Van Bekkum Award

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Striking survival advantage of bad-risk acute myeloid leukemia patients transplanted from haploidentical donors with KIR epitope incompatibility in the GVH direction

In HLA haplotype-mismatched hematopoietic transplants (Aversa et al, NEJM 1998), when KIR epitope incompatibility is in the GvH direction, the post-grafting NK repertoire contains high-frequency donor-vs-recipient alloreactive NK clones which kill pre-transplant cryo-preserved host targets, including myeloid leukemia cells (Ruggeri et al, Blood 1999). The clinical impact of this phenomenon can now be evaluated in 92 transplanted bad-risk acute leukemia patients with a minimum follow-up of one year (1-8 years). Transplants were divided into two groups. In the first (n=58), there was no (HLA-C group 1, HLA-C group 2, HLA-Bw4 alleles) KIR epitope incompatibility in the GvH direction and, incidently, who donors possessed anti-recipient NK clones. In the second (n=34), there was KIR epitope incompatibility in GvH direction, and all 34 donors possessed NK clones alloreactive against recipient target cells. Crucial variables for engraftment and/or GVHD/GVIL, i.e., status of disease at transplant, conditioning regimen, number of stem cells and T cells in the graft, were the same in the two groups. In the absence of KIR epitope incompatibility in the GvH direction, rejection and GVHD rates were 15.5% and 13.7%, respectively. In the presence of KIR epitope incompatibility in the GvH direction, graft rejection and GVHD rates were both 0% (P<0.01). Moreover, while in the absence of GvH KIR epitope disparity relapses occurred in 11/37 AML patients (with a probability of relapse at 5 years of 0.75), zero relapses occurred in 20 AML patients transplanted from donors with KIR epitope incompatibility in the GvH direction (P<0.001). Evidence from mouse models shows alloreactive NK cell kill myeloid leukemia, ablur the host immune system, and eliminate host APCs triggering GVHD (Ruggeri et al, submitted). The combined action of these effects impacts dramatically on the probability of event-free survival of bad-risk AML patients, which is 53% in the absence of KIR epitope incompatibility in the GvH direction, vs a striking 60% in its presence (P<0.001). Choosing donors with KIR epitope incompatibility in the GvH direction offers a striking advantage for survival.

Presidential Symposium

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Chimeric status of gut epithelium after human hematopoietic stem cell transplantation (HSCT)
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A number of groups have reported that bone marrow transplantation (BMT) in mice leads to chimeric status in epithelial tissues of various organs. In humans, BM derived cells have been found to differentiate into mature hepatocytes in the liver. We have investigated whether BM cells contribute to repopulation of the adult gut epithelium. We studied colonic biopsies of 6 female patients, who underwent a sex-mismatched allogeneic HSCT (BMT 4; PBSCT 1; BMT followed by PBSCT 5 months later 1) for the presence of epithelial cells of donor origin. Endoscopic biopsies were taken post transplantation (range 3 weeks to 18 months) and were examined with a combination of fluorescence in situ hybridization (FISH) in order to detect the Y-chromosome, fluorescence immunohistochemistry to detect the epithelial specific marker cytokeratin (CK) and TOTO-3 staining to localize the cell nuclei. By using confocal laser microscopy, serial thin optical sections from 8-20µ thick tissue preparations were collected and the stack of 2-dimensional images was used to generate 3-dimensional (3D) reconstructions. The absence of intraepithelial lymphocytes in the fields previously analyzed by FISH was demonstrated on identical H&E stained sections and on anti-CD45 immunolabelled serial sections. Pts. who received sex matched BM transplants were used as controls and showed high resolution specificity. Y-chrom. pos. / CK neg. cells were found in the gut interstitium of female samples, representing donor derived hematopoietic cells. Y chrom. pos. cells were also found within the gut epithelium. Criteria for the characterization of donor-derived epithelial cells included 3-dimensional colocalization of CK and Y-chrom., presence of the Y-chrom. signal within the TOTO-3 labeled nucleus, CK immunostaining up to the nuclear membrane and absence of intraepithelial CD-45 pos. cells in the serial sections. Using these criteria donor derived epithelial cells were identified in 6/6 patients. Semiquantitative analysis showed higher frequency of donor-derived epithelial cells in the sections taken after HSCT 4 months since transplant. 3D animations of individual CK pos. / Y-chrom. pos. cells will be presented. These data show that after HSCT in humans, donor-derived hematopoietic cells yielded cells that enter the colon and expressed epithelial specific features. The nature and the therapeutic potential of these stem cells and their epithelial differentiating potential demands further examination.

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Acute myeloid leukemia in mice after insertional activation of the proto-oncogene Ev1 by a retroviral gene marking vector

Insertional mutagenesis is a putative risk factor of gene therapy with randomly integrating vectors. In order to assess the safety and efficiency of four different marker genes proposed for human gene therapy, we performed a comparative evaluation in a mouse model of bone marrow transplantation (BMT). The marker genes tested were dLNGFR, the cytoplastically truncated form of the human low-affinity nerve growth factor receptor (LNGFR, well known as p55NTR), two variants of the human CD34 antigen, the full-length protein (ICD34), or a splice-variant lacking most of the cytoplasmic signal transduction domain (tCD34), and EGFP. Methods and Results: Bone marrow (BM) cells from C57BL/6J mice were transduced with cell-free, replication-defective retroviral vectors using MOI of 1.0, and transplanted into irradiated (10 Gy) recipients. Primary recipients showed mostly constant transgene expression levels and had normal blood cell counts and no splenomegaly when examined 28 weeks post BMT. To enable long-term observation of stem cells expressing dLNGFR, ICD34 or tCD34, BM cells were harvested from the primary recipients, pooled according to vector groups and transplanted into a second cohort of irradiated recipients, in half of the cases after MACS enrichment of cells expressing the marker protein of interest. One clone with a single vector integration engrafted all secondary recipients of dLNGFR-marked cells and produced preleukemic lesions which progressed to acute monocytic leukemia. The myeloid transforming gene Ev1 (ectopic viral integration site-1) was activated in malignant cells as a result of vector integration. Monocytic blasts also coexpressed the tyrosine kinase receptor for nerve growth factor, TrkA, and proliferated in response to nerve growth factor. There was no evidence for the activation of replication-competent retroviruses. In addition, none of the secondary recipients marked with tCD34 or ICD34 (n=19) developed a similar disorder during this study. Conclusion: As Ev1 gene expression is not sufficient to cause leukemia in transgenic mice alone, and the human dLNGFR has
been shown to promote fibroblast transformation when co-expressed with Trk receptors, it is possible that the marker protein co-operated with Evi-1 in leukemia induction. This first report of a malignant disorder induced by a single insertion of a retroviral gene marking vector argues for caution in the genetic engineering of stem cells.

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Implantation of allogeneic mesenchymal stem cells results in improved cardiac performance in a swine model of myocardial infarction

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Pathologic ventricular remodeling following myocardial infarction is a major cause of heart failure. We previously demonstrated that autologous mesenchymal stem cells (MSCs) augment local systolic wall thickening and prevent pathologic wall thinning. Based on in vitro studies, we hypothesized that MSCs may be immuno-privileged, and that implantation of allogeneic MSCs could attenuate pathologic remodeling and improve cardiac performance in the swine model of myocardial infarction. Piezoelectric crystals and an LV catheter were implanted in domestic swine prior to a 60-minute LAD occlusion to produce infarction. Following reperfusion, treated animals (n=7) were implanted with allogeneic Dil-labeled MSCs (2 x 10^6 cells in 10ml) throughout the region of infarction. Control (CON) animals (n=6) received vehicle alone. MSCs had been isolated from swine iliac crest bone marrow, expanded in culture, and cryopreserved until the time of implantation. Hemodynamic parameters and regional wall motion were evaluated in conscious animals bi-weekly using trans-thoracic echocardiography (TTE) and sonomicrometry. Animals were sacrificed at multiple time points (6-24 weeks) and tissue harvested for histological examination. Implantation of allogeneic MSCs was not associated with ectopic tissue formation or a significant inflammatory response in any animal. Robust engraftment of allogeneic MSCs was observed in all treated animals. Furthermore, engrafted MSCs were found to express numerous muscle specific proteins, and exhibited morphological changes consistent with myogenesis. When compared to CON animals, MSC treated animals had significant reductions (p<0.05) in LV diastolic pressure (20.7±1.0 vs 30.8±2.0 mmHg in CON) and diastolic wall stress 22.4±2.5 vs 35.9±3.3 g/cm2 in CON) at 6 weeks post-MSC implant. Marked augmentation in both diastolic wall thickness and systolic wall thickening were also observed in treated animals. No significant difference in infarct size was observed between groups. Taken together these data suggest that MSC implantation in infarcted myocardium results in improved global diastolic function. While the mechanism remains unclear, it appears that improved ventricular compliance and diastolic filling are involved. In conclusion, this study suggests that implantation of allogeneic MSCs at reperfusion may be an effective therapeutic option to prevent or reverse the progression to heart failure following myocardial infarction.

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Expanded mesenchymal stem cells (MSC), co-infused with HLA identical hemopoietic stem cell transplants, reduce acute and chronic graft versus host disease: a matched pair analysis


An average bone marrow (BM) transplant contains 10^4/kg mesenchymal stem cells (MSC) while and average peripheral blood (PB) transplant contains no MSCs. In this trial, MSC were harvested from the marrow HLA identical donors, in the context of a prospective protocol investigating their clinical use in the setting of hemopoietic stem cell transplantation: 40 ml of marrow blood, harvested in heparin, were processed by Osiris, USA and MSC selected and expanded to achieve a dose of 1-2x10^6 MSC/kg of recipient. The cells were then cryopreserved and shipped to the transplant centre in liquid nitrogen. Six Centres in the USA and one centre in Europe participated in this study. Eligible for the trial were adults with advanced hematologic malignancies and an HLA identical sibling. Donor and recipient were free of HBV and HCV virus. Evaluable were 31 patients grafted between June 1999 and June 2001. Patients were prepared with conventional conditioning regimen, either CY-TBI, BU-CY or Thioteca-CY. GVHD prophylaxis consisted of CyA-CTX. These patients have been pair-matched with patients transplanted in the Ospedale San Martino of Genova and in the Hospital Saint-Louis in Paris. The adverse events of stem cells was unmanipulated BM in 14 BM and PB in 17 for both study and control patients. The median age and year of transplant for patients receiving MSC versus the controls was 43(19-56) and 48 (23-55) (p=0.02), and the year 2000 (1999-2001) and 1996 (1994-2000) (p=0.0001) respectively. Engraftment was achieved in all patients. Acute GvHD grade 0-1, II, III-IV developed in 14/3, 9/13, 5/10, 2/4, 0/1 for patients receiving expanded MSC and controls respectively (p=0.002). The 6 months incidence of Chronic GvHD was 32-11% versus 67+10% for patients receiving expanded MSC and the controls respectively. In the log rank test at six months the overall difference in incidence of AGVHD and CGVHD is significant (p=0.002) and (p=0.02) respectively. The survival at 6 months is 96% (95%) versus 86+8% (97%) respectively. As seen in Table 1, engraftment was adjusted on all matching factors in multivariate analysis. No significant difference in relapse is observed. These data suggest that the use of expanded MSC is safe and produces a significant reduction of acute and chronic GvHD. Although the follow-up of patients allografted with expanded MSC is still short, the available date warrant further trials with expanded MSC.

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Minor histocompatibility antigens (mHag) HA-1 and HA-2 specific cytototoxic T-cells (CTL) induce complete remissions (CR) after donor lymphocyte infusion (DLI) for relapsed CML or multiple myeloma (MM) after allogeneic stem cell transplantation (alloSCT)


Treatment of relapsed CML after alloSCT with DLI induces molecular CR (mCR) in 70-80% of the cases. Donor T cells may react against leukemia-associated antigens (Ag), over-expressed by the tumors of the recipient. Previously, we have demonstrated that the HLA-A2 restricted mHag HA-1 and HA-2 are expressed exclusively on cells of hematopoietic origin. Thus, when donor and recipient are incompatible for expression of HA-1 or HA-2 in the graft-versus-host leukemia (GVL) direction, these antigens may serve as targets for a GVL reaction. Recently, 3 HA-1 and/or HA-2 positive patients, CML(2) and MM(1), who relapsed after alloSCT were treated with DLI from their donor and/or HA-2 negative donors. Using HLA-A2/HA-1 and HA-2 peptide tetramers we were able to show the emergence of HA-1 and HA-2 specific CD8+ T cells. The appearance of these tetramer positive (Tet+) T cells was associated with a mCR of the disease. Therefore, these antigens may serve as targets for a GVL reaction.
cells present in peripheral blood of the CML patient after DLI were involved in the anti-leukemic response. Furthermore, this implicates that in-vitro generated anti-HA-1 and -2 CTL can be used for adoptive immunotherapy to treat relapsed hematological malignancies after allo-SCT.

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Allogeneic vs autologous stem cell transplantation according to cytogenetic and FAB features in AML patients (pts) <= 45 yrs old in CR1: results of the EORTC-GIMEMA AML-10 trial

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In the AML-10 trial, CR pts received an intensive consolidation course. Subsequently, all pts <= 45 years with a family donor were assigned to undergo allo-SCT, whereas pts without a donor were planned to receive auto-SCT.

