ratio optimal collections/patients obtained with our management (97%/vs90%) could be attributed to the optimisation of the procedure timing and to the excellent cooperation between medical doctors and the nurses in the in-service experience. Concerning the time for an optimal collection, no difference was observed into the two managements (only 7% of the patients needed three or more procedures). The activity of the internal staff has permitted to cut down significantly on expenses in charge of the Institute for the Transfusionist’s performance.

Conclusions: thanks to the constitution of an internal dedicated team, we were able to perform the PBSC collection with similar results and lower cost in comparison with the outsourcing management.

**R1149**

**Factors influencing peripheral blood stem cell mobilisation in patients with haematological malignancies and solid tumours**

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Background: Mobilized peripheral blood stem cells (PBSC) have become the main source for autologous transplantation in patients with haematological malignancies or solid tumours. The aim of the study was to establish the influence of diagnosis, sex, age, number of previous courses of CT, mobilization regimen, bone marrow involvement in the outcome of PBSC mobilization.

Patients and methods: 145 patients with haematological malignancies and solid tumours were included in the study (Hodgkin’s lymphoma, HL (n=24), non-Hodgkin lymphomas, NHL (n=29), multiple myeloma, MM (n=32), acute leukaemias, AL (n=15), solid tumours, ST (n=21)). 79 (54%) were females, 66 (46%) males. 104 (72%) patients were >50 years of age, 41
(28%) patients were an age of less than 50 years. 100 patients (82%) were mobilized with G-CSF 10 mcg/kg for 5 days, a combination of CT and G-CSF (5 mcg/kg) has been used in 21 patients (18%). 46 patients (49%) received more than six courses of CT and 47 (51%) less than six respectively. Bone marrow (BM) involvement was diagnosed in 76 (56%) patients. Apheresis was performed on Spectra v.5.1 (Gambro). The following criteria for the mobilization outcome were used: non-mobilizable patients < 1 x 10^6 CD34+ /kg; poorly mobilizable patients > 1 x 10^6 CD34+ /kg; mobilizable patients > 5 x 10^6 CD34+ /kg (S.Fu et al., 2000).

**Results:** 14 patients (11%) were non-mobilizable; 61 patients (48%)-poorly mobilizable; 52 (41%)-mobilizable patients. According to the diagnosis we observe the following results: HL-9.0±3.4x10^6 CD34+/kg, NHL 6.8±2.2x10^6 CD34+/kg, MM-8.8±2.6x10^6 CD34+/kg, ST-8.6±3.0x10^6 CD34+/kg, AL-5.5±1.2x10^6 CD34+/kg. Comparing all groups between each other we found no significant differences. There were also no influence of age and sex on stem cell mobilization (p= .94, .89 respectively). BM involvement does not seem to be an independent factor with significant adverse influence on PBSC mobilization. Apheresis was performed on ‘Spectra’ v.5.1 (Gambro). The following criteria for the mobilization outcome were used: non-mobilizable patients <1±10^6 CD34+/kg; poorly mobilizable patients >1±10^6 CD34+/kg; mobilizable patients >5±10^6 CD34+/kg. Comparing all groups between each other we found no significant differences. There were also no influence of age and sex on stem cell mobilization (p=. 89 respectively). BM involvement does not seem to be an independent factor with significant adverse influence on PBSC mobilization.

**Stem cell donor**

R1150 Appearance of the cell clumping phenomenon after thawing of the nucleated cell preparations extracted from cord blood
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**Introduction:** The phenomenon of nonspecific cell aggregation (cell clumping) can be observed in nucleated cell preparations obtained from bone marrow, peripheral blood and umbilical cord blood (UCB) following thawing. Such preparations containing populations of both mature as well as immature hematopoietic progenitor cells are obtained by processing whole cord blood and freezing. Different techniques may be applied for the thawing of such preparations.

**Object:** To evaluate the phenomenon of cell clumping, the influence on it was examined at increasing densities of nucleated cell suspensions which had been extracted from cord blood and then frozen. The two selected techniques of thawing were also evaluated and their influence on cell clumping.

**Methods:** The fraction of nucleated cells from the 80 UCB was obtained by sedimentation. Samples containing the suspensions of these cells (5, 10, 20, 50 mln/ml) were cryopreserved. In vitro cultures of the colony forming cells (CFC) were performed before freezing and after thawing.

**Results:** The intensity of the cell clumping phenomenon increased simultaneously with the increasing density of the cell suspension. It increased from approx. 17% in the 5 and 10 mln/ml groups to approx. 43% in the 50 mln/ml group, when thawed accordingly to “the classic” technique. If the solution containing dextran (Rubinstein’s technique) was applied post thaw to dilute the cell suspension, the clumping phenomenon was markedly inhibited. It didn’t exceed approx. 15% in any density group. Independently to the intensity of the aggregation process, the number of the CFC among the whole pool that remained suspended after thawing (per 100 000 cells), maintained a quite stable level of approx. 70% of the pre-freezing value.

**Conclusions:** The intensity of the cell clumping phenomenon is directly influenced by the probability of the cell contact. Nevertheless, the substances like dextran may markedly inhibit this process. This phenomenon is not selective process and affects both early hematopoietic cells (CFC) as well as mature cells, independently of the initial density of the frozen suspension. It seems in order to protect thawed nucleated cell suspensions from clumping and preventing the associated looses, future studies will have to concentrate on the composition of adequate freezing mediums containing clumping inhibitors.

R1151 Adverse effects of PBSC collection in Blood Transfusion Centre of Slovenia, Ljubljana
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Collection of peripheral blood stem cells (PBSC) by apheresis is safe and reliable procedure that is generally well tolerated. Beside the adverse reactions associated with G-CSF or GM-CSF mobilization, some adverse effects related to the apheresis procedure can occur.

In the period from January 2004 to the end of October 2005 we retrospectively analyzed 141 PBSC collections in 74 autologous and 14 allogeneic donors. PBSC collections were performed in the Blood transfusion centre of Slovenia, Ljubljana, by standard volume apheresis procedures (two blood volumes processed) on the Amicus blood cell separator (Baxter). The targeted number of collected CD34+ cells was > 2 x10^6/kg of recipient’s body weight (BW) and > 5 x 10^6 / kg BW in plasmocytoma donors. Peripheral antecubital vein access was established in 117 (83 %), femoral catheter in 22 and subclavia catheter in 2 collections were placed without documented side effects.

We have identified 20 (14.3 %) adverse effects and 5 (3.5 %) instrument troubleshooting. In 11 (7.9 %) collections, the adverse effects occurred during the establishment of venous access (repeated phlebotomy 7%, unsuccessful phlebotomy 1%). During the collection we documented 9 (6.4 %) adverse reactions. The citrate reaction in 5 collections (3.6 %), hematoma in 1 collection (0.7 %), fatigue in 2 collections (1.4 %) and heart arrhythmia in 1 collections (0.7 %). Citrate reactions were mostly present as significant paresthesias and cured by oral calcium tablets and apheresis continued. There were no significant post donation decrease of platelet count and platelet transfusions were not necessary.

The one troubleshooting was due to the unrecoverable stop (0.7 %) an four were during the disposable set instalation (2.9 %).

The data confirmed the presence of mild but relatively common adverse effects in the collection of PBSCs by apheresis especially in the autologous donors. The serious adverse reactions were not documented.

R1152 Predictive factors that affect the mobilisation of CD34+ cells in healthy donors treated with recombinant granulocyte colony-stimulating factor
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No specific characteristics have been identified as predictors of peripheral blood stem cells (PBSC) mobilization in healthy donors.
In this study, clinical characteristics and laboratory data for 122 healthy donors who underwent apheresis on day 5 of treatment with recombinant granulocyte colony-stimulating factor (G-CSF) were retrospectively analyzed for correlations with CD34+ cell mobilization. The variables that were analyzed included age, sex, body weight, basal complete blood count and maximum white blood count (WBC) before apheresis, G-CSF type and dosage. Median age and body weight were 42.5 years (range 16-65) and 72.5 kg (range 47-121), respectively. By univariate analysis, male sex (p= 0.007), body weight (< 70 vs. >70 kg, p= 0.04) and donors age (<50 vs. > 50 years; p= 0.015) were associated with CD34+ cell mobilization. The variables that were correlated with the number of CD34+ cells mobilized. By multivariate analysis, donor’s age and male sex were the only two variables that significantly predicted a high CD34+ cell level. In conclusion, our data suggest that male sex and younger age are the only factors that significantly affect CD34+ mobilization in healthy donors.

R1153
The distribution of HLA-A*19 splits in Russian bone marrow donors
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HLA-A19 is a "broad" specificity, which includes a few serologically defined antigens (spits): A29, A30, A31, A32, A33, A74. The HLA-A19 occurs at a rather high frequency in most human populations, and the A19 splits are found at differing frequencies in different ethnic groups and populations. The distribution of A19 splits is very important for transplantation medicine, especially for bone marrow transplantation. Incorrect assignment of A19 splits is one of a major problems in using serology for typing HLA-antigens. Sometimes serology hardly discriminates A19 splits and the low resolution DNA typing is required. The data of A19 split distribution in Russians are few and ambiguous. The objective of our study was to determine the distribution of A19 splits in Russian donors typed in Research Center for Hematology (Moscow) in 2005.

Methods: 121 donors were typed. Serological typing was performed using commercial sera. In case of HLA-A19 or homozygosity in HLA-A locus revealed by serology the samples of donor blood were retyped using PCR-SSP ("Protrans" or kit of Research Center for Hematology, Moscow).

Results: By serological typing HLA-A19 was found in 24 donors (19.8%). The A19 splits were defined in 16. The HLA-A*19 (by PCR-SSP) was detected in 23 donors (19%). The most frequent among HLA-A19 splits was A*29 (n=7; 5.8%). A*30 and *31 were found with the equal frequency (n=5; 4.1%). A*32 was observed only in 2 cases (1.7%) and A*33 – in 4 cases (3.3%). In our donors we didn’t reveal A*74. In one case serologically defined A19 was not confirmed by PCR-SSP.

Conclusion: These results confirm the studies that DNA typing for HLA class I improves the typing quality. Serological typing is insufficient for HLA-A19 split identification.

R1154
Factors affecting PBPC collection in healthy adult donors: experience of IPO-Porto
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The majority of haematopoietic transplantation are currently performed with Peripheral Blood Progenitor Cells (PBPC). In donors, it is very important to optimize PBPC harvesting to obtain the target CD34+ cell dose required for transplant (>4.0x10^6/Kg body weight of the recipient) with a reduced number of apheresis. In the present study we retrospectively analysed data from PBPC collections of healthy adult donors (n=156, 84M /72F) with a median age of 39 years (range 17 – 73) performed between August 1995 and October 2005.

Donors were mobilized with daily administration of G-CSF (10-16mg/kg) for 5 days, with generally mild to moderate side effects. Total nucleated cells (TNC) and CD34+ cells present on PBPC grafts were evaluated with haematologic counters and flow cytometry respectively. On the collection day, the peripheral blood from most adult donors contained >20 CD34+ cells/µl, previously established as a marker of a good mobilisation. Healthy donors had standard apheresis collection using the Cobe Spectra, with few adverse consequences, mainly related either to vascular access or to metabolic or hemodynamic changes. The majority of donors (n=58%) underwent one apheresis, whilst the remaining 38% and 4% of donors had 2 and 3 apheresis respectively.

For adult donors the PBPC collected yielded a median 15.5 TNC x10^6/kg (2.9 – 168.1) and 8.7x10^6 CD34+ cells/Kg (1.0 –
64.4) body weight of the recipient and only in 3 collections the target number of CD34+ cells was not reached. We found a correlation between age of the donor and the number of CD34+ cells collected per Kg bw of the recipient, being in the older donors more difficult to achieve the required CD34+ cell dose. Our strategy was to increase male donors underwent one apheresis, had significantly higher PB CD34+ cell count prior to apheresis and CD34+ cell/Kg bw recipient collected in comparison to the female donors who had a median of 2 apheresis and had less cells (PB CD34+cells: 79±55 and CD34+ cell/Kg bw recipient 10.7±7.2 for male and female respectively).

There was no correlation between the G-CSF dose used in the mobilization and PB CD34+ cell count prior to apheresis or the total CD34+ cell collected.

Our results show that G-CSF mobilization and PBPC collection in adult healthy donors is feasible and safe for the harvesting of the graft for allogeneic haematopoietic transplantation and the main factors affecting collection are age and gender of the donor.

R1156
Thrombophilic screening in healthy donors treated with recombinant-human granulocyte-colony stimulating factor for mobilisation of peripheral blood stem cells
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The granulocyte-colony stimulating factor (G-CSF) induces an activation state of endothelial cells and coagulation system increasing thrombotic risk. Laboratory testing for the identification of heritable thrombophilia in high-risk subject groups have become common practice.

The objective of this study was to evaluate the effectiveness of thrombophilia screening in healthy donors prior to use G-CSF to mobilize peripheral blood stem cells (PBSC). Thrombophilia screening comprised of testing for Factor V Leiden (FVL) G1691A, prothrombin (PTM) G20210A, thrombolocial variant (C677T) of the methylene tetrahydrofolate reductase (MTHFR) gene, protein C (PC), protein S (PS), Factor VIII (FVIII) and homocysteine (Hcy) plasmatic levels, antithrombin III (ATIII) activity, and acquired activated protein C resistance (APCR).