Between 11.1993 and 12.1999, 2157 pts entered the study, 2018 were evaluable. Among 1123 pts <= 45 yrs, 815 (72.6%) reached CR and 764 received consolidation. 34 pts have not been HLA-typed due to early relapse, refusal or death within a month from start of consolidation. Among remaining 730 patients, 292 had a donor and 438 did not have a donor (40 had no siblings). The median follow-up was 3.6 years; 284 pts relapsed, 66 died in CR1; 285 died.

Allo-SCT was performed in 198 (67.8%) pts with a donor and auto-SCT in 239 (54.6%) pts without a donor. The 4-year DFS rate of pts with a donor was superior to that of pts without a donor (51.4% vs 41.2% p=0.046; HR/hazard ratio=0.80 95% CI 0.643-0.996). The relapse incidence was 31.2% vs 52.9%(p= 0.0001), the TRM 17.3% vs 5.8% (p=0.0001), and the survival rate 58.0% vs 49.4% (p=0.22), HR=0.86 (95% CI 0.68-1.10). In good prognostic FAB subtypes (M2-M4E: 305 pts), the 4-year DFS rates were 55.1% and 52.9% (HR=1.07) and in the other FAB subtypes (418 pts) 48.5% and 32.5% (HR=0.73) respectively.

The 4-year DFS rates for the patients in whom cytogenetics was not done/failed (n=288) was 56.0% for the donor group vs 40.0% in the no donor group (HR=0.68). In 442 pts with evaluable cytogenetics, the following risk groups were considered: good (t(8;21) or inv(16): 121 pts); intermediate (NN or -Y only: 164 pts); bad (all others: 157 pts). In good risk pts, the 4-year DFS rate in pts with a donor vs those without a donor was 58.8% vs 65.4% (HR=1.27); in the intermediate risk pts 45.9% vs 46.3% (HR=1.07) and in bad risk pts 43.8% vs 19.0% (HR=0.59). The decrease in EFS according to type of primary disease, karyotype, highest FAB type or percentage of blasts at SCT. These results indicate that pts with sMDS can successfully be transplanted. We suggest that SCT should be performed as soon as possible once the diagnosis of sMDS has been established.

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Stem cell transplantation (SCT) for children with secondary MDS (sMDS): report from a multicenter study of the European Working Group of MDS in childhood (EWOG-MDS)


sMDS generally carries a dismal prognosis. Here, we report the interim analysis for 23 patients (pts) with sMDS treated on the ongoing SCT trial of EWOG-MDS. Median age at the time of SCT was 11.5 years (5.0 - 26.2). Most advanced FAB-type prior to SCT was RA/RARS in 6 pts, RAEB in 8, RAEB-t in 4 and myelodysplasia-related AML (MDSr-AML) in 5. Four pts had received AML-type therapy prior to SCT, median blast count in the bone marrow at SCT was 13 % (0-88). Karyotype analysis revealed monosomy 7 in 8 pts, complex cytogenetic abnormalities in 3 and other abnormalities in 2. In 8 pts the karyotype was normal and in 2 unknown. Median time from diagnosis of sMDS to SCT was 5.1 mo (0.5-30.3). 13 pts were transplanted for MDS after treatment for a primary malignancy (ALL/AML in 7, others in 6). Median time between primary malignancy and diagnosis of MDS was 2.8 years (1.7-6.1). 10 pts were grafted for sMDS after treatment for acquired aplastic anemia (AA). Median interval between diagnosis of AA and sMDS was 2.8 years (1.6-13.0). Pts were transplanted from an HLA identical or 1 antigen mismatched family (MFD) (n=6) or an unrelated (MUD) (n=17) donor. Myeloablative therapy consisted of busulfan 16 mg/kg, cyclophosphamide 120 mg/kg and melphalan 140 mg/m2. The source of stem cells was bone marrow (n=11) or peripheral blood (n=12). GvHD prophylaxis for transplants with MFD consisted of cyclosporine (CSA), for MUD anti-lymphocyte globulin, CSA and methotrexate was most commonly employed. At time of analysis, median follow was 13 months. 6 pts suffered transplant-related mortality (TRM), 3 relapsed from MDS and 1 from primary malignancy. At 3 years, the probabilities for event-free survival (EFS) of relapse and TRM were 0.57, SE=0.11, 0.14, SE=0.09 and 0.33, SE=0.10, respectively. There were no significant differences in EFS according to type of primary disease, karyotype, highest FAB type or percentage of blasts at SCT. These results indicate that pts with sMDS can successfully be transplanted. We suggest that SCT should be performed as soon as possible once the diagnosis of sMDS has been established.

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Relapse rate after transplantation of allogeneic purified peripheral CD34+ stem cells in children with ALL

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36 pediatric patients with acute lymphatic leukemias (ALL) (CR1=8, CR 2=16, CR3=4, non remission=8), were transplanted with highly purified peripheral CD34+ stem cells from (partially) matched unrelated donors (n=15) or from 1-3 HLA loci mismatched parental donors (n=21) in order to prevent acute and chronic GvHD without any posttransplant pharmacological immunosuppression. After busulfan- or total body irradiation-based myeloablative conditioning regimens the patients received a mean number of 10 Mio stem cells/kg with a median purity of 98.5% CD34+ and only 5000 contaminating T-cells/kg. No pharmacological GvHD prophylaxis was given. T-cell recovery was delayed (median time to reach > 100 T-cells/μl was 106 days) and T-cell response to mitogen stimulation was reduced within 12 months. However, high numbers of Natural Killer (NK)-cells were

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detectable in all patients already in the first month after transplantation. Incidence of GvHD grade 1-2 was 14%, no severe GvHD > grade 2 was observed. All patients being not in remission relapsed. The actuarial risk of relapse of the remission group was comparable with that of a historical group of patients (n=13) from our institution, receiving unmanipulated (non T-cell depleted) bone marrow from unrelated donors and pharmacological GvHD prophylaxis. No significant difference was found (CD34+ enriched group: 42% risk of relapse after 2 years, unmanipulated group: 34%). The 2 year-overall survival was better in the CD34+ enriched group (56%) compared to 31% in the unmanipulated group (p=0.0001). The median follow-up was 2.0 years (range 1.0 - 5.7 year). Furthermore, NK activity was measured in the CD34+ group: most patients showed normal or increased NK activity, which could be enhanced by Interleukin 2 in vitro. Conclusions: transplantation of purified CD34+ cells allows to avoid GvHD effectively even when mismatched donors are used, without increasing the relapse rate in patients with ALL. This could be due to the absence of any posttransplant immunosuppression and also a T cell independent (probably NK cell mediated) GvL effect may exist.

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Stem cell transplantation (SCT) for children with juvenile myelomonocytic leukemia (JMML) and myelodysplastic syndrome (MDS) treated on the EWOG-MDS protocol: a single-center experience

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JMML is a unique disorder of infancy with myeloproliferative and myelodysplastic features. In contrast, MDS is characterized by peripheral blood cytopenia and ineffective hematopoiesis. MDS may be a result of chemoradiotherapy, bone marrow failure disorders. sMDS accounts for about 40% of MDS cases in children, while the remainder is classified as primary MDS (pMDS). SCT is the treatment of choice for patients (pts) with both, MDS and JMML. Here, we report the outcome of 32 pts with MDS/JMML transplanted between 01/97 and 06/01 at the University Children's Hospital in Freiburg, Germany. Pts were transplanted on the ongoing study of the European Working Group of MDS in Childhood (EWOG-MDS). Conditioning regimen consisted of busulfan 16 mg/kg, cyclophosphamide 120 mg/kg and melphalan 140 mg/m². GvHD prophylaxis for pts grafted from an HLA identical or 1 antigen mismatch family donor (MFD) consisted of cyclosporine only, for pts transplanted from an HLA identical or 1 antigen mismatch family donor (MFD) consisted of cyclosporine only, for pts transplanted from an HLA identical or 1 antigen mismatch family donor (MFD) consisted of cyclosporine only, for pts transplanted from an HLA identical or 1 antigen mismatch family donor (MFD)

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Extracorporeal photopheresis for the treatment of chronic graft versus host disease (GvHD) in children

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In children, where the growing organism is particularly vulnerable to the consequences of chronic GvHD itself and or prolonged immunosuppressive treatments, the use of extracorporeal photopheresis (ECP) may be of particular interest. Materials and Methods: ECP was carried out using a Cobe Spectra separator and UV-MATIC irradiator (Vibler Lourmat). A total of 254 ECP procedures were performed in 8 children (median age 10 years : range 5-15).

Results: A peripheral venous single-lumen permanent central catheter access (69% of apheresis) or a dual-lumen permanent central catheter access (26% apheresis) were preferentially used. A median of two patient's blood volume was processed and 5. 10 7 lymphocytes/kg (0.1-50 10 7/kg) was irradiated in each procedure. A median platelet decrease of 17% (p=0.0001) and median hemoglobin level decrease of 15g/l (p=0.0001) were noted following each ECP-apheresis. However, none of the patients had profound thrombocytopenia or anemia. Two minor episodes of catheter related-bacteremia were noted (2310 catheter-days).

All but one patient were treated for more than 6 months and in 3 patients treatment duration exceeded one year. All patients are alive and well. 7/8 experienced a dramatic improvement in their cutaneous status particularly on inflammatory lesions. In scleroderma-like lesions, regression is only partial but important enough to improve patients quality of life. An important residual skin pigmentation is frequently encountered. Mucosal affection completely resolved in the majority of patients. However, our long term data suggest that ECP does not prevent an evolution of oral and genital lesions to atrophic lichen-like plaques. Liver and gut disease resolved completely in 4/6 and 5/5 of patients respectively. A comitant immunosuppressive therapy was stopped (5/6) or considerably reduced (3/8). Five patients with more than two-years follow-up after discontinuation of ECP are in remission with no immunosuppression treatment. They have normal growth rates and normal school activity.

Conclusion: Our study shows that ECP is beneficial, well tolerated and can be safely used for chronic GvHD treatment even in young children with low body weight and a poor performance status. We believe that having a dedicated pediatric environment together with an experienced, pediatric team is of crucial importance for improving patient’s acceptance of this long-term therapeutic program.

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T-cell reconstitution after haplo-identical PBSCT is delayed, comparable with recovery after T-cell depleted unrelated donor BMT but not non-T-cell depleted BMT

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Objective: Previous studies suggest that the kinetics of immune reconstitution after stem cell transplantation is related to the degree of T-cell depletion and the number of CD34+ stem cells infused. We aimed to compare CD3+ T-cell recovery in children after highly purified T-cell depleted and CD34+ selected haplo-PBSCT with that of bone marrow transplantation (BMT) with and without T-cell depletion of the graft.

Methods: A retrospective analysis of prospectively collected data in children undergoing transplantation from different donor groups was undertaken. i.e. HLA-identical, fully matched sibling donors (n=27), matched unrelated, non-T-cell depleted marrow donors (n=13), T-cell depleted unrelated donor marrows (n=14) and haplo-PBSCT (n=7). CD3+ T-cell recovery was measured by FACS analysis at 1,2,3,6 and 12 months post-transplant.
haplo-PBSCT procedures.

Immune deficiency of the intensive T-cell depletion inherent to

3. High levels of infused CD3+ cells do not compensate for the

2. Both show slower recovery in comparison to non-T-cell

1. CD3+ T-cell recovery after haplo-PBSCT does not differ

significantly from that following T-cell depleted BMT.

Conclusions:

1. CD3+ T-cell recovery after haplo-PBSCT does not differ

significantly from that following T-cell depleted BMT.

2. Both show slower recovery in comparison to non-T-cell

3. High levels of infused CD3+ cells do not compensate for the

immune deficiency of the intensive T-cell depletion inherent to

haplo-PBSCT procedures.

Megatherapy/SCT activity in pediatric solid tumors in Europe - the 2001 report

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5192 patients (pts) were registered in the paediatric-adolecent age group (upper limit 18 years) from 243 centres and 33 countries. In 5027 pts minimal data for analysis was available from the EBMT data base with a median observation period (MOP: from date of SCT till 12/2001) of 6.5 yrs (range, 0.3 to 24 yrs.), age:median at MGT, survival % refer to 5-year overall survival (OS 37%) or event free survival (EFS 34%) rates. Neuroblastomas: 2282 pts are registered, 61% are male, age: 3.8 yrs. (0.4 to 18 yrs.). The OS [EFS] is 36% [34%] with a MOP of 7.5yrs. The OS for pts (n=1879) in remission is 38% (CR1/VGPR/PR/SD/PRD) and 28% for relapse (CR2/SR/RR) pts (n=222). The OS rates in compliance with the status at MGT are 53% vs. OS 34% with other regimens (p<0.001) and kindled a prospective European randomised trial for 2001. Ewing tumours: 823 pts up to 18 yrs., 54% are male, age: 12.4 yrs. [range, 0.4-18yrs]. The OS (EFS) rates are 39% [37%]; MOP: 5.8yrs. The OS for 589 first remission pts is 47% and 29% for 261 relapse pts. The OS rates according to status at MGT are 52% for CR1/VGPR (374 pts), 39% for PR (179pts), 19% for SD/PRD (32 pts), 40% for CR2 (132pts), 21% for SR (76pts) and 13% for RR (33 pts) and 0% for UR (14 pts, 10 deaths). Again Busulfan containing regimens resulted in statistically superior survival in first remission pts (OS 50% vs. OS 30% with other regimens, p<0.001). The Euro-EWING 99 Study is currently asking a prospective, randomised question with BU-MEL MGT in selected high-risk patients. The major aim is to start new European collaborations on MGT approaches in high-risk solid tumours based on the high-risk patient definitions resulting from recent multivariate analysis of prospective national trials. Potential candidates for 2001 are: Soft tissue sarcomas: 646 pts, 54% are male, age: 8.8 yrs. [range, 0.4-18yrs], MOP: 6.8yrs. The OS [EFS] rates are 31% [27%]. The OS rate of 379 first remission pts is 34% [31%] and 25% [22%] for 200 relapse patients. Wilms Tumours: 197 pts, 47% are males, age: 6.1 yrs. [range, 0.8-18 yrs.] and MOP: 6.5yrs. The OS [EFS] rates at 5 years are 56% [52%]. The OS was for 57 first remission pts 53% and was 58% in 127 relapse patients. Retinoblastomas: 66 pts, 53% were males, age: 4 yrs. [range,0.05-14.8yrs] and MOP: 7.7yrs. The OS [EFS] rate at 5 years is 66% [59%]. OS in 33 first remission pts was 76% and 55% for 32 relapse pts.

Acute Leukemia

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Comparison of intensive chemotherapy (CHT), allogeneic (ALLO) or autologous (AUTO) stem cell transplantation (SCT) as post-remission therapy in adults with high-risk ALL (HR-ALL). Results of randomized study PETHEMA ALL93


Objective: To analyze the results of prospective randomized trial PETHEMA ALL93 for adults with HR-ALL (one or more of the following: age>30 yr., WBC>25x10^9/L, t(9;22), 11q23 or slow response to induction therapy).

Design: 5-drug induction therapy (VCR, DNR, PDN, ASP and CPM) followed by 3 cycles of consolidation therapy (including high-dose MTX, HD-ARA-C and HD-ASP). Patients with a histocompatible sibling were assigned to ALLO-SCT, whereas the remaining patients were randomized to AUTO-SCT or intensification (the same 3 consolidation cycles) plus maintenance (MP+MTX) therapy for 2 yr.

Patient characteristics: 31 hospitals, 189 evaluable cases, 114 males. Mean (SD) age 23 (13) yr. Phenotype: pro-B 42(22%), pro-A 28(15%), common pre-B 25(14%), common 11(6%), and other 4(2%). The major cytogenetic abnormality was t(9;22) in 29% of patients. Malignant bone marrow blast cells on day 14 of induction treatment) as the only post-remission therapy in adults with high-risk ALL (HR-ALL). Results of randomized study PETHEMA ALL93

Results of therapy: Death in induction 12(6%), resistant disease 25(14%), CR 151(80%). The groups of ALLO-SCT (n=64), AUTO-SCT (n=22), and AUTO-SCT (n=22) and slow response to therapy (>10% blasts in bone marrow blast cells on day 14 of induction treatment) as the only post-remission therapy in adults with high-risk ALL (HR-ALL). Results of randomized study PETHEMA ALL93

Recurrent disease: Death in induction 12(6%), resistant disease 25(14%), CR 151(80%). The groups of ALLO-SCT (n=64), AUTO-SCT (n=22), and AUTO-SCT (n=22) and slow response to therapy (>10% blasts in bone marrow blast cells on day 14 of induction treatment) as the only post-remission therapy in adults with high-risk ALL (HR-ALL). Results of randomized study PETHEMA ALL93

Conclusions: The results and toxicity of PETHEMA ALL93 trial for adults with HR-ALL (one or more of the following: age>30 yr., WBC>25x10^9/L, t(9;22), 11q23 or slow response to induction therapy).

Supported by grants FIS 97/1049, FJU CPTH-01 and FJC P/EF-01
Objectives: The prognosis of pts. with Ph+ALL relapsing after allo SCT is poor. STI571 (Glivec) is an inhibitor of the ABL tyrosine kinase with antileukemic activity in advanced Ph+ALL, although its role in recurring Ph+ALL after allo SCT have not been established.

Patients: 20 pts. with Ph+ALL relapsing after allo SCT were treated with STI571 at a daily dose of 400-600 mg p.o. within two multicenter phase II trials. One pt. had received STI571 prior to allo SCT. Disease status at allo SCT was: CR1 n=10, CR2 n=3, 1st relapse n=3, 2nd or subsequent relapse n=3, primary refractory n=1. Median time interval between transplantation and trial entry was 6 (3-63) mo., median age was 45 (21-62) yrs. 11/12 pts. with informative cytogenetic analysis had additional chromosomal abnormalities.

Results: Complete cytologic remission (CCR) with PB recovery was achieved in 11 pts. (55%) and with persistent cytopenias in 4 pts. (20%) within a median time of 1 (0.5-2) mo.. 5 pts. were refractory, including one early death on day 11 due to leukemic organ infiltration. Clearance of peripheral blasts occurred within 10 days. In CCR pts. Ph+ cells became undetectable by cytogenetic and FISH analysis. Donor chimerism in responding pts. increased from a pre-study median of 91% in PB and 64% in BM to 96% and 82% after 2 weeks and 98% and 96% after 4 weeks. Active GvHD (³ grade III) at trial entry was seen in 4 pts.. No exacerbation of pre-existing GvHD was noted. 1 pt. developed grade III GvHD of skin and gut after 14 days of therapy. Concomitant treatment with immunosuppressive, antiviral and antifungal agents was feasible. 4 pts. were treated with incremental donor lymphocyte infusions without occurrence of GVHD. 10/15 responding pts. relapsed after a median treatment duration of 4 (1.5-7) mo., one pt. died in CR at 3 mo. of disseminated aspergillosis and MOF. 4 pts. remain in ongoing CR after 5, 6, 14 and 21 mo., one pt. remains in complete molecular remission (quantitative RT-PCR, Taqman) after 21 mo. of treatment.

Conclusion: STI571 is highly effective as initial treatment of relapsed Ph+ ALL after allo SCT with a favorable safety profile. However, prolonged CR is only achieved in a small subset of pts., and molecular remissions are rare. Additional therapeutic modalities are required to prevent relapse in the majority of pts. and will be explored in ongoing and future prospective clinical trials.

O103

Fractionated TBI is associated with less T-cell mixed chimerism and increased risk of relapse compared to busulphan in patients with acute leukemia and CML after allogeneic SCT

J. Mattsson, M. Uzunel, M. Remberger, M. Hassan (Stockholm, S)

During the past years the relationship between the incidence of mixed chimerism (MC) and the conditioning regimen has been debated. In the present study we prospectively evaluated MC in the T-cell and myeloid cell lineages and its correlation to busulphan (Bu)- and TBI-based conditioning in 180 patients after allogeneic SCT. Diagnoses were: CML (n=48), AML (n=69), MDS (n=11) and ALL (n=50). Median patient age was 29.5 (0.2-60) years. Conditioning therapy consisted of TBI (10Gy)+Cy (n=76), TBI (4x3Gy)+Cy (n=45) and Bu+Cy (59). Seventy-three patients were grafted with marrow from HLA-identical siblings and 107 from matched unrelated donors. ATG or OKT-3 was given to 113 patients. GVHD prophylaxis was mainly a combination of MTX+Csa. In all patients receiving Bu, mean Bu-concentration was measured during conditioning. The Bu-concentrations were divided into low (<450mg/ml), medium (450-600mg/ml) and high (>600mg/ml). Chimerism analysis was performed using PCR of myeloid lineages. Blood samples were taken day ±14, ±21, ±28 and monthly thereafter. All samples were cell separated for T-cells and myeloid cells followed by DNA-extraction.

113 patients are alive with median follow-up of 28.5 (4-62) months. Acute GvHD grade I occurred in 85 (47%) and GvHD grade II-IV in 39 (33%) of the patients. The incidence of MC in the T-cell lineage was significantly lower in those receiving TBI (22%) compared to those receiving single dose TBI (53%) or Bu (47%) (p=0.005 and p=0.023, respectively). The incidence of myeloid MC did not differ between the three groups. The incidence of T-cell and myeloid MC after SCT did not differ between low (47%), medium (36%) and high (55%) Bu-concentrations measured during conditioning. Patients receiving TBI had significantly higher probability of relapse compared to Bu treated patients (44%vs16%, p=0.01). In multivariate analysis adjusted for disease stage and diagnosis, Bu treated patients showed both a better patient survival (p=0.03) and less probability of relapse (0.026) compared to TBI treated patients.

We conclude that TBI-containing regimen is associated with less incidence of T-cell MC and increased risk of relapse compared to Bu containing regimen in patients with CML and acute leukemia after allogeneic SCT.

O104

Risk factors for relapse after unrelated cord blood transplants in children with acute lymphoblastic leukemia. A Eurocord analysis


GvHD is reduced after unrelated CBT whether this fact increases relapse is unknown. To study risk factors for relapse incidence (RI) we analyzed 165 children transplanted with unrelated CBT for ALL from 94 to 2001. At diagnosis the median number of WBC was 28x10^9/l (7-10000), karyotype was normal in 59 (36%), not available in 35 (21%), abnormal in 71 (43%) patients (49% high, 30% intermediate and 21% standard risk ); 24 patients had T– ALL. At CBT, median age was 6.5 y (0-16); 100 children had a good prognostic status for transplant (CR1 n=27 or CR2 n=73) and 65 more advanced phase. Previous BMT were given to 11 children . The median follow-up was 28 months (0.4-69).

Conditioning regimen varied according centers. As GvHD prophylaxis, 14 patients received CsA alone, 34 MTX containing regimen and 116 patients received CsA-steroids. The median number of nucleated cells infused (NCI) was 3.8 x10^7/kg (0.6-21.3). CB donor was HLA matched in 18 patients and mismatched in the others (5/6 n=67; 4/6 n=66 and 3 or 2/6;n=7). Neutrophil recovery at day 60 was 84% and the median time was 30 days (95CI:21-50). Patients receiving more than 3.7x10^7/kg NC had 93% of probability of neutrophil recovery compared to 75% in the remainders (p<0.001). At day 100, TRM was 34%. Probability of aGvHD II-IV was 38% and cGvHD was observed in 11/89 patients at risk. Estimate 2 year EFS was 33%. It was 40% for patients transplanted in CR1, 34% for CR2 and 29% for the others (p=0.41). Strikingly, RI was 43% in CR1, 35% in CR2 and 32% in advanced phase (p=0.69).Estimate RI at 2 years was 47% in patients with any karyotype abnormalities, 25% in patients with normal and 35% in patients with unknown karyotype (p=0.02).

There was a trend of increased risk of RI: i)T-ALL (p=0.09), ii) presence of MTX (p=0.06), iii) shorter time from diagnosis to CBT (p=0.09) and absence of aGvHD (as a time dependent covariate) (HR: 2.1; p=0.09). Relapse on therapy before CBT was an important factor increasing RI in the group of patients transplanted in CR2 or more (p=0.02). In summary, in a multivariate analysis, the most important factors increasing RI were: i) karyotype abnormalities at diagnosis ( p=0.03), ii) presence of MTX in GvHD prophylaxis ( p=0.007); iii) time from diagnosis to CBT (<25 months).
months; \( p=0.05 \) and the absence of aGVHD \( (p=0.01) \). Since aGVHD influences the RI after CBT for ALL, prospective studies should address the question of GVHD prophylaxis, in other to enhance the GVL effect.

O105

Effect of cell dose on outcome of children with acute lymphoblastic leukemia given an unrelated donor bone marrow transplantation


Previously published studies have suggested that cell dose may play a relevant role on post-transplant outcome of patients given an allograft for acute leukemia. However, no study has specifically focused on children with acute lymphoblastic leukemia (ALL) given allocord-BMT from an unrelated donor. With the aim of investigating the importance of cell dose in these patients, we analysed 187 children with ALL in CR (median age 8.5 years, range 0.6-15.7). Moreover, we investigated the role of other variables on the probabilities of relapse, transplant-related mortality (TRM) and leukemia-free survival (LFS). Fifty-four patients were transplanted in 1st CR, 96 in 2nd CR and 37 in 3rd CR, respectively. In 48 cases karyotype was normal, whereas 78 children had cytogenetic abnormalities. Among this latter group, 35 children had a Ph+ chromosome or evidence of the bcr/abl fusion transcript. A TBI-containing preparative regimen was employed in 162 patients. In 52 children, the graft was T cell depleted. The median number of nucleated cell infused was 3.85x10^8/kg (range 0.3-51). For the purpose of this analysis, we subdivided patients in 3 groups according to the number of cells infused: those receiving <2x10^8/kg, 2-5x10^8/kg and >5x10^8/kg nucleated cells, respectively. Children given a T-cell depleted transplant received a significantly lower number of nucleated cells \( (p<0.01) \).