We investigated prospectively 72 healthy donors, 39 men and 33 women, with a median age of 42 years (range 18-65). Five donors (6.9 %) were heterozygous carriers of FVL G1691A: two healthy donors had the heterozygous PTM G20210A gene mutation; C677T mutation in the MTHFR gene was present in 34 (47.2%) donors in heterozygous and in 7 donors (9.7%) in homozygous. APCR was revealed in 8 donors of the study (11.1 %). The PC plasmatic level was decreased in 3 donors (4.2 %); the PS level was decreased in 7 donors (9.7 %). An elevated FVIII dosage has been showed in 10 donors (13.9 %) and Hyperhomocysteinemia in 9 donors (12.5 %). Concentration of ATIII was in the normal range for all study group donors. The FVL mutation was combined with the heterozygous PTM G20210A in two cases and with PS deficiency in one case; two healthy donors presented an associated deficiency of PC and PS.

The basal screening of thrombophilia permitted to identify healthy donors with a higher risk of thrombotic events. A careful monitoring should be considered in these cases before administer G-CSF. During G-CSF therapy, we administered low-molecular-weight heparin in all donors and folic acid, vitamins B6 and B12 in healthy donors with C677T mutation in the MTHFR gene and a hyperhomocysteinemia plasmatic levels. Our strategy of prophylaxis was correlated with the absence of short- and long-terms thrombotic and hemorrhagic side effects. No complications known to be related to the anticoagulant occurred in this cohort of healthy donors.

Tolerance and rejection

R1157
Differential levels of chimeric tolerance in a single patient. How much change in quantitative chimerism values is enough for clinical significance?
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Chimerism monitoring based on STRs is frequently used following SCT. It is best implemented in terms of long-term tracking since engraftment is a dynamic process. When viewed this way, trends and fluctuations in the chimerism values can be observed. One fundamental question raised by such observations is what is the minimal change in chimeric status that has biological and/or clinical significance. As an indirect approach to this question, we present a case of Thalassemia Major which we were fortunate to follow over a 7 year multi-sample course with two SCTs each with its own stable % chimerism level. Following the first SCT the patient developed a stable chimeric tolerance of approximately 30% [27-35%]. However, he remained transfusion dependent, necessitating a second transplant two years ago. Once again his chimerism stabilized but at a higher plateau of approximately 42% [39-44%]. He is currently healthy, and no longer requires transfusions. In conclusion, this interesting case afforded us the opportunity to compare two different, but stable levels of chimeric tolerance, differing by approximately 10-12%. Since over the long-term, STR-platform error is approximately 2-5%, the patient is likely to have sustained a stable elevation of approximately 10% chimerism that protected him from transfusion dependency. The case raises a number of important questions regarding the biological implications of chimerism values, such as: 1) is 10% a relative or absolute figure; 2) is there a minimum/threshold chimerism value for functionality of the graft in these metabolic diseases; 3) do all patients have the same % chimerism interval/differential and threshold?

R1158
Novel aspect of long-term chimeric tolerance in a patient with severe combined immune deficiency: in utero stem cell transplant suppressed by subsequent paternal iatrogenic transplant
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Severe Combined Immune Deficiency (SCID) is often treated with stem cell transplantation (SCT). The successfully treated patient usually lives in a state of mixed graft-host chimeric tolerance. This relationship, however, may not be entirely static. We report here a case of a child in which a transfusion of maternal stem cells occurred in utero. This was documented by demonstrating a mixed chimerism in the child based on an evaluation of the child’s blood using HLA tissue typing and molecular DNA methods. At age 3 mo the patient manifested SCID, which necessitated an exogenous SCT from the father. Following the paternal SCT, three DNA sources were demonstrated, with four HLA haplotypes. However, in the ensuing period progressive loss of the maternal component of the patient’s tolerance state was observed. Now, after 8 yr post-paternal SCT, the patient is healthy, but there is no significant maternal DNA signal demonstrated in the child, although he does have a stable chimeric level of 25-35%.

In Conclusion, the three-way mixed chimerism was not tolerated, and the in utero maternal stem cell component was ultimately suppressed by the subsequent paternal SCT. This case suggests that even a tri-valent tolerogenic state may be regulated by the dynamic interactions between the host and graft, enabling one set of graft-host interactions to become dominant.
R1159  
**Successful treatment of graft rejection with immunosuppression withdrawal and/or donor leukocyte infusion after early diagnosis based on T-cell mixed chimerism**

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Background: Graft rejection is a serious and difficult to manage complication after SCT. Objective: To evaluate the usefulness of chimerism monitoring for the early diagnosis of graft rejection in different SCT settings, as well as for the follow up after treatment with immunosuppression withdrawal (ISW) and/or DLI. Patients and Methods: 68 SCT (32 ablative, 19RIC, 17 TCD – including 8 haploidentical–). Chimerism analysis was performed by FISH for the sex chromosomes or STR-PCR (sensitivity 1%). Chimerism was analyzed in PB and leukocyte lineages (T lymphocytes CD3+, B lymphocytes CD19+ and myeloid cells CD15+ isolated (purity >95%) by immunomagnetic means, AutoMACS, Miltenyi Biotec), every 2 weeks, starting on day +15 (except in ablative), and until complete chimerism (CC) was achieved. Results: After initial engraftment in all patients, graft rejection was diagnosed in 8 (2.6%) ablative, 2 (10%) RIC, 4 (23%) TCD patients (established (severe pancytopenia and BM aplasia) or incipient (progressive decrease in PB and BM cell counts) a median of 52.5 days (range 23-110) after SCT. All patients showed mixed chimerism (MC) in BM and PB with higher percentages of recipient cells (%R) in PB. In 7/7 patients studied, T cells showed persistent MC with high %R (>50% in 6/7; >50% and >25% in 1/7). B cells showed MC in 5/6 patients studied, with lower %R (<15% in 3/6, >50% in 1/5). Only 2 patients showed MC, transient in one of them, in myeloid cells. 2 patients were not treated due to concurrent multiorgan failure and subsequently died. Reduction of IS in 5 patients obtained 1 response (normal PB and BM counts, and CC). The other 4 patients underwent ISW but no further response was obtained. One of them received a second SCT while the other 3 were treated with DLI, and all of them responded. The last patient (transplanted from a haploidentical family donor) who was not receiving IS, responded to treatment with DLI. Time from therapeutic intervention to response was variable with a median of 2 months (range 1-7). 4 patients developed GVHD+1, which was the cause of death in one and was controlled in the other three. One patient died from sepsis in complete remission (CR). After the transplantation, patients are alive in CR a median of 50 months after SCT (range 16-72). Conclusions: The observation of MC, mainly in T lymphocytes, together with a decrease in PB and BM cell counts, allowed early diagnosis and successful treatment (6/6 patients) of graft rejection.

R1160  
**Immunosuppressive effects of nucleic acids – or how to learn from artefacts?**

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Defibrotide, a single-stranded nucleic acid (ssNA), was already shown to mediate immunosuppressive effects. In our current survey we investigated whether randomly chemically synthesized ssNAs of different length and composition could provide similar effects. For this purpose, purified T-cells were stimulated in the presence of dNTP or ssNA with allogeneic, irradiated PBMC’s, PHA or anti-CD3/CD28 Dynabeads. Cellular proliferation was assessed by incorporation of tritium-labelled thymidine (³H) thymidine, respectively (³H)dAMP or by staining with CFDA (carboxyfluorescein diacetate, succinimidyl ester). After 72h or 120h of incubation, the incorporation of (³H)thymidine, or (³H)dAMP as well as the CFDA distribution was assessed. Cell viability was measured by trypan blue exclusion. T-cell activation was measured after 72h by quantifying the number of CD3+ T-cells expressing the activation markers CD25 and CD69. Cellular uptake of Cy5-labelled NAs was detected by fluorescence microscopy. Moreover, the interference of different NAs or singular nucleotides with nucleoside analogues or T-cell proliferation was tested by CFDA-assays. NA of different length, composition or concentrations (up to 5mM) did not cause cytotoxic effects to lymphocytes. But the incorporation of (³H)thymidine or (³H)dAMP was competed by NA. These effects were found to be dependent on length, concentration and base-composition of the NA. The proliferative capacity of the T-cells, as assessed by CFDA-staining, seemed to be unaffected. Moreover it could be shown, that NAs interfere with nucleoside analogues and antagonized the anti-proliferative and cytotoxic effects of these drugs. The standard approach to detect cellular proliferation by incorporation of tritium-labelled nucleotides or derivatives is not useful to assess changes in cellular metabolism or proliferation in context with NAs. Even more important, treatment approaches using nucleoside analogues like fludarabine, cytarabine e.g. in context with NAs might be critical and diminish the efficacy of these drugs.

R1161  
**Increasing mixed chimerism after haematopoietic stem cell transplantation is related to a brief time marrow relapse and other clinical variables. A perspective analysis with a 16 multiplex PCR-based STRs panel**

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Introduction: Chimerism status (CS) analysis is useful for evaluation of donor (D) cells engraftment after allogeneic haematopoietic stem cell transplantation (HSCT). Adverse events are often described as associated to a loss of chimerism, but a strict correlation between CS and residual disease is still controversial. PCR-based assays analyzing polymorphic Short Tandem Repeat (STR) markers are actually the more employed methods in CS monitoring, even if a standardized specific panel is not still available. Objective: We tested a semi-quantitative method, based on multiplex PCR amplification of 16 STR markers using a commercial kit (PowerPlex 16 System Promega), in order to evaluate its informativity in CS monitoring and the correlation between CS and some clinical variables. Methods: The informativity of the assay was tested on peripheral blood samples from 36 allografted patients (pts) and their related sibling donors; perspective evaluation of CS was performed on 27 pts at 1,2,3,4,6,12,18 and 24 months after HSCT. Pts who showed no evidence of recipient (R) cells were considered to have a complete chimerism (CC), pts who presented each D and R cells were defined as mixed chimerism (MC). Results: The multiplex assay gave at least one high informative marker (range: 1-4, median 2) in all the 36 pts. We analyzed 125 blood samples from 27 pts, 98 (78,4%) presented CC, 27 (21,6%) MC and none autologous reconstitution. Some pts were defined as having an increasing MC (IMC) when they shifted from CC to MC or when in a MC setting the R amount increased in two or more consecutive controls. We evaluated if there was a correlation between CS and some clinical variables: R/D gender, diagnosis, R/D sex mismatch, ABO system incompatibility, conditioning regimen, CD34+ and CD3+ cell dose infused, disease status at HSCT, stem cells source, acute and chronic Graft versus Host Disease (GVHD), marrow relapse. Chi-square analysis demonstrated a significant correlation between ICM and a brief time marrow relapse, moreover a low incidence of chronic GVHD, male D and diagnosis of acute leukemia seem to be associated with increasing level of R DNA. Conclusion: PCR amplification of a panel of 16 STR loci is an informative method to evaluate CS in pts after HSCT. IMC
seems to be useful to predict marrow relapse; some clinical conditions such as male donor and acute leukemia diagnosis seem to limit a complete D engraftment; chronic GvHD is favorable for a stable CS.

Non-haematopoietic tissue repair

R1162

GMP production of autologous CD133+ cells for intratrauma administration after acute myocardial infarction

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Subjects affected by acute myocardial infarction (AMI) with absent angiographic myocardial blush (MB) and lack of ST segment elevation resolution after primary angioplasty, have short- and long-term poor clinical prognosis. We recently started a Phase I/II randomized controlled study based on the hypothesis that, in this target population, after primary angioplasty and stenting, intracoronary injection of CD133+ cells from bone marrow (BM, group A) or mobilized peripheral blood (mPB, group B) could enhance endothelial regeneration and improve heart function compared to controls treated with standard pharmacological therapy alone (group C). The study started in June 2004 and is expected to enroll 15 patients (5 per each randomization group). Up to November 2005, 12 patients have been included. In group A (n=4), BM was processed within 2 hours of collection. In group B (n=3), the administration of G-CSF (5 µg/kg/day for 3–4 days), started from day 6–10 after AMI and leukapheresis was performed following standard procedures. An automated CD133+ stem cell selection was performed with the CliniMACS® plus Instrument (Miltenyi Biotec) in our class B – ISO 6 facility. The mean (±SD) number of CD133+ cells after immunoselection was 8.2x10⁶ (±4.3) in BM samples and 147.7x10⁶ (±182.3) in mPB samples respectively, with a purity of 62% (±8) in the group A and 91% (±8) in group B. The percentages of viable cells (Propidium Iodide) in the post-selection samples were 90 (±1) in group A and 99 (±1) in group B, respectively. The sterility tests for bacteria and fungi on the purified samples were negative. Pulurified CD133+ cells were injected in the culprit vessel using a well-sized over-the-wire angioplasty balloon within three minutes of occlusion. No adverse events have been reported during and immediately after the cell administration. This results show that CD133+ selection is feasible and safe also in AMI patients. The short and long term efficacy of this cell therapy approach in preserving the myocardial viability and function after AMI is currently under investigation using PET-based techniques and echocardiography.