Nineteen patients failed to engraft. In the remaining 168 patients, a cell dose >2x10^8/kg was associated with a faster neutrophil recovery \( (p<0.05) \). The 3-year estimates of LFS for patients receiving <2x10^8/kg, 2-5x10^8/kg and >5x10^8/kg nucleated cells were 26±6%, 44±7% and 61±7%, respectively \( (p<0.0001) \). The 3-year estimates of TRM were 54±8%, 25±6% and 19±5%, respectively \( (p<0.0009) \). In multivariate analysis, patients given a cell dose >2x10^8/kg and those given TBI had a significantly better LFS, whereas results in patients transplanted in 3rd CR were worse. In multivariate analysis, a cell dose >2x10^8/kg was the only factor favourably influencing TRM, whereas TBI was the only variable reducing the risk of relapse. These data suggest that harvesting of a larger numbers of cells reduces the risk of TRM and improves patient outcome. This should be feasible considering that the median age of the donor and recipient was 35 and 8.5 years, respectively. TBI should be preferred as part of preparative regimen, for its favourable effect in reducing the risk relapse and in improving LFS.

O106

Allogeneic hematopoietic stem cell (HSC) transplantation for adult acute lymphocytic leukaemia (ALL) during the last decade in Europe: identification of an easy prognostic score for "rapid" transplant

N. Gorin, M. Labopin, V. Rocha, F. Frassoni on behalf of the Acute Leukemia Working Party of EBMT

From 1990 to 2000, 5141 adult ALL patients were reported to EBM as receiving an HSC transplant: 1671 autologous (CR1: 1299; CR2: 382) and 3470 allogeneic (CR1: 1673; CR2: 757; CR3: 148; Refractory: 682) consisting of: genoidentical: 2445; N. Gorin, M. Labopin, V. Rocha, F. Frassoni on behalf of the EBMT Acute Leukemia Working Party

In addition 45 patients received an unrelated cord blood (UCB) transplant. Patients autografted with Bone Marrow (BM) had a 3 year Leukemia Free Survival (LFS) of 36±2% in CR1 and 22±3% in CR2.

The 3 year LFS for patients allotransplanted in CR1 were 51±1% and 42±5% using an HLA identical family or an unrelated donor respectively. In CR2, it was 35±2 % and 29±4% respectively. By pair matched analysis (all status), the use of Peripheral Blood in unrelated transplants, was associated with a lower LFS (21±7% vs 32± 6%with BM, \( p=0.04 \) ). In 45 recipients of an UCB transplant (all status of disease combined) the 2 year TRM was 53%, the LFS 28% and the overall survival 30%. We studied 1402 patients autografted during this period with an HLA identical sibling in CR1. The median interval from CR1 to allotransplant was 96 days . In patients transplanted less than 96 days after achieving CR1, 3 factors predicted for the outcome by multivariate analysis enabling modelisation of 3 prognostic groups: patient age, CR1 achievement with one or more induction course and the recipient/donor sex combination. These 3 factors overcame the information from Cytogenetics and sex of stem cells .Three prognostic groups could be identified in relation to the outcome following an early transplant (CR1 to transplant < 96 days) : Group 1 (good prognosis) include patients < 35 year old, achieving CR1 with one induction course and to be transplanted with any other sex combination than female to male; group 3 (bad prognosis) with all reverse criteria, and group 2 (intermediate) with one or two adverse factors. In these 3 groups the 3 year TRM were 16%, 34% and 48% respectively, the LFS 58±5%, 48 ± 4% and 29±4% and the OS 65±5%, 53±4% and 29±5% respectively.

Patient age, response to induction and the sex of the HLA identical family donor are the strongest easy predictors of the outcome for an early transplant in an adult patient with ALL. No additional information is mandatory.

O107

Outcome of allogeneic BMT in high-risk childhood T-Cell acute lymphoblastic leukemia (HR-T-ALL) compared to treatment with chemotherapy alone: results from trials ALL-BFM 90 and ALL-BFM 95

A. Schrauder, A. Reiter, M. Zimmermann, T. Klingebiel, G. Mann, K. Welte, M. Schrappe (Hannover, Giessen, Frankfurt, D; Vienna, A)

Background: In trials ALL-BFM 90 and 95 HR-ALL was defined by prednisone poor response (PPR: \( >=1000 \) blasts/mm\(^3\) PB after one week of prednison and one IT MTX), non-remission on day 33 of treatment (NRd33), or translocations (t(9;22) or t(4;11). With a median observation time of 4.7 yrs, we report treatment results of 1065 ALL patients characterized by T-ALL with PPR and/or NRd33 (34.5% of all T-ALL). This group comprised 73% males, median age was 8.8 yrs, and median WBC 142 x 10^9/L. These pts were considered candidates for MFD-BMT. Methods: From 1990 to 2000, 193 HR-T-ALL pts (among 499 pts with HR-ALL) were treated according to trials ALL-BFM 90 (n=106) and 95 (n=87). Event-free survival at 4 years was 29% (SE=4%) for HR-T-ALL in trial ALL-BFM 90, and 48% (SE=6%) in trial ALL-BFM 95 (Log-Rank \( p=0.01 \)).

Results:

1) according to induction response and use of BMT (see table below):

2) Comparison of pDFS (4yrs) between BMT and no BMT (Mantel-Byar-Test):

3) Events (BMT-group; trial 95): In MFD-BMT, relapses were the only type of event (3/13), in contrast to treatment related mortality being the only adverse event in MMFD-MUD-BMT (4/12).

Conclusion:

1) The pDFS at 4 yrs of this unfavorable subgroup of T-ALL has improved significantly from study ALL-BFM 90 to 95 for transplanted and non-transplanted patients \( (p=0.01) \).
2) Allogeneic BMT provides superior results as compared to treatment with chemotherapy alone (p=0.04).

3) The analysis of events in the BMT-group in the most recent trial, ALL-BFM 95, may indicate a biological impact of MUD-BMT in curing HR-T-ALL.

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<td>pDFS (all)</td>
<td>38%</td>
<td>34%</td>
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Legend to table:
a) and remission on day 33
b) in ALL-BFM 95: 12/25 patients MMFD or MUD
c) disease-free survival at 4 yrs (pDFS): Log-Rank p=0.01

O108

Prognostic importance of minimal residual disease and of donor availability in Philadelphia positive adult acute lymphoblastic leukemia - results of an European multigroup prospective study (EIGLE)


The French LALA, the Italian GIMEMA and centers of the EORTC Leukaemia Group conducted a prospective multicentric, multigroup study in adult Ph+ and/or BCR-ABL+ ALL in order to assess the value of stem cell transplantation (SCT) and the predictive power of minimal residual disease (MRD). Patients who reached CR by their groups' induction schedule received a consolidation regimen generally consisting of intermediate or high dose Ara-C and mitoxantrone (HAM). After consolidation patients were eligible for either allografting (if a HLA identical sibling donor was available and if age <= 45-55 years) or autografting (without such donor).

A total of 224 patients were enrolled in this study between 12.1993 and 12.1998. The median duration of follow-up was 4.3 years. At 4 years the overall survival rate was 18.6% and the overall disease-free survival (DFS) rate was 16.4%.

After induction/consolidation chemotherapy, a total of 170 (76%) patients achieved a CR. A SCT was performed in 87.7% of patients with a sibling donor (n=73) and in 52.7% in patients without a sibling donor (n=93). A total of 112 pts relapsed (61 before and 61 after SCT), 25 died in CR (4 before and 21 after SCT) and 33 are still in CCR.

The bone marrow of 107 CR patients was tested for MRD after consolidation. PCR+ patients (n=42) had a longer (p=0.001) DFS than PCR- patients (n=65). The DFS rate at 4 years for PCR+ patients was 46.9% vs 27.9% (hazard ratio (HR) = 0.56; p=0.17), whereas in PCR+ patients these rates were 38.5% and 31% (HR = 0.43; p=0.004), respectively. In 59 of pts who reached CR, an assessment by PCR has not been performed. The median DFS was slightly longer for pts with a sibling donor than for those without such a donor (8.8 vs 4.7 months), but the 2-year DFS rate was approximately 10% in both groups.

Multivariate analyses revealed the following independent prognostic factors for DFS: PCR+ (HR = 2.05, p=0.0056), sibling donor availability (HR = 0.40, p=0.0004), age (> 45 vs <= 45 yrs: HR=1.74, p=0.027), WBC (> 25 vs <= 25: HR=2.08, p=0.0045) and number of courses to reach CR (2 vs 1: HR=2.18, p=0.0071). In conclusion, allogeneic stem cell transplantation from a familial donor is the treatment of choice for Ph+ ALL patients in CR, as shown by uni- and multivariate analyses, using the intent-to-treat principle. Additional consolidation strategy may be necessary in order to increase the number of PCR patients before transplantation.

Lymphoma

O109

Cologne high-dose sequential chemotherapy in relapsed and refractory Hodgkin’s disease - Results of a multicenter phase-II study

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Combination chemotherapy can cure patients (pts) with Hodgkin’s disease (HD), but those with treatment failure or relapse still have a poor prognosis. High-dose chemotherapy (HDCT) and autologous stem cell support (ASCT) can improve the outcome of these pts as shown in the HR-R1 study of the GHSG/EBMT. We designed an intensified salvage program with a final myeloablative course. Eligibility criteria include age 18-60 years, histologically proven primary progressive or relapsed HD and NHL. Treatment consists of two cycles DHAP; pts with PR or CR receive cyclophosphamide 4g/m2; followed by PBSC harvest; methotrexate 8g/m2 plus vincristine 1.4mg/m2; and etoposide 2g/m2. The final myeloablative course is BEAM followed by ASCT. 102 pts (median age 34 years, range 18-63) have been enrolled. So far 99 pts are available for the final evaluation. At 18 months of median follow-up (range 3-34 months) results are as follows: The response rate (RR) after DHAP was 87% (23% CR, 64% PR). The RR at the final evaluation (100days posttransplantation) was 77% (68% CR, 9% PR). PBSC harvest was successful in 96% of pts. Toxicity was tolerable with no treatment related death. FFTF/OS for pts with HD are: early relapse 64%/87%; late relapse 68%/81%; progressive disease: 30%/58%; multiple relapse 55%/88%.

We conclude that this regimen is feasible, tolerable and highly effective in poor risk pts with progressive and relapsed HD. In pts with relapsed HD the CCR1-BMT/EBMT/EORTC is comparing this regimen with relapsed HD the GHSG/EBMT/EORTC is comparing this regimen in a prospective randomized study (activated 01/2001) with 2 cycles DHAP followed by BEAM (HD-R2 protocol).

O110

Vacop-B vs Vacop-B + high -dose sequential therapy (HDS) for aggressive non-Hodgkin’s lymphoma (NHL)

G. Santini, A. Congiu, A. Olivieri, I. Maiolino, T. Chisesi, L. Salvagno, R. Quaini, A. Rubagotti, R. Centurioni, A. Contu, M. Candela, V. Rizzoli for the NHLCSG

In 1997, the Milan Group suggested a statistical improvement in the outcome of pts treated with HDS in comparison with those pts treated with conventional chemotherapy (CT). Only B-cell type, G and H/WF, and BM negative pts were included. CR rate was 96% vs 70%, overall survival 81% vs 55%, and PFS 84% vs 49% in the two arms respectively. In 1998, a randomised study of the NHLCSG compared CT + DHAP in case of persistent disease vs CT + high-dose therapy (HDT) with autologous bone marrow transplantation (ABMT) as front-line treatment for these pts. Pts with BM involvement were excluded. Results were similar in the two arms of pts: CR was 75% vs 73%, overall survival 65% vs 65% and PFS 48% vs 60% in the two arms respectively. We therefore started a new study in which pts with aggressive, advanced stage NHL were randomised to receive VACOP-B +
HDS in case of persistent disease vs VACOP-B + HDS (CY, 7 gr/m²; VP 16, 2 gr/m² and BEAM + PBPC rescue) in all cases. Aims: a) to confirm the Milan Group’s data; b) to evaluate possible use of HDS only when necessary. 223 pts were registered and 200 are now evaluable for response. Second interim analysis shows 65% and 69% of CR respectively. With a median observation time of 36 months, actuarial curves show a 6-yr probability of survival and of PFS of 50% and 47% respectively, with no difference between the two arms. When only B-cell type, G and H/WF NHL without BM involvement were analysed, probability of survival and of PFS improved to 70% and 82%, and to 64% and 71% respectively, in the two arms respectively. Pts with T-cell type NHL and with BM involvement showed poorest results. When pts with BM involvement were excluded, the probability of survival and PFS were 66% and 68%, and 49% and 57% in the two arms respectively. This 2nd interim analysis seems to confirm the Milan Group’s data, in a selected group of pts and suggests that results achieved with CT + HDS are similar to those observed with CT + ABMT. There is no apparent difference in using HDS after CT in all cases or only in the case of persistent disease.

O111
Mega-CHOEP: A phase II/II study of the German High Grade NHL Study Group For Primary Treatment of Aggressive Non-Hodgkin's Lymphoma: efficacy of dose level 1 + 2 and feasibility of dose level 3
B. Glass, M. Kloss, W. Berdel, A. Engert, M. Bentz, L. Trümper, M. Löfler, M. Pfleundschuh, N. Schmitz on behalf of the German High Grade Non-Hodgkin's Lymphoma Study Group

To optimize high dose therapy for aggressive NHL, we evaluated a new treatment strategy comprising of four to six cycles of dose escalated CHOEP + Etoposide chemotherapy requiring stem cell transplantation after three of these cycles. Patients with newly diagnosed aggressive NHL, 18-60 years of age, and LDH above normal were included. Dosage at respective dose levels (DL) were as follows: DL1, Cycle1: cyclophosphamide (CY) 1500 mg/m², Adriamycin (ADR) 70 mg/m², vincristine 2 mg, Etoposide (ETO) 450 mg/m², and prednisone 500 mg/m².: Cycle II and III : CY 4500 mg/m² and ETO 600 mg/m², cycle IV: CY 6000 mg/m² and ETO 1000 mg/m². At DL2 ETO was further intensified: 600, 960, 960 and 1480 mg/m² at cycles 1-4, respectively. At DL3 patients were randomized to a four or six cycle variant of Mega-CHOEOP with the following dosages of CY: 1500, 6000, 6000, 6000 mg/m² ETO 600, 1480, 1480, 1480 mg/m² (arm A) vs. CY 1600, 1600, 1600, 4500, 4500, 6000 mg/m², ETO 600, 600, 600, 960, 960, 1480 mg/m² (arm B). From February 97 to November 01, 249 pts were enrolled in the study. 47 at dose level 1, 77 at dose level 2 and 125 at dose level 3. Eight cases (3%) of treatment related mortality did occur. So far, no case of secondary leukemia was reported. 101 / 110 eligible patients at dose level 1 and 2 were evaluable. In 100 / 101 pts, sufficient numbers (> 2 x 106 CD34+ cells / kg BW) of PBPC could be harvested for all scheduled cycles of stem cell transplantation. Recovery of leukocytes (> 1/ ni) was achieved after 12, 14, 15 and 16 days, recovery of the platelets (> 80/ni) occurred at day 14, 16, 17, 20 (medians) after courses 1-4 of MEGA-CHOEOP. 75 % of the patients achieved CR or CRu 3 months after last cycle of therapy. At two years, overall survival was 69.6% and freedom from treatment failure was 66%. Actuarial median observation time is 21 months. At September 01, 74 patients at DL3 could be evaluated; results were different in arm A and B. 4 / 28 eligible patients in arm B showed progression under therapy and 2 patient did not mobilize sufficient numbers of stem cells. This was not observed in 46 patients treated in arm A. Therefore Mega CHOEP DL3A (four cycles) is the basis of the current protocol combining Rituximab with MEGA-CHOEP therapy. MEGA-CHOEP is feasible (DL1-3) and effective (DL 1+2) as primary treatment of aggressive NHL. MEGA-CHOEP will be included in future Phase III studies of the German High Grade NHL study group for pts with high risk features.

O112
Prospective tandem autologous stem cell transplantation (ASCT) in patients with refractory and unfavourable relapse from Hodgkin's lymphoma (HL)
R. Bouabdallah, F. Morschauser, M. Diviné, V. Leblond, A. Stamatoulas, C. Bélanger, P. Colombat, P. Brice for the intergroup GELA/SGM

From 01/95 to 12/97 a pilot study demonstrated the feasibility of tandem ASCT in 43 patients. Our present protocol stratifies patients according to the characteristics at HL relapse/progression. Induction failure (IF) or early (CR < 12 mo) and disseminated (or previously irradiated site) relapse, received 2 course of salvage chemotherapy (IVA or MINE) and non progressive patients received ASCT1 after CBV + mitoxantrone (30 mg/m²) then ASCT2 (cytarabine 6 g/m², melphalan 140mg/m² and total body irradiation (TBI) at 12 Gy or busulfan 12 mg/kg). The protocol activated in 01/98 has included 56 patients and we present the results of 99 patients from this ongoing study. Patients characteristics : males : 63%, mean age : 33 years, nodular sclerosis : 80%, stage III/IV : 55%, all patients had received chemotherapy (MOPP/ABV : 34%, ABVD : 25% and others), previous radiotherapy : 47%. Relapse characteristics, induction failure : 49%, Extraneural relapse : 50%, B-symptoms : 46%, staging histology at relapse : 70%. Treatment: 69% of the patients received the IVA and 25% the MINE regimen. For ASCT2, 40% received TBI in the conditioning regimen.
Results: After salvage chemotherapy the response rate (CR + PR > 50%) was at 65 % and at 50% for patients with IF, 92% of the patients received ASCT1 and 72 % the 2 ASCT at a mean interval of 63 days, the main reason not to receive ASCT was disease progression. 5 patients had a bone marrow transplantation due to failure of leukaphereses collection. Toxicity was within normal range for leukocytes (GCSF in 70%) and platelets surgery but a delayed platelet recovery was observed after ASCT2 when busulfan was used. Two toxic death occurred: one venoocclusive disease and one ARDS. In intent to treat analysis at the end of the protocol, the response rate is at 66% (similar in IF or unfavorable relapse).With a median follow-up of 3 years event free and overall survival are respectively at 50 and 75% for all patients and at 70 and 85% for those receiving the 2 ASCT.
Conclusion: In this very unfavorable group of progressive HL, a program with a tandem ASCT is feasible without unexpected early toxicity but some refractory patients (25%) could not receive the procedure.

O113
Autografting followed by nonmyeloablative allografting for advanced lymphoma: a higher than expected disease control rate
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Autografting followed by nonmyeloablative conditioning prior to autografting is an attractive strategy for reducing lymphoma burden. Since immune-mediated graft vs lymphoma (GVL) effects can produce remissions in advanced lymphoma patients, we designed a strategy to debulk advanced lymphoma patients using autografting and then induce GVL using nonmyeloablative autografting. We report here the outcome data of this combined procedure. A total of 44 patients (HD-24; Hg-NHL: 12; Lg-NHL: 8) were treated. Median age was 38. Patients received high-dose therapy (BEAM protocol) followed by autologous mobilized peripheral blood stem cells (PBSC). At a median of 90 days, the patients received fludarabine and cyclophosphamide followed by mobilized allogeneic peripheral blood stem cells. At the time of allograft transplant, 10/24 HD patients had chemosensitive disease with 7/12 Hg-NHL and 4/8 Lg-NHL. AcuteGVHD prophylaxis consisted of cyclosporin A plus methotrexate. Neutrophil and platelets never reached values < 1x10⁹/L and < 50x10⁹/L. At 100 days, 32
patients achieved full donor chimerism and 10 patients mixed chimerism, of whom 5 pts progressed early of their disease. At a median of 14 months, 17 HD patients are alive and 9 of them are disease-free, 7 of Hg-NHL patients are alive and 6 of them are disease-free, 5 Lg-NHL are alive with 4 patients disease free. One HD patient, one Hg-NHL and 3 Lg-NHL patients died of Non-relapse Mortality in the first year from transplant (GVHD : 3 patients, infections: 2 patients). Patients with HD had lower NRM and higher PFS despite the high-risk characteristics of patients. The best results were achieved in patients achieving full chimerism and developing acute/chronic GVHD. Continuing accrual of patients will help to better evaluate these preliminary findings.

O114
Adaptive transfer of human T-cells modified to express a CD19-specific chimeric immunoreceptor and CD28 eradicates pre-established lymphoma in a murine model

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Disease relapse is the leading cause of treatment failure after autologous/allogeneic stem cell transplant in patients with acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL). Adoptive immunotherapy with T-cells engineered to express a chimeric receptor represents an attractive means of achieving a graft-versus-leukemia (GVL) effect. In the EBMT registry we identified 352 patients with either Hodgkin’s disease or Non-H Hodgkin’s lymphoma who received a second transplant as salvage therapy for recurrent lymphoma between 1983 and 1999. 218 of them received ASCT and 134 an (non-mini) allogeneic transplant. The median age at second transplant was 39.4 years for pts. receiving an autologous and 35.8 years for those receiving an allogeneic transplant. Distribution of pathology was as follows: AlloSCT: HD 22.9 %, Indolent NHL 40.5 %, aggressive NHL 31.3 %; autoSCT: HD 34.3 %, Indolent NHL 27.8 %, aggressive NHL 29.2 %. In both groups, the majority of patients received one salvage regimen (85% pts allo, 123/141 pts auto) between first and second transplant. In comparison to patients receiving and autologous graft, those receiving an allogeneic graft had a shorter duration of disease at second transplant (2.6 vs. 3.2 years, p=0.047), a shorter time interval between first and second transplant (471 vs. 655 days, p=0.0026) and more frequent bone marrow involvement (41 % vs. 18 %, p=0.003). At time of second transplant, resistant relapse was significantly more frequent in allografted than autografted pts.(40 % vs. 28 %, p=0.04). 43.3% of allografts and 79 % of autografts consisted of PBPC. Five years after transplant, overall survival was comparable in both groups, (34 % allo vs. 29 % auto), however lymphoma was the leading cause of death in autografted pts (68.0 %) whilst toxic death was far more common in allografted pts (74.7 %, p < 0.001). The relapse rate at five years was significantly lower after allogeneic than after autologous transplant (39% vs. 78%, p < 0.001).

In summary, younger patients with earlier and frequently resistant relapse tend to be treated by HDT and allogeneic SCT as salvage for progressive lymphoma after a first transplant. Compared to ASCT, allogeneic SCT seems to have superior anti-lymphoma activity which is, however, outweighed by its high toxicity. New strategies should focus on methods to achieve graft-versus-lymphoma-effect with less toxicity.
Multiple Myeloma

O117
Transplantation outcome in rare myelomas
C. Morris, A. Hagman, B. Bjorkstrand, G. Gahrton on behalf of Myeloma Subcommittee of Chronic Leukaemia Working Party

IgD, IgE, IgM in myeloma are each rare myelomas accounting for less than 1% of all cases and sometimes thought to have a worse prognosis than “usual” myelomas (IgG, IgA and light chain myeloma). Non-secretory (NS) myeloma is also an uncommon condition which has also been associated with a poor prognosis.

We have used the EBMT Data Registry to study the survival of these patients treated with transplant strategies. Seventy-three IgD patients who had autologous transplantation were compared with 4905 patients with usual myelomas and were noted to have significantly higher B2M at diagnosis (p=0.006), more patients who received TBI in their conditioning regimen (p=0.005) and less responsive disease at the time of transplant (p=0.02). The median survival post transplantation was 28 months compared with 52 months for the usual group (p=0.002). In contrast in the 174 NS (non M-protein producing) myelomas there were significantly more cases of stage III disease (p=0.02) but less TBI usage than in the usual myelomas (p=0.014). Median survival was 45 months (p=0.49) indicating the outlook for NS myeloma treated by transplantation is no different to usual myeloma. With regard to IgE (2 patients) and IgM (6 patients) there was insufficient data for statistical analysis although scrutiny of the data suggested that both IgE and IgM may have similar survival to IgD myeloma. Review of the allogenic database shows that 19 NS patients were transplanted with a median survival of 27 months which compares favourably with median survival for “usual” myelomas with allogeneic transplantation of 26 months. In the IgD patient group with allogeneic transplantation survival is variable. Two IgM patients also had allogeneic transplantation. These results indicate that NS myeloma responds to transplantation therapy as if they were secretory (usual) myelomas while the confirmation of poor prognosis in IgD myeloma indicates the necessity to look for additional or alternative therapeutic strategies.

O118
Transplantation in plasma cell leukemia
M. Drake, C. Morris, A. Hagman, B. Bjorkstrand, G. Gahrton, J. Apperley on behalf of Myeloma Subcommitteee of Chronic Leukaemia Working Party

Primary plasma cell leukaemia (PCL) is an infrequent disorder, reportedly about 50 times less common than multiple myeloma (MM). Resulting from the expansion of immature plasma cells, the condition requires prompt and vigorous treatment, which has improved the poor response seen with standard alkylating agent based therapies. We report EBMT Registry data on a series of 56 patients with PCL and compare this with 5157 patients with usual myelomas (MM) undergoing autologous haemopoietic stem cell transplantation (HSCT). Patient groups were comparable with regard to gender and M protein at diagnosis. 1903/5157 MM patients and 24/56 PCL patients were Stage III at diagnosis (p=0.1621) and disease status pre-transplantation was not significantly different. PCL patients had higher B2 microglobulin at diagnosis (p = 0.0002) and comprised a significantly younger group at time of transplantation (median age 51 years v. 59 years, p = 0.04). Transplantation of PCL patients occurred earlier after diagnosis with median time from diagnosis of 7 months v. 8 months for MM patients, p = 0.006. Conditioning included TBI in 2653 (51.4%) of MM patients and 27 (48.2 %) of PCL patients (p = 0.8059). There was no difference between groups in the use of bone marrow, peripheral blood stem cells or a combination as source of HSC (p = 0.096). However, outcome after HSCT was significantly different between the groups, with median survival dramatically lower for PCL patients (26 months v. 51 months for MM patients) and high attrition in the 1st year post – transplant. Review of the allogeneic transplant database shows 15 patients with PCL transplanted; their characteristics are similar to the autologous group but with a median survival of under 1 year. Treatment of PCL is often disappointing with only a subgroup of patients surviving initial therapy. High dose therapy offers significant long-term survival but outcomes are significantly worse than for comparable patients with myeloma, despite younger age and earlier transplantation from time of diagnosis. Further investigation of conditioning and supportive measures is necessary to improve the outlook in this aggressive disorder.