R1163

In vitro monitoring of defibrotide prophylaxis for endothelial complications in allogeneic stem cell transplantation

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Defibrotide (DF) is a polydisperse mixture of 90% single-stranded polydeoxyribonucleotides with anti-thrombotic, pro-fibrinolytic and anti- apoptotic functions. DF is already successfully used in the treatment of hepatic veno-occlusive disease in allogeneic stem cell transplantation (SCT). Our observation that DF can also protect endothelial cells (EC) from conditioning (Fludarabine)-mediated apoptosis (1) prompted us to apply it prophylactically to patients (pts) at risk for endothelial complications. Pending on the magnitude of risk, pts received 200-800mg every 6h in 2h-infusions, usually from day (d) -7 until d+21 post SCT.

Circulating EC (CEC) as a marker of conditioning-mediated endothelial toxicity (2) were detected by magnetic bead separation of CD146+ cells from EDTA blood of SCT pts (20 DF, 13 non-DF) and co-staining with Ulex Europaeus antigen lectin 1. CEC maxima until d+100 post SCT were compared between the two groups. An overlap of maxima was significantly lower maxima of CEC than untreated pts (1142 ±1082) in the DF treatment group vs. 2508 ±1987 CEC/mL in non-DF pts, respectively, p=0.008). Similarly, when CEC maxima were compared in the time period of DF prophylaxis, again, DF pts had less cell counts (499 ±615) vs. 1498 ±1709 CEC/mL in control pts, respectively, p=0.01). In an overlapping cohort of 52 pts (21 non-DF, 31 DF) serum was assayed for its induction of apoptosis in a human microvascular endothelial cell line (HMEC), a monitoring approach that had been found to correlate with episodes of GvHD and severe microangiopathy (3). Apoptosis was determined by flow cytometric analysis of the cellular granularity of propidium-iodide-negative indicator HMEC. Similar to the CEC measurements apoptosis inducing maxima until d+21 were compared between DF and non-DF pts and turned out to be significantly different (Apoptosis by pts’ sera normalized to untreated control HMEC: 4.4 ±2.0 in DF vs. 5.9 ±4.2 in non-DF pts, p=0.04).

These preliminary analyses suggest the protective efficacy of DF prophylaxis in the course of SCT. The final proof of principle is to be validated in long-term clinical follow-ups.

1. G. Eissner et al., Blood 100, 334-340 (2002).
2. A. Woywodt et al., Blood 103, 3603-3605 (2004).
3. A. Gaon et al., Bone Marrow Transplant. 33, 355-357 (2004).

Aplastic anaemia

R1164

Treosulfan, cyclophosphamide and anti-thymocyte globulin for allogeneic haematopoietic stem cell transplantation in severe aplastic anaemia

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The major cause of failure after alloSCT in severe aplastic anaemia is Graft rejection, which is major cause of failure after alloSCT in severe aplastic anaemia (SAA), when cyclophosphamide is used as a single agent for conditioning. To reduce the risk of this complication we decided to intensify the preparative regimen by adding reduced dose of treosulfan – an alkylating agent possessing both immuno- and myeloablative properties. Between 2003-2004 six patients (age: 21(14-25) years) were treated in a single institution with alloSCT from either HLA-identical sibling (n=3) or an unrelated volunteer (n=3). Conditioning regimen consisted of treosulfan 10 g/m²/d on days -7, -6, cyclophosphamide 40 mg/kg/d on d. -5, -4, -3, -2, and anti-thymocyte globulin (thymoglobulin, Genzyme) 2 mg/kg/d on d. -3, -2, -1. Each bone marrow and peripheral blood was used as a source of stem cells in 3 cases. All patients engrafted with the median time to neutrophil >5x10⁹/L and platelet >50x10¹⁰/L recovery of 16 (14-22) days and 21.5 (12-29) days, respectively. Complete donor chimerism was achieved on day +30 in all cases. None of the patients developed grade III-IV acute GVHD, one patient experienced grade II acute GVHD. At one year the cumulative incidence of extensive chronic GVHD equaled 33%, overall cGVHD – 50%.
At the median follow-up of 12.5 (12-25) months all patients remain alive and disease-free with complete donor chimerism. At one year the Karnofsky index equaled 100% in 5 patients, 70% - in one case.

We conclude that treosulfan + cyclophosphamide + antithymocyte globulin is a well-tolerated preparative regimen and allows stable engraftment in SAA patients. The use of treosulfan allows intensification of the conditioning without providing an additional non-hematological toxicity.

R1165
Clinical outcome in adults with severe aplastic anaemia. A retrospective single-centre analysis
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Introduction: Allogeneic bone marrow transplantation (BMT) is the treatment of choice in young patients suffering from severe aplastic anaemia (SAA). Due to improved, less toxic conditioning regimens and advances in prophylaxis against graft-versus-host-disease (GVHD) survival has improved steadily. Nonetheless, long-term side effects, such as chronic GVHD, occur in up to 30% of patients requiring treatment and lead to an increased mortality. Here we present the analysis of for patients with SAA comparing. Patients and methods: Between 1987 and 2005, Twenty one patients (13 male, 8 female) with SAA and one patient (female) with paroxysmal nocturnal haemoglobinuria (PNH) were transplanted in our centre. The median age at transplantation was 24.5 years (range 17 – 46). Fifteen patients were transplanted with stem cells from their HLA-identical related donor, 5 patients from HLA-identical matched unrelated donors and 2 patients were transplanted from a syngeneic donor. In 19 cases stem cell source was bone marrow (BM), 1 patient received BM and peripheral stem cells (PBSC) and 2 were transplanted with PBSC. Conditioning regimen consisted of cyclophosphamid (Cy) alone (n=14), or in combination with either total nodal irradiation (n=1), or with Flu darabin (Flu, n=3). One patient was treated with Cy and total body irradiation (TBI) while1 patient received Flu, Cy and TBI. GVHD-prophylaxis was cyclosporin (CsA) and methotrexate (MTx) in all but the 2 patients transplanted from syngeneic donors. All patients engrafted. Acute GVHD developed in 9 patients (41%) and was readily controlled by immunosuppression. Chronic GVHD occurred in 9 patients (36%; 6 limited, 2 extensive) within a median follow-up of 3 years (range 0.1-15.5 years). Twenty one out of 22 patients are alive and free of haematological disease, one patient died because of toxoplasmosis before day +50.

Conclusion: The incidence and severity of acute and chronic GVHD is similar other studies. Our data suggest that BMT is a favourable therapy for young patients with aplastic anaemia, showing good engraftment, controllable complications and a good clinical outcome.

R1166
Stem cell transplantation and immunosuppressive treatment of severe aplastic anaemia: single-centre experience
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Background: Stem cell transplantation (SCT) from an HLA-identical fully matched sibling donor (MSD) is the best treatment option for severe aplastic anaemia (SAA). Patients without suitable MSD should be treated with immunosuppressive therapy (IST).

Patients and methods: between 10/1995 and 11/2005 26 patients with newly diagnosed SAA were treated either with SCT from MSD (11 patients) or with IST (15 patients). There were performed 12 allogeneic SCT in 11 patients. All donors were HLA-identical sibling (1 donor was identical twin). Source of stem cells was bone marrow in 9 (one with second transplant) and peripheral stem cell in 3 SCTs. Conditioning regimens were based on cyclophosphamide (Cy) with ATG in 19 cases and Fludarabin with Cy and ATG in 3 SCTs. 13 patients received combined IST with antithymocyte or antilymphocyte globuline (ATG/ALG), cyclosporine A and steroids and 2 patients ATG with steroids. The median interval from diagnosis to ISH was 30 days (range 23 to 510).

Results: engraftment was documented in 10 patients with allogeneic SCT (1 patients died without engraftment). One patient developed acute GVHD grade 3-4 and died after 48 days, and the other had pneumonitis interstitialis (CMV+) and died after 60 days. Till November 2005 7 of 10 patients (70 %) are alive with sustained engraftment. Median survival from SCT is 44 months (range 6 to 104). Considering IST, 13 of 15 patients (86.7%) achieved response (4 had two or three cycles of IST). One patient relapsed 1 year after IST. Two patients from IST group died, major causes of death were infection and hemorrhage. Overall survival in the IST group is 73.3% (11/15 patients) after a median follow up of 38.7 months (range 6 to 121).

Conclusion: Our results confirm significant improvement in outcome of SAA patients during last decades in due to modern induction front line therapy including allogeneic

Autoimmune diseases

R1167
Successful treatment of autoimmune thrombocytopenic purpura after bone marrow transplantation with anti-CD20 antibody: a case report
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We describe a case of persistent, severe autoimmune thrombocytopenia, refractory to prednisolone but responsive to chimeric monoclonal antibody anti-CD20 (Rituximab). A patient transplanted for Hodgking disease (UPN 576), developed autoimmune thrombocytopenia with severe bleeding, 150 days after unrelated bone marrow transplantation: at the same time there were no signs of graft-versus-host-disease, cytomegalovirus infection, sepsis or microangiopathic process. High titer of antibody against platelet antigens was found. During treatment with prednisolone 1 mg/Kg, for two days, the platelet count remain below 5000/µL. In spite of increasing the dose of cyclosporine and methylprednisolone (2 mg/Kg/daily) with addition of intravenous immunoglobulin for five days, only a transitory partial response (platelet 40000/µL) was observed. After four days from last dose of immunoglobulin, the platelet count fell again below 5000/µL; antiplatelet antibodies still highly positive.

After two weeks of therapy with steroid, we started with anti-CD20 antibody with first dose of 375 mg/m² followed by dose of 100 mg/m² once weekly for 3 weeks. Complete response was achieved after 6 weeks from therapy initiation, with a complete normalization of platelet count and with disappearance of antiplatelet antibody in peripheral blood. No apparent toxicity, or side effects that could be attributed to Rituximab were observed. The patient is in complete response 5 months after therapy with anti-CD20. Rituximab induced complete response in approximately 50% of the patient with immune thrombocytopenic purpura refractory to prednisone or splenectomy; it’s also effective in patients with secondary ITP. To our knowlege, only few cases of ITP after bone marrow transplantation have been successful treated with Rituximab. We suggest for an early use of anti-CD20 in similar cases.
Malignant forms of multiple sclerosis (MS) are rare cases characterized by very aggressive demyelination and rapid progression to disability leading to death within 5 years from onset despite treatment, which fails to control the disease. We report a case of a young male patient of 19 years old with aggressive MS who was treated with a high-dose immunosuppressive regimen (HDIS) using myeloablation followed by autologous blood stem cell transplantation (ASCT) that has induced a dramatic and long-lasting remission of the disease. The patient was diagnosed with MS in June 2000. At that time he had minor disability, his EDSS score being 3.5, and active disease seen on MRI showing 10 gadolinium-enhancing (Gd+) lesions. He was on steroids and interferon-beta which, however, had no effect, while disability was rapidly accumulating. By February 2001, i.e. within 8 months, the EDSS score rose to 6.5 and the patient was unable to walk unaided for more than 20 metres. On MRI, Gd+ lesions increased to 18, as did their volume. In March 2001 we decided to treat him with HDIS and, in order to mobilize blood stem cells, he received cyclophosphamide (CY) 4 g/m² plus GCSF 10 µg/kg. Six days after CY infusion he had a disease flare with worsening of EDSS score to 7.5, and further increase in the number and volume of Gd+ lesions. The patient was treated with steroid pulses for 3 days and showed some improvement which allowed the continuation of G-CSF and subsequent stem cell collection. Two months after CY infusion, he underwent ASCT with busulfan 16 mg/kg over 4 days plus antithymocyte globulin 7.5 mg/kg for conditioning. One month post ASCT the EDSS score dropped to 3.5 and no Gd+ lesions were detected. The patient continued to improve over the following years. The last assessment at 52 months after ASCT showed nearly absent disability (EDSS score; 1.5) and, again, no Gd+ lesions on MRI. The spectacular responses of this case and also of the three similar cases reported in the literature support the role of ASCT in rapidly evolving, so-called malignant, forms of MS.

R1169
Treatment of a malignant form of multiple sclerosis with immune ablation and autologous stem cell transplantation
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Objective: Scleromyxedema is characterized by cutaneous deposition of mucin, dermal fibrosis and monoclonal gammopathy. The response to treatment has been variable and no Gd+ lesions on MRI. The spectacular responses of this case and also of the three similar cases reported in the literature support the role of ASCT in rapidly evolving, so-called malignant, forms of MS.

Conclusion: HDT and ASCT may be an alternative treatment in the amelioration of lesions related to scleromyxedema.

R1168
An obvious amelioration in a case of scleromyxedema after successful double autologous peripheral blood stem cell transplantation followed by thalidomide and bortezomib consolidation
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Objective: Scleromyxedema is characterized by cutaneous deposition of mucin, dermal fibrosis and monoclonal gammopathy. The response to treatment has been variable after several treatment modalities including high-dose treatment (HDT) and autologous stem cell transplantation (ASCT).

Methods: We report a case of scleromyxedema who achieved amelioration after double HDT and ASCT, followed by thalidomide and bortezomib consolidation. A 38-year-old male patient was admitted with persistent pruritis. He was treated with anti-inflammatory and antidepressive drugs with diagnosis of neurodermatitis; however, the skin was thickened and papular lesions appeared. A skin biopsy revealed scleromyxedema. He was treated with topical corticosteroids and retinoid acid preparations. However, no amelioration was noted. Topical cyclophosphamide, systemic corticosteroids were used for three months, then the patient has discontinued all the medications. Low-dose interferon-a treatment (1.5 mU) was initiated for three times weekly; however, the treatment was stopped due to an acute and severe rhabdomyolysis after the third administration. On his admittance in our department, he had papular lesions of 0.3 mm without pain, erythema or desquamation all over his body. He had also some nodular formations of 1.3 x 1.5 cm, on his face and neck. The whole blood count and biochemical analysis were normal. Erythrocyte sedimentation rate (ESR), CRP were within normal limits and all viral markers were negative for hepatitis, EBV, CMV, Toxoplasma, HIV as well as rheumatologic markers. Serum immunoglobulin (Ig) levels were within normal ranges except for Ig G and Ig light-chain lambda. The peripheral blood smear and bone marrow aspiration and biopsy revealed no pathology. He then underwent an ASCT after conditioning with melphalan (200 mg/m²) and a second transplant was done four months later using the same conditioning regimen (140 mg/m²).

Results: After the transplantation, the immunoglobulin levels have partially regressed. Physical appearance has been ameliorated and the skin biopsy revealed a regression in mucin deposition in dermis. Consolidation treatment was initiated with thalidomide followed by bortezomib. He is still on his follow-up without any progression for three years.

Conclusion: HDT and ASCT in rapidly evolving, so-called malignant, forms of MS.
transplantation. These results demonstrate that GMP production of CD34+ cells for autologous transplantation is feasible, safe and could efficiently support a transplantation program for patients affected by SS.

R1171
High-dose immune immunoablative therapy and autologous stem cell transplantation in severe resistant Crohn's disease: profound response for 18 months followed by treatable relapse
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We report the case of a 25 year old female with severe resistant Crohn’s disease (CD) treated with High-dose immune immunoablative therapy (HDIT) and autologous stem cell transplantation (SCT). Diagnosed with CD at age 11, initial disease control had been achieved with azathioprine and steroids, but, at age 14, colectomy and ileostomy were performed for a severe flare. From age 22, increased disease activity was unsuccessfully controlled with azathioprine, steroids, infliximab, methotrexate, combination rifabutin/metronidazole/clarithromycin, thalidomide and tacrolimus. From Nov 2000-Jan 2003, 5 surgical episodes had resulted in resection of 1.25m small bowel. Dietary modifications had included an elemental diet, but from 2002 the patient was dependent on home total parenteral nutrition. Severity of symptoms had resulted in recurrent and prolonged inability to work and to warrant care under a palliative medicine specialist. Based on poor quality of life, risk of life threatening complications, and inability to control the disease effectively, the option of autologous transplant was pursued after examination of the case and proposed treatment protocol by the local Research Ethics Committee, review by two independent gastroenterologists and one transplant haematologist, and obtaining informed written consent.

Treatment commenced in Sept 2003 with mobilisation using cyclophosphamide (Cy) 2g/m² and G-CSF. In Nov 2003 the patient was treated with Cy 200mg/kg, rabbit ATG 6mg/kg and methylprednisolone 1gx5 followed by 3.0 x10⁶/kg isolex enriched CD34+ cells. Treatment was complicated by neutropenic sepsis, orpharangal and stomal mucositis. Engraftment time was within normal limits. Discharge was on day+16. Pre- and 12 months post-treatment data are summarised in the table. CD remained inactive until March 2005 with the development of increased stoma output and abdominal pain. Relapse was confirmed by ileal biopsy. In contrast to pre-SCT, disease control has been achieved with immunosuppressants and surgery, permitting ileal reanastomosis, pouch formation and reversal of ileostomy in Sept 2005. The patient remains stable as of Nov 2005.

In conclusion, HDIT and autologous SCT may be an effective therapy for medium term control of severe, treatment resistant CD. It remains to be seen whether post-SCT relapse is easier to control than CD activity before SCT, as suggested by data in other autoimmune diseases. In addition to randomised trials, future studies could look at means of prolonging response, such as maintenance treatments.

Chronic leukaemia

R1172
Hyperkalaemia induced by cyclosporin in bone marrow transplantation patients
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Hyperkalaemia is one of the side effects of cyclosporin (CSA). The mechanism of hyperkalaemia is unclear. Apparently many factors in pathogenesis of hyperkalaemia may be involved. Nephrotoxicity of CSA that significantly impairs renal perfusion, glomerular filtration reduced K+ excretion and secondary hypoaldosteronism seemed to be the reasons for CSA-associated hyperkalaemia. There are publications about hyperkalaemia in patients after renal transplantation but only few reports devote this phenomenon in bone marrow transplantation patients. We report about 2 cases of hyperkalaemia with bradyrhythmia during CSA administration in patients with CML in 1 CP, male 27 and 46 years old undergoing BMT from HLA - identical siblings. Hyperkalaemia (6.2 - 6.5 mmol/l) and bradyrhythmia (40-50 /min) in 42-68 days after transplantation was observed. At that time a serious worsening in renal function (increase serum creatinine and serum urea, decrease filtration rate) and in serum CSA level rise was found in both. After discontinuation of CSA treatment all these symptoms disappeared. These observations suggest that CSA may be a cause of hyperkalaemia associated with bradyrhythmia in bone marrow transplantation patients. Careful monitoring of CSA level in blood and renal function may be important in prevention these complications.

R1173
Thalidomide therapy for refractory chronic lymphocytic leukaemia after allogeneic stem cell transplantation
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Objectives: Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is the only curative therapy for patients with chronic lymphocytic leukaemia (CLL). However, transplant-related mortality remains relatively high and relapse is still a major problem. There are only few anecdotic reports of the use of thalidomide, an immunomodulating agent, in such patients.

Case report: 48-year old patient with resistant CLL is presented. He was treated unsuccessfully with several cycles of different agent combinations, started with chlorambucil-methylprednisolone then fludarabine-cyclophosphamide and also with monoclonal antibody rituximab. As he has HLA matched sister we proposed allo-HSCT.

On admission before transplantation patient was presented with massive lymphadenopathy, anemia (Hb 76 g/L), WBC 24.8 x 10⁹/L with absolute lymphocytosis (lymphocytes 98%) and thrombocytopenia 23 x 10⁹/L. Pre-transplant conditioning consisted of high-dose cyclophosphamide and total body irradiation. Combination of monoclonal antibody alemtuzumab (Campath-1H), cyclosporine A and short methotrexate were given for the prevention of acute graft versus host disease (GVHD). The patient received 2.4 x 10⁶/kg stem cells. There were no serious complications in post transplant period. Lymphadenopathy completely disappeared. Patient was discharged 4 weeks after transplantation, without GVHD and with normal complete blood counts (CBC). On bone marrow examination there was still residual leukaemic infiltration (70%). Two months later acute GVHD developed, with skin involvement only. At that time lymph nodes massively enlarged again and high WBC with absolute lymphocytosis reappeared. Cyclosporine immunosuppressive therapy was stopped and thalidomide 100 mg/day was introduced combined with methylprednisolone 8 mg three times per week. During period of 4 months treatment he was readmitted.
onc for lung aspergillosis which responded well to voriconazole. Improvement appeared slowly, with regression of lymphadenopathy. After 4 months he still has residual leukemic marrow infiltration – about 30%, but normal CBC without absolute lymphocytosis.

Conclusion: Our patient is an example that thalidomide may have significant antileukemic effect in refractory CLL. Due to this and anti GVHD effect perhaps in the future it could be incorporated as a first line GVHD prophylaxis regimen in patients transplanted for CLL.

R1174

Intensive combination therapy and autologous PBPC for blast crisis CML revisited

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Imatinib is the most active therapy for chronic myeloid leukemia (CML) in all phases of the disease. Overall survival of patients with blastic phase CML treated with Imatinib monotherapy however is 6 months only. The reason for Imatinib resistance in advanced phase CML is mostly due to BCR-ABL independent mechanisms. Therefore a combination therapy of Imatinib with conventional high dose chemotherapy is often used for remission induction and allogenic stem cell transplantation is performed if a suitable donor is available. Autologous stem cell transplantation has drawn new attention in the treatment of CML since sufficient numbers of peripheral stem cells (PBPC) can be mobilized under concomitant treatment with Imatinib and collection of BCR-ABL negative autologous peripheral stem cells has been reported.

We tested in a pilot trial of 3 patients the feasibility of a treatment consisting of Imatinib 600mg qd combined with 4 cycles of cytarabine 1000mg/m² x 7d in patients with CML in blast crisis (BC) who do not have a HLA-matched stem cell donor. PBPC were mobilized after the 4th cycles of cytarabine with G-CSF (filgrastim) 10µg per day. PBPC were cryopreserved and reinfused after a conditioning therapy with either cyclophosphamide 200mg/kg and busulfan 16mg/kg (BuCy)(table 1: patient 2 + 3) or cyclophosphamide 120mg/kg plus 12 Gy TBI (Cy/TBI)(table 1: patient 1)

The stem product of all 3 patients contained sufficient numbers of CD34+ cells after mobilization with G-CSF after the 4th cycle of high dose cytarabine and Imatinib given through. The autologous PBPC graft of one of the patients was BCR-ABL negative, a second graft had detectable BCR-ABL at low level in the Q-RT-PCR (table1). No data on the presence of BCR-ABL is available for the third graft. Engraftments of the transplants were in the expected range. No excessive toxicity was recorded. Treatment of CML in BC with Imatinib combined with high dose cytarabine and autologous PBPC after high dose chemo-/radiotherapy is feasible and may result in sustained complete molecular remission.

At the time of imatinib era in CML treatment HSCT became a disputable issue. In this study we compared the outcome of unrelated donor transplantation to that in sibling donor setting in CML patients receiving reduced toxicity conditioning. 19 patients received HSCT from matched sibling donors, 30 patients from unrelated donors. Reduced toxicity consisted of: Busulfan 8mg/kg, Fludarabine 120mg/m² (allo sib) or 150mg/m² (MUD) and ATG. Donors for unrelated transplant were matched for 10 specificities at following resolution levels: i) loci A, B, C at intermediate or high resolution and DR at high resolution level only. Patients were stratified into 3 groups: i) ALLO-SIB ( HLA matched ; n=19), ii) MUD fully matched (10/10 match at intermediate or high resolution level for HLA.

R1175

Alemtuzumab and autologous SCT in CLL, experience in a small centre

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Alemtuzumab (AL) as a single agent or in combination with chemotherapy is an effective treatment for Chronic Lymphocytic Leukemia (CLL) in refractory or relapsing patients (pts) and has been shown to induce complete molecular responses. Autologous Stem Cell Transplantation (SCT) induce prolonged and durable remission in many hematological malignancies but its role in the treatment of CLL is controversial. We included AL in the treatment of refractory, relapsed or high risk CLL pts prior to stem cell collection and high dose chemotherapy consolidation of the response obtained. We treated 7 pts with Binet stage III CLL in partial remission (PR) or stable disease (SD) after 1 to 3 lines of chemotherapy, including fludarabine and 1 pt with advanced B-lymphocytic lymphoma in PR. Chemotherapy debulking prior AL was necessary in pts with high disease burden and large nodal involvement. Disease status before AL treatment was complete remission (CR) 2, PR 4, and SD 2. AL was given subcutaneously, treatment duration was 4-12 weeks and total dose was 180-1000 mg. Results after AL were CR 5 and PR 3, all 8 pts had become normal lymph node remission in marrow cytogenetic counts below 20%; 5 out of 7 CLL pts had CD5/CD19 expression in the marrow below 1%. After AL 5 out of 8 pts were mobilized: 4 with cyclophosphamide 5-7 gr/mq and 1 with additional chemotherapy followed by AraCytin; mobilization is planned in the last pt. Stem cell collection was adequate: 4-40 CD34x10⁶/Kg, in 1-4 apheresis procedures. Two pts were not mobilized after being treated for cytomegalovirus (CMV) infection at the end of AL treatment, they maintain CR without further treatment 11 and 18 months after AL. Four pts undergo SCT: engraftment and clinical course were normal, 1 pt progressed before transplant. Of the 4 transplanted pts 3 are in CCR at 24, 12 and 6 months post SCT, 1 pt progressed 13 months post SCT and was retreated with AL with minor response. CMV reactivation occurred in 6 out these 8 pts and antiviral therapy was necessary in 4. AL was effective in inducing significant clinical response in these high risk pts, stem cell collection was feasible and autologous SCT could be performed without significant early or late complications. CMV reactivation occurs in the majority of AL treated pts and must be carefully monitored. The results in this very small group of patients are encouraging but we can not draw any conclusion about the general application of this program in CLL.

R1176

Optimal matching at 5 HLA loci level improves outcome of unrelated HSCT in CML patients transplanted with the use of reduced-toxicity conditioning

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At the time of imatinib era in CML treatment HSCT became a disputable issue. In this study we compared the outcome of unrelated donor transplantation to that in sibling donor setting in CML patients receiving reduced toxicity conditioning. 19 patients received HSCT from matched sibling donors, 30 patients from unrelated donors. Reduced toxicity consisted of: Busulfan 8mg/kg, Fludarabine 120mg/m² (allo sib) or 150mg/m² (MUD) and ATG. Donors for unrelated transplant were matched for 10 specificities at following resolution levels: i) loci A, B, C at intermediate or high resolution and DR at high resolution level only. Patients were stratified into 3 groups: i) ALLO-SIB ( HLA matched ; n=19), ii) MUD fully matched (10/10 match at intermediate or high resolution level for HLA.
class I and high resolution for class II; n=11), iii) MUD mismatched (at least 1 allele mismatched; n=19).