O119
Complete remission at transplant and in vitro purging of the graft translate into a better event-free and overall survival in multiple myeloma
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We report the results obtained in 60 newly diagnosed Multiple Myeloma (MM) patients who after high dose chemotherapy (VAD x 3. Cyclophosphamide, 7 gr/m2 + G-CSF 5 mcg/kg) were randomized to either unmanipulated (31 patients) or in vitro purged (29 patients) peripheral blood stem cells (PBSCT) in a tandem autologous program. Patients and results were as follows: 32/28 male/female; median age 56 years; stage II-A (n= 4) and stage III-A/B 49/7 patients. Median serum Beta 2 microglobulin (B2M) was 3.45 mg/L (range 1.3-16.8), median calcium was 9.4 mg/dL (range 7-14.7). Cytogenetic analysis was successfully performed in 29 patients. After each transplant the haematologic engraftment and the immunologic reconstitution were rapid and comparable in both arms and no transplant related mortality was observed. Minimal residual disease (MRD) before and after in vitro purging, was evaluated by Real Time (RT) PCR analysis. The unmanipulated aphereses contained a heavy plasma cell contamination and a significant (3-4 log) reduction of tumor cells was obtained by our in vitro purging technique. With a median follow-up of 30 months (range 11-61) the event free survival (EFS) and overall survival (OS) at three years is 44% and 87% in the control arm and 73% and 85% in the experimental arm. By a Cox time-dependent multivariate analysis, a reduced risk of disease progression was associated with being in CR at time of first transplantation (p= 0.003) and the use of a purged graft (p= 0.005). Interestingly, at three years the EFS of patients achieving early CR after VAD or high dose cyclophosphamide was 86% versus 44% of patients not achieving early CR (p= 0.004). Among these latter patients, (23 in the purged arm and 17 in the control) the role of purging appears to be even more important, since at three years the EFS was 62%, in patients transplanted with a purged graft versus 21% in patients transplanted with unmanipulated PBSCT (p= 0.02). The achievement of CR at the time of transplantation also affected survival since OS at three years was 100% for patients achieving CR as compared to 67% (p= 0.03) observed in patients not in CR before transplantation.

O120
Intensified conditioning therapy with total marrow irradiation, busulfan and cyclophosphamide followed by PBSCT results in a high CR rate and prolonged EFS in patients with advanced multiple myeloma

The overall survival of patients with advanced multiple myeloma (MM) undergoing high-dose chemotherapy and autologous stem cell transplantation (SCT) was found to depend mainly on the
quality of response. Thus, to improve the response rate, a new intensified high-dose chemoradiotherapy was evaluated in a phase I/II study.

89 patients (median age of 51, range 32 - 60 years) received an intensified conditioning regimen consisting of total marrow irradiation (9 Gy applied in 2 fractions over 3 days with shielding of the lung and liver), high dose busulphan and cyclophosphamide 120 mg/kg (TMI/Bu/Cy) followed by PBSC. Prior to chemoradiotherapy an induction and mobilization chemotherapy (4 g/m² cyclophosphamide) were administered. Isotypes were IgG in 48, IgA in 23, Light chain disease in 14, IgD in 3 and a non-secreting MM in 1 patient. Stage III MM was diagnosed in 52, stage IIIB in 18, stage IIA in 16, stage IIB in 3 patients. 56% patients were pretreated at the time of inclusion in the study (35 with 1, 21 with > 1 line of pretreatment). 27 patients had received conventional therapy for > 1 year prior to high dose chemoradiotherapy. Regimen-related toxicity according to WHO criteria and response rates as defined by the EBMT/IBMTR criteria were analysed. TRM was 2% with 1 patient dying of invasive aspergillosis. Three patients developed reversible hepatic veno-occlusive disease (VOD). The main toxicity observed was mucositis WHO grade III/IV in 76%, and fever WHO grade > I in 75% of patients. Among the patients with de novo and pretreated MM a CR rate of 48% and 41%, respectively, was documented. With a median follow-up of 35 months, the actuarial median duration of progression-free survival (PFS) and overall survival (OS) of the whole patient group after transplant were 31 and 58 months, respectively and for patients with de novo MM 35 and > 60 months, respectively.

Thus, administration of this new intensified conditioning regimen was associated with a tolerable toxicity and a high response rate. Due to these promising data, conditioning therapy with TMI/Bu/Cy is currently compared with tandem high dose melphalan in patients with advanced MM in a phase III study.

O121
Second high-dose melphalan autografts for myeloma patients relapsing after one autograft: results equivalent to tandem transplantation

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One of the potential treatment options available for myeloma patients relapsing after one autograft is another cycle of high-dose therapy and a second autograft. 96 patients (34-77 y, median 55) underwent second autografts (Tx2) after 200 mg/m² melphalan (HDM200) for myeloma relapsing after one autograft (Tx1). The interval between Tx1 and Tx2, Tx1 and low day <100 mg/m², ATG (3x10mg/kg) and allografting from related (n=7) or unrelated n=7) donors to induce a graft versus myeloma effect. The median interval between autologous and allogeneic transplantation was 119 days (range 60-210).

Results: After dose-reduced allografting all patients became neutropenic (< 0.2 x109/L) and required platelet transfusions. No graft failure was observed. The median time for leukocyte (>1 x 109/L) and platelet (>20 x 109/L) engraftment was 16 (range 13-22) and 24 days (range 14-43), respectively. Complete donor chimerism was detected in all patients after a median of 30 days (range 19 – 38) post allografting. Acute GvHD occurred in 4 patients (30%). Severe grade III GvHD was observed in two patients (15%). Two of nine evaluable patients developed chronic GVHD (22%). Grade II toxicity was primarily mucositis (50%) and liver toxicity (42%). One patient died with multiorgan failure after septicemia and pneumonia on day 22 after allogeneic transplantation. Overall transplant related mortality at day +100 was 7%. 70% achieved a complete remission (CR) with negative immunofixation and 4 patients have PR with still decreasing monoclonal bands.

Conclusion: The tandem auto-allo-transplant-protocol provides rapid engraftment with complete donor chimerism, manageable toxicity and 7 day 100 treatment related mortality. The high rate of CR is encouraging but a longer follow-up is needed to determine late mortality and late relapse.

O123
Allogeneic transplantation following relapse after autologous transplantation in multiple myeloma

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Background: Although notable advances in therapy for multiple myeloma have been achieved, most patients relapse despite autologous stem cell transplantation. Unfortunately, survival is very limited after post-transplantation relapse, and chemotherapy is unable to establish long-term remission. Allogeneic transplantation offers a potential for cure but is also associated with high transplant-related morbidity and mortality (TRM). Non-myeloablative transplantation has been shown to reduce TRM but the benefit for patients having relapsed after autologous transplantation has not yet been proven. Therefore, we performed a retrospective analysis comparing non-myeloablative transplantation versus conventional chemotherapy in this situation.

With a median follow-up of 35 months, the actuarial median survival is 61% at 5 y and the median survival is 6.4 y. The latter is comparable to that reported with tandem autotransplantation suggesting that salvage autotransplantation at relapse produces results that are equivalent to those seen with tandem autografts. We conclude that HDM200 and a second autograft is a reasonable treatment option in selected patients relapsing after 1 autograft.

O122
High complete response rate of tandem transplant consisting of autograft followed by dose-reduced allograft in patients with multiple myeloma


Purpose: To evaluate the toxicity, engraftment, chimerism, acute graft-versus host disease (GVHD) and response of a dose-reduced allograft after cytoreductive autograft for patients with multiple myeloma (MM).

Patients and Methods: 14 patients with advanced stage II/III MM first received autografting after melphalan (200 mg/m²) followed by a dose-reduced regimen consisting of fludarabine (180 mg/m²), ATG (3x10mg/kg) and allografting from related (n=7) or unrelated n=7) donors to induce a graft versus myeloma effect. The median interval between autologous and allogeneic transplantation was 119 days (range 60-210).

Results: After dose-reduced allografting all patients became neutropenic (< 0.2 x109/L) and required platelet transfusions. No graft failure was observed. The median time for leukocyte (>1 x 109/L) and platelet (>20 x 109/L) engraftment was 16 (range 13-22) and 24 days (range 14-43), respectively. Complete donor chimerism was detected in all patients after a median of 30 days (range 19 – 38) post allografting. Acute GvHD occurred in 4 patients (30%). Severe grade III GvHD was observed in two patients (15%). Two of nine evaluable patients developed chronic GVHD (22%). Grade II toxicity was primarily mucositis (50%) and liver toxicity (42%). One patient died with multiorgan failure after septicemia and pneumonia on day 22 after allogeneic transplantation. Overall transplant related mortality at day +100 was 7%. 70% achieved a complete remission (CR) with negative immunofixation and 4 patients have PR with still decreasing monoclonal bands.

Conclusion: The tandem auto-allo-transplant-protocol provides rapid engraftment with complete donor chimerism, manageable toxicity and 7 day 100 treatment related mortality. The high rate of CR is encouraging but a longer follow-up is needed to determine late mortality and late relapse.
Patients and Methods: From 8/96 to 9/01 28 patients with relapsed MM were treated with conventional chemotherapy alone, whereas 16 received a related (n=10) or unrelated (n=6) allogeneic stem cell transplantation following induction chemotherapy. For conditioning, 3 patients received 2 Gy TBI alone, in the other patients fludarabine was combined with 2 Gy TBI. Posttransplant immunosuppression consisted of cyclosporine and MMF.

In the chemotherapy group, patients were treated with varying regimens, including VAD, MP and dexamethasone. All relapsed patients received thalidomide, usually in combination with cyclophosphamide (400mg/m²), etoposide (40mg/m²), dexamethasone (40mg). Both groups were well matched for age, initial disease stage, and time from diagnosis to relapse.

Results: The median overall survival was 24 months in the allogeneic group compared to 22 months in the chemotherapy group. A Kaplan-Meier estimate of survival showed a 3-year survival of 50% for both groups. When allogeneic transplantsations from related versus unrelated donors were analyzed separately, patients who received grafts from a related donor had a prolonged survival expectancy of 70 % at 36 months, in contrast to 50 % for the chemo-group and 30 % for patients grafted from unrelated donors.

Conclusion: Our results suggest a beneficial effect of allogeneic transplantation from a related donor after reduced intensity conditioning in patients relapsing after autologous transplantation. A prospective randomized study is needed to confirm this observation. In addition life-quality assessment is needed to define the role of allogeneic transplantation in this poor prognosis group of patients.

O124
An update of allogeneic transplantation with peripheral blood stem cells (PBSCT) as compared to bone marrow (BMT) in multiple myeloma
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Allogeneic transplantation with peripheral blood stem cells (PBSCT) has been increasingly performed in multiple myeloma at EBMT centers since 1994. So far PBSCT for multiple myeloma has not proven to be superior to bone marrow transplantation (BMT). In the present update in March 2001 of 521 allogeneic transplants (PBSCT: n=224; BM: n= 297) an attempt was made to compare results of PBSCT versus BMT as well as in prognostic subgroups. The two groups PBSCT and BMT were well matched for patient age, gender, stage at diagnosis, time from diagnosis to transplant, treatment lines before transplant and response to pretransplant regimens. However, the follow up time was significantly shorter for PBSCT, due to the increasing frequency of PBSCT with time.

The overall response rate was similar in both groups (complete hematologic remission (CR): 54% and 55 % at 2 years respectively). Survival tended to be better in the BMT group (PBSCT: 44% and BMT: 53 % at 3 years; p = 0.05). Transplant related mortality was not significantly different (PBSCT: 33% and BMT: 28 % at 1 year; p = 0.14). Acute GVHD was similar in both groups (absent: 30 % and 36 %; grade I: 25% and 26 %; grade II: 30 % and 26 %; grade III/IV: 15% and 12%; p= 0.8). Chronic GVHD in the PBSCT and BMT groups respectively was 26% and 25% at 6 months, 38% and 33% at 12 months and 59% and 40% at 24 months. This difference was not statistically significant (p = 0.09). There was no significant difference in progression free survival or relapse rate.

Analysis of PBSCT versus BMT in subgroups i.e. high or low age, male or female, one or >one lines of therapy, responsive-no responsive, stage III/III indicated that PBSCT may be preferential for survival in patients diagnosed in stage I/II (p=0.06) with no significant difference in other subgroups. Thus, although overall survival tends to be superior following BMT, patients diagnosed in stage I/II seem to have a somewhat better survival with PBSCT with no significant difference between PBSCT and BMT in other subgroups.