Cumulative proportion survival was: i) 78% at the end of 4 y follow up period in sib H SCT, ii) 65% in patients transplanted from MUD donors fully matched in 10 specificities and iii) 35% in patients transplanted from donors mismatched in at least one allele.

(Figure)

Our results document, that optimal matching in five loci benefit the outcome of transplantation, which in unrelated donor transplantation can be similar to that obtained with sibling donors providing 10/10 matched donor at high resolution level.

Haemoglobinopathy

R1177
Refactory graft-versus-host disease after stem cell transplantation in thalassaemia: pentostatin is safe and effective treatment
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Toxicity, graft rejection with return of the thalassemia and graft-versus-host disease (GVHD) are the main causes of failure after stem cell transplantation (SCT) in thalassemic patients, particularly in those at poor prognosis (i.e. class 2 and 3).

Steroid refractory GVHD is associated with a not negligible non-relapse mortality. Few data are actually available on the use of pentostatin for refractory GVHD in thalassemic transplanted patients.

We analyzed four children, 2 females and 2 males, aged 8-13 (median 9.5 years), transplanted for beta-thalassemia major (class I, 1 class 2, 2 class 3). Two patients (class 1 and class 2) received unrelated SCT (1 bone marrow, 1 PBSC). The two other children (both class 3) were transplanted from HLA identical sibling after previous transplantation procedures, having rejected twice and once, respectively.

Three patients (2 unrelated and 1 HLA identical SCT) developed refractory acute GVHD grade III-IV with multiple organ involvement at +29, +35 and +58 days, respectively. One patient had chronic extensive GVHD. In the patient receiving the third allogeneic HLA identical SCT both acute and chronic refractory GVHD occurred. Patients affected by acute severe GVHD failed to respond to primary treatment with cyclosporine A and methylprednisolone at doses varying from 2 to 5 mg/kg/day and thus received salvage therapy with pentostatin 1.5 mg/sm for 3 consecutive days. One presenting with acute multorgan GVHD (skin, liver and gastrointestinal tract) had short term response. Two patients responded: one developed a secondary, chronic extensive, GVHD. This child as well as the other with chronic severe extensive GVHD (involving skin, liver, eyes and oral mucosa in one case and skin and mouth in the other) starting at +256 and +526 days from transplant were treated with pentostatin (4 mg/sm i.v. every 2 weeks) for 3 and 4 months. They are alive with significant improvement of skin and mouth symptoms and tapered concurrent immunosuppressive treatment. No toxicity or impairing chimerism due to pentostatin were observed.

Pentostatin thus appears as safe and effective treatment for acute and chronic severe GVHD after SCT for thalassemia. Supported in part by AIL Pesaro Onlus

R1178
Pure red cell aplasia after MUD stem cell transplant in thalassaemia major: successful treatment with rituximab
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Pure red cell aplasia (PRCA) is a not infrequently observed complication of allogeneic SCT performed across ABO complex and often refractory to standard strategies. The peculiar recovery of erythropoiesis after SCT in thalassaemia major could be a possible factor confounding for a correct diagnosis, as well as for the therapeutic choice. A young male patient was transplanted from ABO incompatible (donor B Rh+; recipient 0 Rh+) HLA-identical unrelated donor for beta thalassaemia major. After standard myeloablative conditioning regimen (Bu-Cy-Thio), he received cyclosporine A and MTX as GVHD prevention prophylaxis. The post-transplantation course was characterized by an incomplete haematological recovery. A poor graft function was observed at day +200. The bone marrow was hypocellular with the apparent absence of erythroid precursors, and a possible rejection of the graft was suspected; nevertheless, FISH analysis of Chromosome y for the engraftment showed 94% of donor cells, with a normal count of the beta chains (100%); no evidence of haemolysis was recorded. All DNA virus were negative. The diagnosis was PRCA. EPO 10.000U/every other day was started by day +201; additionally we submitted the patient to plasmapheresis (3 procedures, last at day +210) without hematologic response (3-5 units RBC/weeks, 1-2 platelets for weeks). At day +215 a first dose of Rituximab (RTX) (375 mg/sm) was administered; we observed a progressive increase in reticulocytes and platelet counts (respectively 10600/mm³ and 52000/mm³) 19 days after the infusion of RTX. Insorgence of cystitis and pielonephritis (P.Aeruginosa) delayed the administration of a following dose of RTX with a progressive, increasing transfusion dependency. After the resolution of the infective complications, a second dose of RTX was administered on day 272, and a third dose on day +299. Two weeks after the last dose of RTX a response was observed: reticulocytes count increased, and the level of Hgb slowly normalized. The patient is now in complete remission 18 months after transplantation, and with a complete hematologic take. Rituximab could thus be a promising agent for treatment of not infrequent cases of PRCA, in transplanted thalassemia patients, refractory to standard therapies.

Inborn errors of metabolism

R1179
A successful T-lymphocyte engraftment achieved by megadose CD34+selected peripheral blood stem cell
transplantation in a T-B-NK+ SCID case without using conditioning regimen
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Severe combined immunodeficiency (SCID) is a genetic disorder characterized by profoundly defective or absent T and B cells functions. Allogenic stem cell transplantation (SCT) is to date the only curative therapeutic option for SCID. Here we report a T-B-NK+SCID patient who was transplanted by megadose peripheral blood stem cells (PBSC) collected from his father without conditioning regimen.

Case: A 6 months old boy referred to us with the diagnosis of T-B-NK+SCID. His physical examination showed disseminated hyperkeratotic papular skin lesions. Immunohistochemical investigation of the skin biopsy revealed CD34+ cells with acryloid and foscarnet combination, but skin lesions didn’t resolve. Thus he received a megadose CD34+ selected (CliniMACS, Miltenyi Biotec) PBSC (62x10^6 CD34+cells/kg) transplantation from his father without conditioning regimen. He received cyclosporine for GVHD. He engrafted at posttransplant day 34. Detection of chimerism performed by STR (short tandem repeat) PCR analysis and whole blood samples showed mixed chimerism with 100% donor T cells. Acute GVHD grade I developed at day 56 rapidly resolved with systemic corticosteroid treatment. His chronic hyperkeratotic papular herpetic lesions completely recovered at second month after SCT. He is doing well with successful immunoreconstitution five months after SCT. To our knowledge he is the first successfully engrafted SCID case with T-B-NK+ phenotype following unconditioned haploidentical PBSC transplantation.

R1180 Dysregulation of MRP-8/MRP-14 proteins in a child with severe anaemia and neutropenia
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Myeloid-related proteins 8 (MRP8) and 14 (MRP14), both S100 proteins, are the major calcium-binding proteins expressed in phagocytes during specific stages of differentiation. They form stable complexes and are present in circulating neutrophils and monocytes, representing the first cells invading inflammatory lesions. The protein complex is found in inflammatory fluids in distinct inflammatory conditions, including rheumatoid arthritis, allograft rejection, inflammatory bowel disease, and lung disease. Prerequisite for its secretion is the contact of phagocytes with extra-cellular matrix proteins or inflamed endothelium, resulting in elevated intracellular calcium levels and activated protein kinase C. MRP8/MRP14 is thereby released specifically at inflammatory sites and leads to increased serum levels in correlation with the degree of inflammation, indicating an extra-cellular role of these molecules in inflammatory processes. We report a 4-year-old girl with: a) severe anaemia, b) neutropenia, c) inflammation and d) severe growth failure. Bone marrow examination showed moderate dyserythropoiesis. We did not detect hemolysis, iron deficiency, hemoglobinopathies, immunological diseases or any autoantibody. Serum levels of copper and ceruloplasmin were within normal range, although serum Zn concentration was markedly increased (310 µg/dL). Urinary Zn excretion and erythrocyte Zn concentrations were within normal range. Family studies demonstrated normal Zn and Cu levels and plasma levels. Patient's plasma calprotectin concentration showed a 6000-fold increase (2900mg/L) compared to normal values. Calprotectin concentration is known to be elevated in many inflammatory conditions but is generally below 10mg/L and thus far below the levels reported in this patient. We describe this case as an inborn error of zinc metabolism caused by dysregulation of calprotectin metabolism, which mainly presented with the features of chronic microcytic anemia and inflammation. We suggest that bone marrow transplantation could be the best clinical intervention for this new disease.

Lymphoma

R1181 Sequential autologous peripheral blood stem cell transplantation with BEAM conditioning as salvage treatment for refractory high-grade lymphoma
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T(8;14) mature B-cell (Burkitt's) lymphoma/leukaemia (BL) is classified as one entity in the World Health Organisation (WHO) classification. BL is a poor-risk, aggressive non-Hodgkin lymphoma. Despite significant improvements in the treatment of BL, outcomes of adults are generally inferior to those of children. Further intensification of the chemotherapy regimens and the inclusion of up-front, high-dose therapy and autologous peripheral blood stem cell transplantation (ASCT) has significantly improved the duration of response and survival. Strategies to improve survival in these poor-risk patients also include sequential ASCT, and ASCT followed by non-myeloablative allogeneic transplantation.

We present a 54-year old male who was diagnosed with BL with a double translocation t(8;14) and t(14;18). He was in remission after cycle 1 of the HOVON 37 study protocol (Prednisone, vincristin, daunorubicin, asparaginase) but relapsed after the second cycle (Ara-C, mitoxantrone). He was then treated according to the Hoelzer protocol but after initial good response proved progressive after the second block, with increasing abdominal mass, acute renal failure and metabolic encephalopathy. Salvage therapy was initiated with an autologous peripheral blood stem cell transplantation (PBST) with BEAM (carmustine, cytarabine, etoposide, melphalan) conditioning resulting in a very good partial remission, which was then consolidated at day + 29 by a second autologous PBST after BEAM conditioning. He was then planned for an haploidentical allogeneic PBST according to the Perugia Protocol on day +51 after the second PBST but died unexpectedly of complications of an acute gastrointestinal bleeding before the conditioning (TBI, thiopeta, fludarabin, ATG) could be started.

We present what appears to be the first reported case of tandem ASCT with BEAM conditioning in an adult patient with Burkitt's lymphoma/leukemia. This intensive therapy proved feasible and relatively well tolerated, certainly in view of the bad condition of the patient at the start of the first conditioning. The use of Palifermin (Keratinocyte Growth Factor) in the future could further increase the tolerability of this regimen. BEAM has been proved to be a highly effective treatment in lymphoma but provokes a severe mucositis. Nevertheless, tandem BEAM and autologous PBST should be further developed as a salvage regimen in the treatment of patients with poor-risk aggressive lymphomas.

R1182 An association between angioimmunoblastic T-cell lymphoma and HCV infection: therapeutic difficulties
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Angioimmunoblastic T cell lymphoma (AIL) is a rare lymphoproliferative disorder characterized by systemic lymphadenopathy, hepatosplenomegaly, fever, liability to
infections, skin eruption, polyclonal hypergammaglobulinemia, hemolytic anemia. Clinically the disease runs a fatal course in the majority of patients even after multiagent chemotherapy, interferon α, cyclosporine A, corticosteroids, danazol and recently purine analogues. Fewer than 20% of patients survive 5 years after diagnosis. High-dose chemotherapy (HDCT) followed by autologous bone marrow transplantation represents a promising new treatment modality for patients with this type of lymphoma. We present a case of 48 year-old woman with association of AIL and HCV+ infection with high replication of virus and interesting clinical course of her disease (CNS infiltration and complication with bacterial meningitis). It is widely thought, but not yet explained that there might be a pathogenetic link between the infection of hepatitis C virus and onset of B non Hodgkin’s lymphoma (NHL). In our case we have association with T cell lymphoma. We discuss our treatment difficulties (4 courses IMVP-16, 4 course for brain type lymphoma), following by Fludarabine. There is an evidence that AIL is susceptible to HDT and autologous stem cell transplantations should be considered to the patient.

R1183
A case of autologous stem cell transplantation in refractory HIV-associated diffuse large cell lymphoma

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Recent data suggest that high dose chemotherapy (HDCT) and autologous stem cell transplantation (ASCT) may be of benefit in patients with Aids-related lymphoma (ARL). We report a patient with refractory ARL successfully salvaged by HDCT and ASCT. In August 2004 a 43-year-old man presented with a right-lower quadrant abdominal mass. He was known to be HIV-positive since 1984 (CDC category C3). Highly active antiretroviral therapy (HAART) was initiated in 1997. His latest CD4 cell counts was 232/µl and his HIV-load <50 copies/ml. Abdominal CT-scanning confirmed a lesion of 10 x 7 cm in lower quadrant abdominal mass. He was known to be HIV-infected with T cell lymphoma. We discuss our treatment difficulties (4 courses IMVP-16, 4 course for brain type lymphoma), following by Fludarabine. There is an evidence that AIL is susceptible to HDT and autologous stem cell transplantations should be considered to the patient.

R1184
Angioimmunoblastic T-cell lymphoma: treatment in two cases

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Introduction: Angioimmunoblastic T-cell lymphoma (AIL) is one of the mature T-cell neoplasm defined in the REAL- and WHO-classifications. Although patients with angioimmunoblastic T-cell lymphoma (AIL) have a poor prognosis with conventional treatment, there are no generally accepted treatment guidelines of proven effectiveness because of low frequency. In that way, it would be necessary to determine new lineage-treatment to improve unfortunately evolution.

Methods: Between 2002 and 2005 we reported the good development with high-dose chemotherapy (HDCT) and autologous haematopoietic stem cell transplantation in two patients with refractory in our centre.

Results: The age at transplantation was 53 and 55 years-old respectively. Treatment prior to bone marrow transplantation (MMT) in one case was initially prednisone alone and Cladribine, Cyclofosfamide and Prednisone for 3 cycles, and the other one was chemotherapy based of increased dose of schedule EPOCH; In this case was necessary secondary treatment with Ifosfamide and Etoposide IFE for 2 cycles. CD34+ selected autologous peripheral blood stem cell transplantation was given like third line of treatment in the two cases. The regimen for the mobilization of peripheral blood stem cells (PBSC) included Ifosfamide, Etoposide and G-CSF. In one case the median yield of PBSC was 7,55 x10^6 CD34+cells/kg and 1.2 x10^5 CD3+cells/kg and the other one was 4,15 x10^6 CD34+cells/kg and 0.8 x10^6 CD3+cells/kg. The conditioning treatment consisted of BEAM regimen. There was none treatment-related death. Post-TASPE complications were herpes zoster infection and bicalon gammopathy IgG kappa and IgG lambda in one case, and no evidence of acute complications in the second patient. The patients remain in complete remission after a following time of 29 and 5 months respectively and there is no evidence of relapsed.

Conclusions: Our cases confirm previous experience that AIL is susceptible to high-dose chemotherapy and CD34+ selected autologous peripheral blood stem cell transplantation, but more cases and longer observation time as well as better selection of patients with refractory AIL would be necessary to determine the standard treatment.

R1185
BEAM CAMPATH – a novel conditioning regimen in autologous peripheral blood stem cell transplantation

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Introduction: We describe the novel use of BEAM Campath conditioning in an autologous setting. We used Alemtuzumab (Campath 1H) in combination with BEAM conditioning in two patients who underwent autologous peripheral blood stem cell transplantation for aggressive T cell lymphoma.

Case A: A 42 year old lady of Carribbean origin presented with HTLV-1 driven ATLL. She was treated with 6 courses of CHOP + Dacluzimab with good PR. She received DHAP salvage therapy with stem cell collection followed by a BEAM Campath 1H autograft. She developed CMV reactivation treated with Valganciclovir. Her day +90 re-staging showed CR with significant reduction in HTLV-1 proviral load from 77 copies to 5.82 copies/100 PBMCs.
Case B: A 33 year old South American presented with massive splenomegaly & pancytopenia. He was diagnosed with gd hepatosplenic lymphoma and was started on CHOP 14 chemotherapy regimen with initial response followed by progression after 6 cycles. He received DHAP chemotherapy followed by autologous peripheral blood stem cell transplant with BEAM Campath 1H conditioning. Post transplant, he developed CMV reactivation managed with Valganciclovir. He received splenic irradiation 6 weeks post transplant and at 14 months post transplant, he remains in complete remission.

Role of Alemtuzumab (Campath 1H)- Campath 1H targets all lymphocytes expressing CD52. This includes gd T cells. HTLV-I predominantly infects CD4+ T cells (90% to 99% of the proviral load). The expression of CD52 on HTLV-1 infected cells has not been studied in humans but in NOD/SCID mice developing HTLV-1 positive tumours, the ‘leukemic’ cells express CD 52 to a high level.

Conclusion: The use of BEAM Campath 1H conditioning regimen in an autologous setting is a novel approach in selected patients with aggressive T cell lymphomas. It merits further investigation.

R1186
Unrelated bone marrow transplantation in a child with high-risk anaplastic large cell lymphoma: case report
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The use of allogeneic stem cell transplantation in Non-Hodgkin Lymphoma patients is not yet clearly defined, especially in children and adolescents. For patients who are in need of BMT, to have a chance for human leucocyte antigen (HLA) identical sibling to serve as an allogeneic donor is only 30%. For others, transplant from a matched unrelated donor (MUD) is an alternative option but with higher risk for GVHD and graft rejection as well as infectious complications and organ toxicity. Here, we describe a case of ALCL treated with unrelated cord blood transplantation.

5 year old boy admitted with the complaint of mass in right medial thigh region was diagnosed as ALCL and chemotherapy was started. At the beginning of second course, because of progression, chemotherapy was changed and Alemtuzumab was added. By the beginning of third chemotherapy course, he was in remission and chemotherapy was continued while an unrelated donor was being searched since HLA matched sibling donor was not available. At the end of forth course, one antigen mismatched cord blood was found and following marrow recovery transplantation was performed. The patient was conditioned with total body irradiation (total 1200 cGy) given in six fractioned doses in association with etoposide (40 mg/kg) and cyclophosphamide (90 mg/kg). He was infused with 1.79x10^7/kg MNC and 0.2x10^5/kg CD34+ cells. Cyclosporine, metotrexate and ATG were used for GVHD prophylaxis. Engraftment was achieved for neutrophil and thrombocyte on posttransplant day 24 and 39, respectively. On posttransplant day 15, venoocclusive disease developed and it was treated with defibrotide. He has not been experienced any sign of GVHD and he has been still in remission by the end of posttransplant three months.

He was the first pediatric ALCL case to our knowledge who was treated with unrelated cord blood transplantation. The increasing number of volunteer stem cell donors and although in very limited cases successful results published in the literature regarding the unrelated stem cell transplantation in this group of patients, make this therapeutic option acceptable in the treatment of high risk or treatment failure patients although more data and long term follow up in larger series are needed.

R1187
High-dose chemotherapy and autologous stem cell transplantation for malignant lymphoma: influence of disease status at time of transplant
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High-dose chemotherapy with autologous stem cell transplantation (ASCT) could improve the survival of patients (pts) with malignant lymphoma, but the optimal time of ASCT is still not clear. This therapeutic method is considered as the optimal strategy in high risk patients in remission and relapsing malignant lymphoma patients. However the potential role in chemoresistant disease and primary refractory relapse is still controversial and optimal timing and indication is still to be assessed.

Methods: We analyzed 25 consecutive adult patients with malignant lymphoma who underwent ASCT in a period 2000-2005. M.Hodgkin (HD) was diagnosed in 12 pts, non-Hodgkin lymphoma (NHL) in 13 pts. The graft was non-purging, peripheral stem cell. The median follow-up was 30 months (from 1 to 60 months).

In HD group the conditioning regimen was BEAM in all cases. Status at ASCT was complete remission (CR) in 2 cases, chemo sensitive relapse in 5 cases and primer refractory disease in 5 cases. Patients in complete remission were patients with adverse prognostic factors for Hodgkin disease.

In NHL group the conditioning regimen was BEAM in 11 cases, and other chemotherapeutic regimen in 2 cases. High grade histology was diagnosed in all patients, diffuse large B-cell 9 pts, anaplastic large T-cell 3, lymphoblastic 1 pts. Status at ASCT was complete remission (CR) in 2 cases, chemo sensitive relapse in 5 cases, and refractory disease in 6 cases. According to the International Prognostic Index (IPI) which is validated scoring system predictive of survival in various types of de novo aggressive non-Hodgkin lymphoma patients with CR has been evaluated as high risk patients.

Results: Permanent complete remission was achieved in 4 out of 4 pts (75%) with CR at ASCT in 5 out of 10 pts (50%) with chemo sensitive relapse. Complete remission could not be achieved in refractory disease.

Conclusion: The retrospective analysis of our data showed that the ASCT in patients with malignant lymphoma is an effective salvage therapy. Status of the disease at the time of ASCT is prognostic factors which strongly influenced the outcome of ASCT. The study suggests that the best results are achieved if ASCT is performed in complete remission compared with refractory/relapsed disease. Patients with malignant lymphoma and initial high risk prognostic factors should be transplanted in first complete remission.

R1188
Autologous stem cell transplantation after radioimmunotherapy with ibritumomab-tuxetan (Zevalin) followed by high-dose cyclophosphamide is feasible in lymphoma patients with end-stage renal disease
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We report on a 51 year old male patient suffering from follicular lymphoma grade II, stage IIIA, first diagnosed in 2004. Initial therapy consists of 8 cycles R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) with partial response. In April 05 patient became symptomatic with abdominal pain and a Ct scan demonstrates enlarged lymph nodes paraaortocaval and a left hydronephrosis necessitating ureter stent. After failure of lymphoma relapse two cycles of Dexambeam (dexamethasone, BCNU, etoposide, ara-C, melphalan) chemotherapy were initiated followed by stem cell harvest and as lymphoma shows progression we added one cycle of R-DHAP (Rituximab, dexamethasone, high dose ara-c, cisplatin) again without any
response. Due to further lymphoma progression patient became haemodialysis dependent as creatinine level increase to 621 µmol/l and urea level to 29.8 mmol/l. Furthermore, he suffers from oliguria, ascites and severe edema of the legs, scrotum, and abdomen. As no standard chemotherapy with a clear cure of disease was feasible we decided to start a high dose chemotherapy followed by autologous stem cell transplantation. Conditioning regimen consist of radio-immunotherapy with ibritumomab-tiuxetan (1.2 GBq) 22 days prior to high dose cyclophosphamide (60 mg/kgBW/day) on days -3 and -2. On day 0 1.99 x 106 CD 34+ cells per kg BW were given. Platelet engraftment was observed on day +15 (20,000/µl) and day +31 (500,000/µl) respectively, as neutrophil exceed 500/µl on day +10. Patient did not show any problems associated with either radio-chemotherapy or neutropenia. As edema and ascites disappear during neutropenic phase kidney function resolve 3 weeks post transplant. MRT scan shows a very good partial response with residual lymph node paraaortocavally. From this case we conclude that refractory follicular lymphoma radio immunochemo-therapy followed by high dose cyclophosphamide as conditioning regimen even in the case of renal failure necessitating haemodialysis is feasible and highly effective.

R1189 Long-term survivors among non-Hodgkin lymphoma patients who underwent high dose chemotherapy rescued by blood stem cell transplantation

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Twenty patients (male:female=13:7, ages ranging 15-62 with a median of 38) received high dose chemotherapy followed by peripheral blood stem cell transplant (PBSCT) at Inha University Hospital. Their diseases consisted of diffuse large B cell lymphoma (DLBCL, n=7), peripheral T cell lymphoma (PTCL, n=5), lymphoblastic lymphoma (LBL, n=2), anaplastic large cell lymphoma (n=2), Burkitt lymphoma (n=1), nasal type extranodal NK/T cell lymphoma (n=1), mycosis fungoides (MF, n=1), and nodal marginal zone lymphoma (MZL, n=1). Most of them were in stage VIA (n=9) or VIb (n=5), and others were in stage IE (n=1), bulky IIa/bulky IIIE (n=4), or IIIa (n=1). Three patients had disease extended to CNS at the very beginning of the treatment. Chemotherapy including CHOP, R-CHOP, ProMACE/CytaBOM, COPBLAM-V, DHAP, high-dose methotrexate, or regimens for acute lymphoblastic leukemia (patients were exposed to a median of 9 courses of chemotherapy ranging 6-20) yielded CR1 (n=11), PR1 (n=1), CR2 (n=5), CR3 (n=2), and PD (n=3) right before PBSCT. A total of 24 (3 allogeneic and 21 autologous) PBSCT were performed for 20 patients. Allogeneic PBSCT was carried out in case of disseminated MF, recurrent PTCL after autologous PBSCT, and recurrent PTCL in CR3 after tandem autologous PBSCT. A patient with LBL received double autologous PBSCT. The conditioning regimen was CBV (cyclophosphamide, BCNU, etoposide) for most autologous PBSCT, CyTBI (cyclophosphamide and total body irradiation) for MF, fludarabine based chemotherapy in other allogenic settings. Radiotherapy was given before or after PBSCT in 6 patients (brain in 3, abdomen in 1, mediastinum in 1, and nasal cavity in 1). A fatal veno-occlusive disease developed in MF patient who died even after orthotopic liver transplant. Fatal septicemia in 3 patients at immediate post-PBSCT period hampered proper evaluation of the treatment efficacy. A median disease free survival duration was 6 months (range 0-75+), and overall survival duration 16.5 months (range 1-75+). All the patients died of disease who had metastatic disease in their brains. As of this writing, 9 of 20 (45%) are alive disease free at a median of 26 months (range 4-75). It is of note that, among them, a PTCL patient who received triple PBSCT is alive disease free at 54 months post-transplant, a patient with DLBCL in CR3 at 66 months, a patient with disseminated MZL in CR1 at 70 months, and a patient with PTCL in CR1 at 75 months.

R1190 Haematopoietic cell transplantation in NHL

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The non-Hodgkin's lymphomas (NHLs) are cancers of the cells that populate lymph nodes. It is classified according to its histology, its immunophenotype, cytogenetic and molecular biology. Most NHLs are cancers of B-lymphocytes. Although some of patients with NHL are cured with chemotherapy with or without radiotherapy, the ones who relapse and those with primary refractory disease have poor outcomes with salvage regimen. Over the past 17 years, several clinical trials using high dose chemotherapy or chemoradiotherapy with autologous stem cell transplantation or allogeneic stem cell transplantation in this setting have been reported. Approximately 50% of patients appear to be cured using this approach. High dose therapy/Autologous stem cell transplantation is standard therapy in two scenarios, in relapsed or refractory non-Hodgkin's lymphoma and in patients with refractory aggressive lymphoma whose disease is relapsing with second-line chemotherapy. Here we report 65 patients with NHL, who have undergone high-dose chemotherapy (HDCT) followed by hematopoietic cell transplantation (HCT). Of all the 65 patients 47 (72.3%) were male, and 18(27.7%) were female. The median age was 27 years old; with minimum and maximum of 10 and 51 y/o respectively. The majority of patients who had immunophenotype study were diagnosed with B-cell lymphoma (62.9%), 54 patients (83.0%) received autologous stem cell transplantation and 53 ones (81.5%) had peripheral blood as graft type. Before (HCT) 52.3% of patients were in first complete remission. (Including CR with salvage therapy) The conditioning regimen for the majority of patients (34.85%) was CCNU, Etoposide, Ara-C (Cytarabin) and Melphalan. The median duration of hospitalization for autologous transplanted patients was 24 days which was 37 days for allogeneic transplanted ones. Transplant mortality rate in the first 100 days was 4%. The median follow-up duration was 253 days, with minimum and maximum of 2 and 3103 days respectively and during this period the overall survival (OS) is 70.83% and the disease free survival rate (DFS) was 60.08%.