Autoimmune Disease
O125
Autologous hematopoietic stem cell transplantation in secondary progressive MS: clinical, MRI and laboratory findings

In MS five phase I/II studies have been published, which demonstrate that AHSCST is feasible and is associated with a stabilization of the disease course in the majority of cases. Recently, a phase II study evaluating the effect of the treatment on MRI enhancing activity has been organized in Italy. 18 patients with secondary progressive MS, with EDSS between 5 and 6.5, unresponsive to conventional treatment and with clinical and radiological signs of active disease, were mobilized with CY 4 g/m² followed by Filgrastim 5 µg/Kg; the ablative regimen was BEAM, followed by rabbit ATG (Thymoglobulin, Sangstat) 10 mg/Kg. Patients were then submitted to monthly MRI, for the first six months, then every three months until month 24, then every six months. Neurophysiological, neuropsychological tests and CSF examination were also performed. Mobilization was successful in all cases with a median number of CD34+ collected of 8,74 (3,51-26,02). In one case, with a follow-up of 4 months, hemorrhagic cystitis occurred after mobilization. The conditioning regimen was well tolerated but infections were common. In 5 cases we observed a CMV reactivation in the first month after AHSCST. Immunological recovery after transplantation showed a prolonged decrease of circulating CD4+ count as compared to CD8+, thus resulting in a marked inversion of CD4+/CD8+ ratio up to one year. During the first year, a very low number of cells with a CD4+45RA+ phenotype was also detected.

The median follow-up is now 18 months (range 2-42). The number of Gd positive areas dropped to 0 in 14 out of 16 cases in the months following the conditioning therapy. After 1 year there was a decrease of T2 total lesion load and volume of black holes of 8% and 19% respectively. Brain atrophy was still present and ongoing, but it appeared to slow after +180.

At month 6 after AHSCST, patients developed multiple oligoclonal IgG bands (OB) in the serum, and many of them were also present in the CSF. After 12 and 24 months OB were still present in the serum and additional OB were detected in the CSF, but an accumulation of clonally related B cells, present before treatment, was no more detectable. Clinically patients were stable or slightly improved. The clinical efficacy has to be demonstrated in a phase III study comparing ASCT to standard immunosuppressive therapy in order to assess if the clinical efficacy of AHSCST justifies the associated mortality and toxicity.
Autologous hemopoietic stem cell transplantation (HSCT) in rheumatoid arthritis (RA): a report from the EBMT and IBMT


Since 1996, autologous HSCT has been used to treat severe RA. Published reports have been of individual cases or series containing relatively small numbers. This study aimed to combine the worldwide experience in one analysis. The EBMT and IBMT registries were used to identify patients with RA treated with autologous HSCT. Further information relating to patient and treatment specific parameters was obtained by questionnaire. Seventy-six patients were registered from 16 centres and 73 patients had undergone autologous HSCT. Transplanted patients (median age 42, range 22-63 years, 74% female, 86% rheumatoid factor positive) had been previously treated with an average of 5 (range 2-9) disease modifying anti-rheumatic drugs (DMARDs). Significant functional impairment was present with a HAQ disability score of 1.4 (range 1.1-2.0) and Steinbrocker score mean 2.59 (SD 0.58). The conditioning regimen was cyclophosphamide (Cy) alone in 62 (85%) patients, mostly 200mg/kg. Seven patients received Cy 200mg/kg + ATG, two BuCy, one Cy 200mg/kg + TBI + ATG, and one fludarabine + ATG. One patient received bone marrow but the rest received PBSC. Some form of graft manipulation, mainly CD34+ selection, was performed in 45 patients (62%). Median follow up was 16 months (range 3-55 months). Responses were measured using American College of Rheumatology (ACR) criteria. Forty-nine patients (67%) achieved at least ACR 50 at some point following transplant. There was a significant sustained reduction in HAQ score (p<0.005). Most patients (60%) were re-started on DMARDs within six months for persistent or recurrent disease activity, which provided disease control in about half the cases. Response was significantly related to seronegative RA (p=0.02), but not to disease duration, number of previous DMARDs, HLA-DR4, mobilisation chemotherapy or graft manipulation. One patient died from infection and incidental non-small cell lung cancer 5 months post BuCy and selected autograft. In conclusion, autologous HSCT is a relatively safe form of salvage treatment in severe, resistant RA. Profound responses are achieved in most patients and, although the procedure is not curative, recurrent or persistent disease activity may be controlled with DMARDs post transplant. Clinical trials are necessary to develop this approach.

Relapsing/refractory Evans syndrome treated with reduced intensity allogeneic HLA-identical bone marrow transplantation (Bmt)

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Refractory Evans syndrome (ES) and AIPT that relapse after splenectomy and do not respond to corticosteroids are associated with substantial morbidity and mortality because of the combined effects of disease and treatment. Results following autologous SCT have been controversial. We report here the case of a 21 year old male patient who was treated with a reduced intensity conditioning regimen and BMT from his 12 year old HLA-identical sister. The disease started in 1992 with autoimmune hemolytic anemia (AIHA), and autoimmune thrombocytopenia (AIPT) supervised soon after. Splenectomy was performed in 1996, with a short-lived remission. AIPT worsened and was complicated with polyneuritis. Antinuclear immunity was never detected. Because of deceiving results with autologous transplants for severe AIPT 1 on November 3, 2000 he received unmanipulated BMT from his 12 years old HLA-identical sister, following a regimen consisting of Thiopeta 10 mg/kg followed by CY 100 mg/kg. CyA dosage was 1 mg/kg. Five platelet transfusions were necessary, but a rise of Plt starting on day +12 peaked to 700 x 10^9/L on day +30. This dramatic platelet peak is superimposable to those reported following autologous transplants2, and is thought to consist of an initial autologous reconstitution. There was subsequently a level of platelets up to 400 x 10^9/L, for about one month after which a further decline with positive immune tests. Cytogenetic, FISH and STR showed mixed myeloid-lymphatic chimerism with gradual decline to recipient patterns on day +160. Five incremental DLI were given between day +135 and day +245, and full chimerism was finally achieved. Grade II gastrointestinal GvHD supervened, but responded to corticosteroids. Platelets are now >300 x 10^9/L, and autoimmune are no more detectable. Corticosteroids have been tapered to 8 mg/day of 6-methylprednisolone, but it remains to be seen whether CR will be maintained after discontination The object of allogeneic transplants for marrow and blood is not to establish chimeric myelopoiesis, but donor immunoipoiesis with (hopefully) eradication of the autoimmune clones (GVA) and achievement of tolerization, although the abrogation of autoantigenic peptides must also be considered.


A high incidence of autoimmune thyroid disease and autoimmune cytopenia after T-cell depleted allogeneic peripheral blood progenitor cell transplants: clinical features and analysis of risk factors

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The relationship between allogeneic stem cell transplants and autoimmune disease is complex. On the one hand autoimmune disease can be cured by an allogeneic stem cell transplant, on the other hand de novo autoimmune diseases can be induced by the transplant procedure. Over the past 5 years we have performed 160 T cell depleted (TCD) peripheral blood progenitor cell transplants (PBPCCT) in patients with hematological malignancies and have observed a high incidence of autoimmune diseases, especially autoimmune thyroid disease. Here we report the clinical features and risk factors for the development of this complication.

The cohort consisted of 160 patients (pts), 87 men and 73 women with a median age of 42 y (range 17-63 y).The conditioning regimen consisted of TBI 12 Gy or Bu 12,8 mg/kg i.v.+ CTX 120 mg/kg +/- Thiopeta 10 mg/kg, 52 high-risk pts were additionally treated with a Rhe-188 labelled monoclonal anti-CD66 antibody. The following donors were used: HLA-identical sibling n = 80, matched unrelated donor n = 45, mismatched family donors n = 5. In vivo T cell depletion was performed with ATG (n = 108) and Campath 1H (n = 52). Three methods of ex-vivo TCD were employed: CD34+-selection with the SEPRATE device + CD2/CD3 depletion (n = 47) or with the CliniMACS device (n = 62) or Campath 1H in the bag (n = 52). 24 pts (15%) developed autoimmune disease after a median of 9 mo postTX (autoimmune thyroid disease n = 14, AIHA n = 4, ITP n = 4, autoimmune neutropenia n = 3, autoimmune pancytopenia n = 1).The cohort of pts with autoimmune disease consisted of 12 males and 12 females with a median age of 32 y (range 20-60 y). All 14 pts with thyroid disease developed autoimmune thyroiditis with hypothyroidism. An univariate analysis of possible risk factors revealed no influence of age, hematological malignancy, type of donor, HLA-haplotype, method of T cell depletion or the presence or absence of GvHD. A comparison with a cohort of 111 pts receiving a bone marrow T cell depleted with Campath 1M demonstrated a strong influence of the stem cell source. In the BM group only 2/111 (1.8%) developed autoimmune disease.These differences in the incidence of autoimmune disease are statistically highly significant (p<0.0005 and p<0.0021 respectively).These data suggest that the high incidence of autoimmune disease after T cell depleted PBPCCT is not due to T cell depletion per se but due to the use of PBPC as stem cell source.
**O129**

**Immunobiological therapy with PBPC support with in vitro or in vivo T-cell depletion in patients with poor risk multiple sclerosis**


The immunobiological therapy with hematopoietic stem cell transplantation represents an encouraging method for patients with intractable multiple sclerosis (MS).

Nineteen patients with multiple sclerosis were included to the phase I/II clinical trial involving the high dose chemotherapy with autologous peripheral blood progenitor cell (PBPC) rescue. All patients were in secondary progressive phase of MS, not responding to several salvage regimens, including interferon-beta, mitoxantrone, cyclophosphamide, methylprednisolone in most of them. Fifteen patients underwent high dose conditioning BEAM. T cell depletion in vitro was performed in 9 grafts, in vivo with ATG in 6 patients. The toxicity of the transplantation differed in each individual; one serious respiratory infection has been observed during neutropenic period after the transplantation in a patient with significant bulbar symptomatology. No mortality was noted. Median EDSS and SNRS of grafted patients at the time of inclusion was 6.6 (6.7-5.5) and 59 (40-66), respectively. Median follow-up is 20 months (4-38). One patient (7%) improved significantly (by 1.5 point) on EDSS, 6 patients (40%) improved not significantly (by 0.5 point). Four patients (27.0%) did not change their EDSS. One patient continuously worsened and died 31 months after the transplantation from disease progression (EDSS 10.0). Three other patients (20%) worsened not significantly (by 0.5 point) on their EDSS. Ten patients (67%) improved their SNRS. Improvement was observed after several months after discharge from the transplantation unit. We observed no serious late infection, no myelodysplasia occured. We conclude that high dose immunobiological therapy is a feasible procedure for patients with MS. However, it can bear risk of infection complications in some patients with advanced MS. The results are promising. However, further follow up is needed to finally assess clinical effectiveness of immunobiological therapy in this study. Careful selection of patients with advanced forms of MS is needed to avoid possible infection complications.

**O130**

**Phase I/II trials of autologous peripheral blood stem cell transplantation (ASCT) in autoimmune diseases (AID) resistant to conventional therapy: preliminary results of the Spanish experience**


Objectives: ASCT is an experimental therapy for AID resistant to conventional treatments. The aim of this study is to report the preliminary results of the active pilot studies in Spain. Methods: Twenty eight patients (median age:33; range:21-54) are reported from ongoing multicentre open phase I/II studies: 22 (78%) multiple sclerosis (MS), 3 (11%) rheumatoid arthritis (RA), 2 (7%) systemic sclerosis (SSc), myositis (MY) and lupus erythematous (LE). Objectives: To determine the feasibility, tolerance and efficacy of autologous HSC transplantation in the treatment of systemic lupus erythematosus.

Conclusions: 1) CD34+ selected ASCT seems to be an effective therapy for AID previously resistant to conventional treatments. 2) TRM was 3.5% in this series. 3) These results, along with other published data, warrant comparative phase III studies for selected patients and diseases.

**O131**

**Autologous hematopoietic stem cell transplantation in the treatment of systemic lupus erythematosus**

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Objective: To evaluate the efficacy, safety and feasibility of autologous hematopoietic stem cell transplantation in the treatment of systemic lupus erythematosus.

Methods: Among 18 patients, 6 were in ABMT and 12 were in APBSCT. Conditioning regimens composed of fractional total body irradiation 8-12 Gy, CTX 160mg/m2 and 50mg/kg/3-2,1 ATG (Fresenius, rabbit-anti-human) 20mg/kg(+1,+2). Mobilization for PBSCs was CTX 2.0g/m2 and G-CSF (Lenograstim) 250µg/d. The median progenitor cell reached a median number of MNC 2.91×10^8/kg(2.67-4.28), CFU-GM 3.49×10^5/kg(0.82-9.53), CD34+ cells 3.01×10^6/kg(1.92-5.43) in PBSCs and MNC 1.10×10^8/kg(0.83-1.27) in BM.

Results: All patients were engrafted. The median time of hematopoietic reconstitution was 15 days (12-18). Immune reconstitution showed that the number of CD4 CD19 cell have been reduced markedly and CD8 CD16+56+ increased, this characteres stretched over 8-12 months. The median follow-up duration was 12 months (3-26 months). Of the 18 patients, 12 cases recehieved CR, 3 cases PR, 3 cases NR. 2 patients relapsed at 3, 4 month after transplant. In 13 patients, Renal function have stabilized at a creatinine level 41-109mg/g, 24-hour urine protein 0.1-0.5g. Anti-ds-DNA, ANA and C3, C4 are normal. No patient died as a result of the transplant.

Conclusion: HSCT has been suggested as effective therapy for severe autoimmune disease. This data indicated the safety and feasibility of autologous HSCT for SLE although it was necessary for a long-term to take an observation. In this study, ATG was administered after stem cell infusion for the purpose of T-cell depletion in vivo. It may be of benefit in the setting of autologous HSCT with unmanipulated grafts.