R1191 Zevalin therapy of a non-Hodgkin relapsed lymphoma patient following an autologous peripheral stem cell transplantation

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The yttrium-90 (90Y) - labelled ibritumomab tiuxetan (Zevalin, IDEC-Biogen, San Diego CA) is an accepted therapy for relapsed, or therapy-refractor B-cell non-Hodgin lymphomas, but it is not officially recommended in patients who have failed an autologous stem-cell transplant. Patients with recurrent lymphoma following an autologous transplant have limited treatment options. 11 cases so far only have been described in the literature whose relapse following autologous peripheral stem-cell transplantation (AP SCT) has been treated with Zevalin. In the present paper the authors discuss the case of a 53 year-old male patient, who underwent lymph-node biopsy due to generalised lymphadenomegaly. The histology test proved positive for CD20 follicular lymphoma. Following an 8 cycle CHOP (cyclophosphamide, doxorubicine, oncovin, prednison), a 4 cycle FND (fludara, mitoxantron, dexamethasone), later a 3 cycle DHAP (dexamethasone,
high-dose Ara-C, cisplatin) therapy, autologous peripheral stem-cell transplantation was performed. Six months later due to a relapse a 4 cycle R-CEPP (rituximab, cyclophosphamide, etoposide, prednisin, procarbasine), then a 5 cycle hyper-CVAD therapy was applied. The patient (had no compatible sibling) proved to be therapy-refractory, therefore Zevalin therapy was performed after a preparatory rituximab therapy 1180 MBq Zevalin was applied). The Zevalin treatment did not caused any unusually serious side effect. During the 5 months since the start of the Zevalin therapy the patient has been in a complete haematological remission. In NHL, in case of a relapse following APSCT Zevalin therapy has proved to be a good alternative.

**R1192**

The use CD-20 monoclonal antibody in the treatment of B-cell non-Hodkin lymphoma with autologous stem cell transplantation

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Introduction: Recent trials have shown that CD-20 monoclonal antibody, Rituximab (R) may be effectively associated with autologous stem cell transplantation (ASCT) in the treatment B cell non-Hodkin’s lymphoma (NHL).

Aim: To analyze the efficacy of the incorporation of R at different steps of autologous transplantation (R-ASCT) programs in patients (pts) with high-risk NHL.

Methods: Between March 2000 to December 2004 R+ASCT was applied for the treatment of 7 pts with B cell NHL (2 low grade, 5 diffuse large cell lymphoma - DLCL). ASCT were performed after CHOP induction chemotherapy (CT) with R (R-CHOPx4) in 4 pts or without R in 3 pts. In the time of ASCT complete remission (CR) had 2 pts and others pts had partial remission (PR). Regimen mobilization were G-CSF with Cy ± VP-16 in 3 pts, ESHAP CT in 1 pt, R+Cy in 2 and MegaR-CHOEP in 1 pt. A single infusion of R (375 mg/m²) used as in vivo purging 3 days prior Cy or 0 day of therapy MegaR-CHOEP. The average number of collected mobilized CD34+ cells was 5.7x10⁶/kg BM (range 4.1-8.2). All pts received CVB conditioning regimen. The posttransplant immunotherapy consisted of a single dose R every 3 months (m) started 2 m following ASCT in 6 pts. Sequential monitoring of minimal residual disease (MRD) during and after treatment was performed by PCR. Five of seven pts are available for MRD.

Results: At a median follow-up of 36 months (6-50), 6 pts are alive. After R+ASCT treatment, 3 out of 5 available pts became PCR negativ (low grade lymphoma-1; DLCL-2). Four pts with DLCL are still in complete remission (CR) and two of them in molecular remission (MR) 45 and 46 months (m). The both pts with low grade lymphoma relapsed 23 and 24 m after transplantation, the one of them never attained PCR negativity and second pt reverted to PCR positivity after 15 m. This therapy is well tolerated, with no adverse effects on hematological recovery of incidence of infections.

Conclusion: This therapy is effective in the subset of pts with high risk DLCL. R+ASCT treatment is able to eliminate MRD, whereas PCR positivity is associated with a high risk of relapse.

**Multiple myeloma**

**R1194**

Double syngeneic transplantation in plasma cell leukaemia

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Introduction: Plasma cell leukemia (PCL) is a rare disorder, characterized by circulating clonal plasma cell. It accounts for less than 1% of all plasma cell dyscrasias and has a fatal prognosis. It can be primary or secondary, when there was a previously diagnosed plasma cell dyscrasia. The median survival is 7-12 moths in the first case and 2 moths in the second.

Case: We present a 54 years old man, diagnosed in November 2003, with multiple myeloma IgA kappa, Bence Jones +, that presented weight loss, retinal hemorrhages, respiratory distress, hepatomegaly, splenomegaly, osteolytic lesions, the cariotype showed hyperploid, chromosome 13 monosomy, translocations t (1,12) and t (4,14). First, he was treated with tree cycles of a drug’s combination with melphalan, carmustine, vincristine and dexametasone with no response, therefore, was changed to cyclophosphamide, Adriamycin, vincristine methotrexate y citarabine. After two cycles, the patient got complete remission. The patient had a twin brother, and we decided, to do a double transplantation to consolidate the response. The first transplantation was conditioned with carmustine, etoposide, citarabine and melphalan (BEAM), and the second with cyclophosphamide and total body irradiation; the patient remained in complete remission.

Eight months after second transplant was admitted to the hospital with disorientation, Bradipsiquia, headache, and sensorimotor loss of lower extremity. Laboratory examination showed differential count of leukocytes, haemoglobin, splenomegaly, and platelets were normal, LDH increase, absence of monoclonal gammopathy in blood and urine by immuno fixation, and the brain’s computerized tomography showed multiple intraparenchymatous lesions, with peripheral edema, in both cerebral hemispheres, confirmed by magnetic nuclear resonance, all of that suggested a neoplastic disease. These lesions were biopsied with the result of multiple myeloma lambda.

The patient died one day after biopsy because intracranial hypertension.

Conclusion: Plasma cell disease has poor prognosis, and transplantation could be a good option for some patients, but in our case we only achieve to extend life a few months.

**R1195**

Delayed engraftment following autologous stem cell transplantation: risk factors apart from stem cell dose

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Background: The use of mobilized peripheral blood stem cells results in prompt engraftment of all three cell lines in both autologous and allogeneic transplantation compared to bone marrow. The stem cell (CD34+) dose is known to be a major factor for bone marrow recovery. Although reinfusion of ≥ 5x10⁶ CD34+ cells/kg is considered more than adequate in terms of rapid engraftment, there are still patients who engraft after day 21, despite sufficient CD34+ cell dose. We looked for other factors contributing to delayed engraftment apart from the stem cell dose.

Methods: Retrospective data on lymphoma and multiple myeloma patients with delayed engraftment (absolute neutrophil count >500/uL after day 21) after autologous transplantation were analyzed. Factors including stem cell dose, infectious complications and outcome were evaluated.
Results: A total of 466 patients underwent an autologous stem cell transplantation for lymphoma and multiple myeloma at the Rambam Medical Center between the years 1995 and 2005 (238 and 228 respectively). Indications for transplantation included a chemosensitive relapse in lymphoma high risk patients and rarely also for refractory patients. In myeloma auto transplants were performed for patients achieving good response upon initial chemotherapy. Patients with lymphoma received the BEAC/BEAM conditioning and patients with myeloma were give melphalan 200 mg/m². 12 patients showed a delayed engraftment, 9 of them were patients with multi ple myeloma and only 3 with lymphoma. Median time to engraftment was 26 days (range 21-41). In 11 patients the CD34+ cell dose was >10^5/kg and in one patient it was 6x10^5/kg. 2 patients died of severe infections, one with lymphoma on day 36 without engraftment, the other with myeloma although engrafted on day 21. The outcome of other patients was uncomplicated. There were no detectable differences in the clinical course or toxicity among those who engrafted early or the later engrafters. Conclusion: The use of mobilized peripheral stem cells shortens time to engraftment. Using high doses of stem cells is safe, but there remains a significant risk of delayed engraftment in 4% in myeloma compared with <1% in lymphoma patients. Considering the homogeneity of the groups and the high stem cell dose infused, it is likely that bone marrow microenvironment, known to be impaired in multiple myeloma, has a role in facilitating engraftment.

R1196
A single-centre experience in autologous stem cell transplantation for patients with multiple myeloma
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From Oct. 1996 to Aug. 2005 we performed 121 autologous stem cell transplantsations (ABCT) in 71 multiple myeloma (MM) patients (age 58 (median:37-67) years; female:31, male: 40). Following conventional chemotherapy 33 patients (transplanted between 1996 -1999 or patients not eligible for a tandem transplantation concept due to late infections (n=2) or toxic side effects (cardiotoxicity (2), neurotoxicity (1), dermatitis (1), sMDS (1)) underwent a single course of ABCT and 38 patients multiple courses of transplantsations (26 double and 12 triple ABCT).

No significant differences between both groups were seen according to age, sex distribution or stage of disease at time of transplantation. The patients who relapsed after conventional treatment (15 vs. 5 pts) were included in the single ABCT group. The conditioning chemotherapy consisted of MEL 200 mg/m² for single and double ABCT and 100 mg/m² for triple ABCT. All patients were transplanted with peripheral stem cells. The time to granulocyte recovery > 0.5 G/l lasted 8-13 days (median:11) and to platelet recovery > 50 G/l 8-55 days (median:13) without a difference between the consecutive numbers of transplantsations.

All patients but one in each group responded to transplantation: CR 13 pts., PR 19 pts., TRM 1pt. (single ABCT group) and CR 16 pts., PR 21 pts., failure 1 pt. (multiple ABCT group), resp. In the single ABCT group 22 pts. (67%) relapsed after 7-73 months (med: 17 mo), in the multiple ABCT group 16 pts. (42%) relapsed after 6-44 months (med: 16 months). The median observation time is shorter in the multiple ABCT group (22 vs. 40 months). The median PFS and OS in the single ABCT group is 20 and 69 months, resp. In the multiple ABCT group median PFS lasted 16 months. The median observation time is 16 pts. (42%) relapsed so far after 6-44 months.

No significant differences between both groups were seen according to age, sex distribution or stage of disease at time of transplantation. The patients who relapsed after conventional treatment (15 vs. 5 pts) were included in the single ABCT group. The conditioning chemotherapy consisted of MEL 200 mg/m² for single and double ABCT and 100 mg/m² for triple ABCT. All patients were transplanted with peripheral stem cells. The time to granulocyte recovery > 0.5 G/l lasted 8-13 days (median:11) and to platelet recovery > 50 G/l 8-55 days (median:13) without a difference between the consecutive numbers of transplantsations.

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R1197
Autologous haematopoietic stem cell transplantation for the treatment of multiple myeloma
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High dose therapy with autologous stem cell transplantation (ASCT) is the treatment of choice for patients (pts) with multiple myeloma (MM). We report here our centre experience in pts with MM who have undergone ASCT. Between December 1998 and September 2005 27 pts (19 M/8 F) with a median age of 49 years (range 38-62) were transplanted. Most pts (74%) were in stage III (Durie-Salmon). Before transplantation, 20 pts have received 1 line of chemotherapy (4-6 cycles VAD). At the time of autograft 75% pts were responders (partial remission or very-good partial remission) and the others had minor response/refractory disease. All pts received peripheral blood stem cell support after conditioning with melphalan (5 pts) or melphalan associated to cyclophosphamide and busulfan (ByCy2+M). Three months (m) after ASCT 16 pts received interferon (3 MU/s.c. t.i.w) and 4 pts thalidomide (100-200 mg daily) as maintenance therapy. After transplantation a complete (CR) or very-good partial response was achieved in 11/25 pts (44%); all other treated pts experienced a reduction of M-component >50%. With the median follow up of 21.5 m (range 2-72), 63% pts were alive. To date, in CR are still 8 pts with the median duration of remission from ASCT of 24 m. Seven pts relapsed or progressed during 8 to 26 m after ASCT. Two pts died from transplant related complication. ASCT is safe and effective procedure not only in chemotherapy sensitive pts with MM but also in resistant cases. Our date confirm that ASCT is the current gold standard therapy for many pts with MM.

R1198
Dose intensity and efficacy of treatment in patients with multiple myeloma
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63 MM patients were included in our study. Induction therapy was VAD-D or IdaD-D-D. 23 patients with MM were undegone intensification DexaBEAM. 14 patients received 1 cycle DexaBEAM. 6 was sensitive to the first line therapy, 1DexaBEAM resulted in 4 (66 %) CR, 1 (17 %) nCR and 1 (17 %) PD. 8 patients was unsensitive and the resulte was 3 (37 %) CR, 3 (37 %) PR, 1 (13 %) SD, 1 (13 %) NA.

2 cycles DexaBEAM received 9 patients, 2 of them were in PR after the first line therapy, 4- in SD, 3- in progression of disease. 2 (29%) sensitive patients achieved nCR, 7 patients, resistant to the first line therapy achieved 2 (29 %) CR, 4 (57 %) PR, 1 (14 %) - SD.

28 patients of MM underwent 1 ASCT,18 patients (64,2 %) after conditioning regimen of melphalan 200 mg/m², 5 patients (18 %) - melphalan 180 mg/m², 4 patients (14,2 %) - 140 mg/m².

6 patients with MM received tandem ASCT. 14 patients received 1 cycle DexaBEAM, 6 was sensitive to the first line therapy, 1DexaBEAM resulted in 4 (66 %) CR, 1 (17 %) nCR and 1 (17 %) PD. 8 patients was unsensitive and the resulte was 3 (37 %) CR, 3 (37 %) PR, 1 (13 %) SD, 1 (13 %) NA.

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28 patients of MM underwent 1 ASCT,18 patients (64,2 %) after conditioning regimen of melphalan 200 mg/m², 5 patients (18 %) - melphalan 180 mg/m², 4 patients (14,2 %) - 140 mg/m².

6 patients with MM received tandem ASCT. 14 patients received 1 cycle DexaBEAM, 6 was sensitive to the first line therapy, 1DexaBEAM resulted in 4 (66 %) CR, 1 (17 %) nCR and 1 (17 %) PD. 8 patients was unsensitive and the resulte was 3 (37 %) CR, 3 (37 %) PR, 1 (13 %) SD, 1 (13 %) NA.

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3 years DFS in group of the patients with tandem ASCT was 65 % in comparison with 15 % in group of the patients with single ASCT. p = 0.03. The conclusion: Our preliminary results are that the increasing of dose intensity improves the efficacy of treatment of patients with multiple myeloma.

Myelodysplasia

R1199
Successful myeloablative allogeneic haemopoietic stem cell transplantation in a patient with end-stage renal failure on haemodialysis
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Introduction: End stage renal failure (ESRF) has conventionally been considered a relative contraindication to HSCT. Although increasing numbers of patients undergoing autologous procedures with HD are being reported, documented experience in the allogeneic HSCT setting remains extremely limited. To the best of our knowledge only three previous case reports have been published. Case report: A 38 year old male, treated with regular HD for ESRF from 2003, was diagnosed with myelodysplastic syndrome associated with monosomy 7 (IPSS INT-2) in 2004. After extensive counselling with the patient, careful consideration of donor issues, and collaboration between BMT and renal teams, a decision was made to proceed with allogeneic HSCT with curative intent. In April 2005, the patient underwent myeloablative conditioning with total body irradiation 12 Gy and cyclophosphamide 120 mg/kg followed by transfusion of G-CSF mobilised allogeneic PBSC from his HLA and ABQ-matched sister (CD34 cell dose = 6.8x10^6/kg). Recipient and donor CMV serology was negative. During conditioning, HD was performed on a daily basis to optimise biochemistry. The patient was closely monitored for ciclosporin levels and echocardiography. Oral mesna was used to prevent haemorrhagic cystitis. HD was subsequently maintained at the echocardiography. Oral mesna was used to prevent

Liver complications in hematopoietic transplantation (HCT) setting may be life threatening, and hepatitis B virus (HBV) infection increases the risk of hepatic complications in patients undergoing HCT. We describe a 63 year old white women who was treated 5 years before with chemotherapy for non-Hodgkin disease achieving complete remission. She acquired HBV infection because of a blood red cell transfusion. Five years after complete remission she developed anemia, piastrinopenia and leucopenia. Bone marrow biopsy histology excluded lymphoma relapse but revealed multilineage myelodysplasia with excess of blasts. Cytogenetic examination detected 7q-deletion. According to IPSS score myelodysplasia was classified as high risk. Since an high viral B load (450560 copies/ml), despite normal ALT and AST values, the patient underwent lamivudine prophylaxis.

HCT was considered and his brother resulted full HLA match. He was successfully mobilized with G-CSF. Before transplantation viral B load did not decrease so antihelatitis B prophylaxis was changed from lamivudine to adefovir dipivoxil that was also employed as the only antiviral prophylactic treatment along all the procedure. Patient underwent reduced conditioning regimen: e.v. Busulfan (7,2 mg/Kg weight) plus Fludarabine (150 mg/m²) and rabbit ATG Fresenius (25 mg/Kg).Graft versus host disease (GVHD) prophylaxis consisted in cyclosporin and metotrexate. 5,83 x 10^6/kg Fludarabine (150 mg/m²) and rabbit ATG Fresenius (25 mg/Kg).Graft versus host disease (GVHD) prophylaxis consisted in ciclosporin and metotrexate. 5,83 x 10^6/kg CD34 monocleated were infused. Neutrophil engraftment was at +13, while platelet engraftment at +12. The patient developed steroid sensitive cutaneous grade II acute GVHD. Complete chimerism was achieved at + 21. At day 60 she developed abrupt ALT and AST increase (>600 IU/L), without bilirubin increase and a concomitant viral B load more than 10^7 copies/ml. Lamivudine was reintroduced and adefovir was continued. One week later transaminases began to decrease and at day 85 both ALT and AST were under 100 IU/ml, while viral B load was 1 x 10^4 and the patient is well at day 100. A reduced conditioning regimen with fludarabine and e.v. busulfan together with rabbit ATG revealed to be safe and efficacious in this 63 year old patient. Moreover lamivudine and adefovir association was able to control HBV replication. Adefovir alone successfully avoided every other viral infection (HSV, CMV etc.)along transplant procedure and it did not impair engraftment.

R1201
Haematopoietic stem cell transplantation in poor prognosis MDS and sAML: prolonged patients survival is achievable, but refining of infections management and reduction of treatment-related toxicities is required to improve patients outcome
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Introduction: allogeneic hematopoietic stem cells transplantation (HSCT) is the therapy of choice for poor prognosis MDS and sAML patients (pts); a HLA identical, related (Allo) or unrelated (Mud) or haploidentical familial (Haplo) donor is available for most pts. Autologous HSCT is an alternative for pts without a donor. Prolonged overall survival (OS) and disease free survival (DFS) are reported in a minority of MDS/sAML pts after HSCT. Major drawback of Allo, Haplo and Mud HSCT is the high incidence
of lethal infections and toxicities, of Auto HSCT is the high incidence of disease relapse.

Aim: to retrospectively evaluate treatment related toxicities and survival after HSCT in MDS/SAAML pts, treated at our Institute.

Methods: 29 MDS and sAML pts, period 4/1999-7/2005. Median age at HSCT: 56 (40-72), 8 pts > 60. Diagnosis: MDS=16, sAML=13. Twenty-seven pts received a median of 2 (1-4) chemo cycles before HSCT; 2 pts received only red cells transfusions. HSCT: Auto 7, Allo 7, Haplo 11, Mud 4. Status at HSCT: CR 15, RA 5, RAEB 7, sAML 1, not evaluable 1. HSCT conditioning: myeloablative 20 (7 Auto, 2 Allo, 10 Haplo, 1 Mud), reduced intensity 9 (5 Allo, 1 Haplo, 3 Mud), HSCT source: PBSC 26, BM 3. Diagnosis of aspergillosis pre-HSCT:11/29 (37.9%).

Results: 19 pts (65.5%) died after HSCT. Transplant related mortality (TRM): 8 (27.5%) pts; 1, 1, 6 and 0 after Auto, Allo, Haplo and Mud, respectively. Causes of death: infection 8, disease progression 10, ARDS 1, other 1. All pts evaluable for Haplo and Mud, respectively. Causes of death: infection 8, mortality (TRM): 8 (27.5%) pts; 1, 1, 6 and 0 after Auto, Allo, Haplo and Mud, respectively. Five pts died in CR (4 Haplo, 1 Allo). Median OS from HSCT is 258 (19-1466) median. At last update 10 pts (34.5%) are alive in CR after a median follow-up of 705 days (125-1467); 3, 3, 1 and 3 after Auto, Allo, Haplo and Mud, respectively.

Conclusions: prolonged OS and DFS are achievable with HSCT in poor prognosis MDS and sAML pts, also in the elderly. Prevention of pts contamination before HSCT, mainly from aspergillus, and reduction of early TRM, mainly in the Haplo subset of pts, could improve pts survival. Trials for primary/secondary anti-fungal prophylaxis are ongoing and reduced-toxicity conditioning regimens are under investigation at our Institute. Auto in CR is an alternative if HSCT from a donor is not feasible; to reduce the relapse rate after Auto an experimental maintenance treatment should be proposed.

Solid tumours

R1203 Improved survival in neuroblastoma by autologous peripheral blood stem cell transplantation: a single-centre experience

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Objectives: Neuroblastoma is the most common extracranial solid tumor of childhood, and its outcome in advanced cases has been very poor. In this study, the author evaluated the treatment outcome and prognostic factors in advanced neuroblastoma.

Methods: The study group comprised of 48 patients who were diagnosed and treated with neuroblastoma at Chonnam National University Hospital from January, 1996 to May, 2005. Data were obtained from the retrospective review of the medical records. Patients were classified according to the Evans group. The conventional treatment including surgery, radiotherapy, pre- and post-operative chemotherapy was given to Stage I, II, and III patients. For Stage IV, relapsed patients, high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (PBSCT) was administered. The chemotherapy consisted of cisplatin, doxorubicin, etoposide, and cyclophosphamide (CCG 3881, 3891). Conditioning for PBSCT was modified VAMP-TBI(cisplatin, doxorubicin, etoposide, melphalan, and total body irradiation). All patients who completed cytotoxic therapy were then either received no further therapy or treated with 13-cis-retinoic acid for six months.

Results: Among 48 patients, 27 were males and 21 females. The median age at diagnosis was 36.5 months (range, 1-167 months). The primary sites were the adrenal glands in 25 patients, followed by retroperitoneum in 10, and thoracic cavity in 10. Most of the patients were in advanced stages: Stage III in 11; Stage IV in 26. Autologous PBSCT was done in 14 cases. The 5-year event-free-survival (EFS) rate was 41% in all study patients with 100% for Stage I, 67% for Stage II/III, 35% for stage IV, 50% of IV-S. In cases with stage IV neuroblastoma, the EFS rate at 4 years after diagnosis was better among the patients who underwent autologous PBSCT than among the patients who received chemotherapy (51 % vs. 20 %; P = .05). Also, EFS was better in patients who received 13-cis-retinoic acids after PBSCT than those who did not (100% vs. 14%; P < .005).

Conclusion: Treatment with high dose chemotherapy and autologous PBSCT improved EFS among children with advanced neuroblastoma. In addition, treatment with 13-cis-retinoic acid was beneficial for patients who underwent transplantation. Prospective randomized study is warranted to further improve survival for subset of advanced patients who might fail to current management strategies.
Results of high-dose chemotherapy followed by autologous stem cell transplantation in children with advanced neuroblastoma in paediatric bone marrow transplant centres in Poland from 1995 to 2005

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Purpose: Postransplant morbidity and clinical outcome in children with advanced neuroblastoma (nb) who underwent high dose chemotherapy followed by autologous stem cell transplantation were investigated.

Patients: The total 80 children with stage III/IV neuroblastoma (nb) who underwent high-dose chemotherapy followed by autologous stem cell transplantation were investigated. Median age of children was 4.95 years (range 1-14.5 years). 48 children were transplanted in 1 complete/partial remission (CR/PR), in 32 patients megachemotherapy was a part of the treatment of relapse (>1 CR/PR). Aphaeresis was done in 64 patients. Bone marrow was collected in 10 pts; bone marrow and stem cells were transplanted in 6 patients. Reinfusion of CD34 cells followed myeloablative chemotherapy with Busulfan + Melfalan in 63 patients; Treosulfan + Melfalan in 2 pts.; Melfalan + Etoposide + Carbo in 10 pts.; Melphalan in 3 pts.; Thiotepa + CTX + Carbo in 1 patient and Thiotepa + Topotecan + Carbo in 1 patient.

Results: 47 (57.5%) children are still alive at median observation time 16 months. 34 (42.5%) children died, 27 of them due to disease progression, 7 patients died due to postransplant complications: VOD (3 pts), MOF (2 pts); EBV-LPD (1 patient), post varicella zoster complications (1 patient). Overall survival (OS) at median observation time 16 months is 0.7; disease free survival (DFS) is 0.6; expected 5-year OS and DFS is 0.42 and 0.4 respectively. OS at 16 months in the group of children transplanted in 1 CR/PR was 0.74; DFS 0.61. OS and DFS expected at 3 years were 0.54 and 0.52 respectively. In children transplanted > 1 CR/PR estimated OS was 0.74, DFS 0.58 at 16 months and expected 3-year OS and DFS were 0.25 and 0.42 respectively.

Conclusions: Megachemotherapy followed by auto-HSCT in patients with advanced neuroblastoma has not many adverse effects. Estimated 5-year OS and DFS are higher in the group of children transplanted in 1 partial/complete remission than in children transplanted after relapse.