**O132**

**Autologous hematopoietic stem cells (HSC) transplantation in scleroderma, myositis and lupus erythematosus: the French experience**


Objectives: To determine the feasibility, tolerance and efficacy of autologous HSC transplantation with CD34+ selection in severe scleroderma (SC), myositis (MY) and lupus erythematosus (LE).

Methods: We used a national, non randomized, open phase I/II trial. Mobilization of HSC used cyclophosphamide (CYCLO) (4g/m2) + G-CSF (5microg/kg/day) or G-CSF alone (10 microg/kg/day) if left ventricular ejection fraction (LVEF) was < 40% until last apheresis to obtain at least 2.5x 10^6 CD34+ cells/kg. Conditioning using CYCLO (187 mg/kg) ± melphalan (140 mg/m2) if LVEF was < 40%, prior to CD34+ HSC reinfusion. Patient's evolution, hematological and immunological reconstitution were recorded up to the end of the aplasia and...
every 3 months thereafter. We analysed: 1) failure of the procedure, defined by failure of either HSC mobilization, CD34+ selection, or hematological reconstitution or procedure related death; 2) toxic events, according to WHO classification and 3) response to therapy, i.e. regenerating major, partial and no response, disease progression or relapse.

Results (median, range): 13 SC + 3MY + 2 LE, age 32 years (17-26), were included in 30 months, meanwhile 10 SC + 2 MY + 1 LE candidates died from their disease. HSC mobilization was successful in 12 SC + 2 MY + 1 LE. It led to death from acute necrotizing myocarditis a 23 yr old LE patient and it did not yield enough HSC to allow CD34+ selection in 1 SC and 1 MY patient. After 2 (1-3) apheresis, the total number of CD34 + collected for each patient was 11 x 106 CD 34+/kg (4-34). Purity was 98.6% (89.3 - 99.8). CD34+ cells yield was 61.5 % (46.4 - 82).

Autologous peripheral (n = 11 SC + 3 MY + 1 LE) or bone marrow (n = 1 SC) transplantation was performed with 1 procedure related death in 1 SC and 1 death from original disease in 1 MY. Median time to neutrophils > 500/mm3 and platelets > 25000/mm3 was respectively 12 (9-15) and 10 (7-13) days. After 14 (range 1-20) months (1 - 50) (p value = .03). PFS of the transplanted patients are alive and disease free, 4 (10.0%) are alive with disease, 3 (7.5%) died for disease and 12 (30%) died for transplant- related complications. OS of early vs late transplants was 84.0 % and 34.5% at median time from transplant 12 months (1 - 47) and 7 (1.5 - 34) (p value = .01).

Conclusions: In our experience, the probability of identifying a donor was higher than 60% at a median time from search activation of about 4 months. The PFS of the transplanted patients was higher than that of non - transplanted ones (p value = .03). Moreover, transplants performed early in the course of the disease gives an advance in term of OS and PFS.

O134

The importance of identifying a back-up donor for stem cell transplantation

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The importance of identifying a back-up donor, once a primary suitable matched stem cell donor has been found, is often underestimated. Transplant centers seem to rely on unrelated volunteer donors to be willing, available and medical fit for actual donation. According to our data (all unrelated work up procedures, initiated for Dutch patients from 1987 to 2002) in every one out of 12 workups the primary requested donor fails to donate stem cells due to various reasons (personal or medical problems).

In total 492 work up procedures for an unrelated donor worldwide have been initialized. There were 43 donor related cancellations, which include 35 donors who were deferred for medical reasons, from which 70 % were female donors (p = 0.001). In 50% of the cases for which a back up donor was available, the patients were transplanted with less than 2 weeks delay.

To deal with these problems the transplant physicians should inform the patient properly about the donor work up procedure. The information should include the discussion of the likelihood that the identified suitable donor may not proceed to actual donation (i.e. donor’s personal situation, donor’s medical fitness etc) which can be very disappointing to the patient, especially when no back up donors are available.

Therefore we strongly encourage implementing a search for at least one back up donor in the primary search. The availability of a back up donor can save precious time and complicated logistic re-scheduling.

O135

Only one of three patients qualifying for an unrelated stem cell transplant receives one. Ways and means to improve this

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Registries of unrelated stem cell donor volunteers have been in operation since the early 70’s and have shown a spectacular growth since the late eighties. Worldwide almost 7.5 million stem cell donors are available, while in the year 2.000 for over 17.000 patients a search was initiated on www.BMDW.org.

The annual report of the World Marrow Donor Association (WMDA) reports however that less than 5.000 patients received a stem cell transplant from an unrelated donor. This would imply that less than 1/3 patients eligible for an unrelated stem cell transplant actually received one. This observation is in accord with figures which can be extracted from the annual reports of the EBMT transplant activities (A.Grathwohl et al 1991-1999).

A preliminary analysis indicates that the failure of 2/3 of the patients to reach transplantation is due to a number of causes such as: incomplete HLA typing of the donors, under usage of (mismatched)donors, cord bloods and members of the extended family and inability to cope with the complexity of HLA and microchips.

A proposal will be presented to improve the present situation. This is a matter of some urgency not only because more patients should and can be helped, but also because of the gigantic
financial investments made in the registries and cord blood banks (over 3 billion EURO).

**O136**

Activity in a cord blood bank after 100 units delivered for transplantation: the Barcelona experience

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Introduction: Cord blood (CB) tissue can reconstitute bone marrow, providing GVL effect. Since its permissive immunity makes it possible to perform unrelated HLA non-identical transplants through some degree of HLA mismatching, CB potential advantages are: higher probability to find a donor, ready-to-use product, known cell specifications and lower transmissible disease frequency.

Objective: To assess the variables that influence the use of CB units at Barcelona CB bank (bcB).

Material and methods: Activity at Barcelona CBB (October 1995-October 2001), performed in 8 maternities and 2 processing laboratories (CBTB and IRO).

Results: After 73 months, 8134 units have been collected and 5036 validated (62%). CB units accepted had a mean of 14.04x108 NC in 106 mL at start, and 10.67x108 NC in 29.3 mL at final product. NC recovered after HES sedimentation is 76%. Mean progenitor content of stored units is 3.51x106 CD34+ cell, and 8x106 CFUs. Information was requested for 615 patients (1.26 units per patient). Patients’ countries were USA (45%), Europe (40%, one half from Spain), and 15% from rest-of-world (one half from Australia). Patients’ median age was 14 years, and weight was 48 kg. Most frequent patients’ diagnosis was acute leukaemia (56%) and CML (14%). Finally, 100 units have been delivered for transplant (ratio CB shipped per CB unit stored is 0.33% per year). Half of the transplanted patients belong to Spanish centres, 24% to Europe centres and 20% to USA centres (only 8% of those initially studied). The median time elapsed from information request to reservation, shipment and transplant is 24, 55 and 70 days, respectively. The mean NC stored of transplanted units is 19.81x108, indicating that cell number is a relevant parameter considered for CB unit selection. Fifty-two transplants (50% adults) have been evaluated after 1-year follow-up. Probability of 60-day neutrophil engraftment is 78%, overall survival is 33% (42% for pediatrics and 22% for adults). GVHD II - IV incidence is 39% (88% were HLA non-identical).

**O137**

Collections of rhG-CSF mobilized blood stem cells from 395 unrelated donors

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A U.S. National Marrow Donor Program prospective trial is evaluating feasibility and safety of peripheral blood stem cell (PBSC) donations by unrelated donors. We report on 395 donors with at least 1 month follow-up (f/u). PBSC collections were facilitated by 50 donor centers and 64 apheresis centers. Donors, 18 to 60 y/o (median: 37) and 45% female, received rhG-CSF at ~10 mcg/Kg d1 to d5. Bone pain was the most common side effect with 331 donors (85%) reporting pain; 38% moderate, 5% severe. 44 donors (11%) reported 70 instances of severe (grade 3-4) CALGB toxicity. 28 reported 1 severe toxicity and 16 reported 2-5. Common severe toxicities were insomnia (23 reports) and headache (22 reports). 1 wk post-donation, all donor symptoms had returned to baseline (BL). Target donor blood volume was processed by recipient body wt. 74% of donors had 2 apheresis collections; median blood volume processed of 12L (range: 4-24) on d5; (range: 2-21) on d6. Central venous access was required for 41 donors (10%). The majority were female (37 of 179 females (21%) and 4 of 216 males (2%), p<0.001). A single laboratory enumerated donor and product CD34+ cells. d5 median donor blood CD34+ cell count was 54x10(6) CD34+ cells/L (range: 5-680). The median CD34+ yield/L of donor blood processed on d5 and 6 was 21x10(6) and 22x10(6), respectively (range d5: 2-151 and d6: 1-133), White blood cell (WBC), hemoglobin (Hb) and platelet (PLTS) counts were determined at BL, mobilization, collection, and f/u. With each apheresis procedure, PLTS dropped significantly (mean decline d5: 87x10(9)/L (range: -98-225), p<0.001; mean decline d6: 53x10(9)/L (range: 15-211), p<0.0001). After d6 apheresis, 100 of 288 donors (35%) had PLTS<100x10(9)/L; no bleeding occurred. Hb levels were less affected (mean decline d5: 1.2 gm/dL (range: 1.4-5)), p=0.0001 and d6: 1.1 gm/dL (range: -1.7-5.4), p<0.0001. At 1 month post-donation, blood counts remained below BL (mean decline PLTS: 12.3x10(9)/L (range: -48-123), mean decline WBC: 0.7x10(9)/L (range: -5.8-6.9), mean decline Hb: 0.5 gm/dL (range: -2.5-2.6), p<0.0001 for all). For 111 donors with 1-yr f/u, PLTS, WBC and Hb were unchanged from BL. We conclude that PBSC donation by unrelated donors is feasible and safe, even with complex logistics. Thrombocytopenia is frequent following apheresis: in 1 in 5 females may require CVA. Donor blood counts remain minimally depressed 1 month after donation, but seem to recover completely by 1 yr.

**O138**

Intermediate versus standard dose of G-CSF for stem cell mobilization in healthy donors for allogeneic transplantation

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We compared in a retrospective analysis two doses of recombinant human granulocyte stimulating factor (G-CSF) for stem cell mobilisation in 90 healthy donors for allogeneic stem cell transplantation. Group I (n=46) received 10 µg/kg G-CSF (Filgrastim) given as 5 µg/kg twice daily, and group II (n=44) received 16 µg/kg, given as 8 µg/kg twice daily with a 12 hour interval. The groups were well-balanced for age and body-weight. G-CSF application was performed on an outpatient basis, and leukapheresis was started in all donors on day 5. The most frequent side effects of G-CSF were bone pain grade I/II, headache grade I/II and fatigue grade I/II in both groups, whereas grade III of bone pain, headache and fatigue occurred in the 2 x 8 µg/kg group only. One serious non-fatal event with non-traumatic spleen rupture occurred in the 2 x 5 µg/kg group. The CD34+ cell count in the first apheresis of all donors was 5.1 x 10(6)/kg donor weight (range, 1.5-19.3). The CD34+ cell harvest was higher in the 2 x 8 µg/kg group than in the 2 x 5 µg/kg group (7.1 x 10(6)/kg vs. 4.9 x 10(6)/kg; p=0.09). The target of collecting > 3.0 x 10(6) CD34+ cells/kg donor weight with one apheresis procedure was achieved in 95% of group I and in 82% of group II, respectively. The target of collecting > 5.0 x 10(6) CD34+ cells/kg in the first apheresis was achieved in 45% and 61%, respectively. Administering G-CSF at a dosage of 8 µg/kg twice daily leads to a higher CD34+ cell yield than a dosage of 2 x 5 µg/kg, but is associated with increased toxicity and higher cost.
O139
Long-term follow-up and safety of normal peripheral blood progenitor cell (PBPC) donors treated with filgrastim: a cancer center experience
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Background: Data on long-term safety of filgrastim administration in normal PBPC donors are scarce. In the view of the known biologic activity of filgrastim, the main theoretical risk is believed to be the possible, late development of acute or chronic leukemia.

Material and Methods: We conducted a survey of the PBPC donors enrolled and registered in the PBPC collection protocol at our institution between 1994 and 1998. This time period was selected to ensure adequate long-term follow-up. The study was approved by the Institutional Review Board and the participants provided informed consent. A total of 396 PBPC donors were included in the database. They underwent filgrastim mobilization (for 3-5 days on average) and PBPC apheresis. The donors were interviewed by telephone between December 1998 and February 2000. Fifty-three donors were excluded from the survey (forty-nine were not living in the US, three had blank records/no donor name available, and one person did not actually donate).

Results: 343 donors, 281 (82%) were interviewed. The reasons for the lack of interview (n=62) were: no current contact information available n=36 (10%), declined n=10 (3%), no response despite multiple contacts n=14 (4%), and two had died (one suicide, one grand mal seizure, both seemingly unrelated to the donation). The mean age at donation was 44 years (range 5-77), 47% male and 53% female. Seventy-six percent were Caucasian, 17% were Hispanic, 5% were African-American, and 1% other ethnicities. The donor was usually the sibling of the recipient (96%), with 4% being some other blood relative. The median follow-up after PBPC donation was 39 months (range 7-80), 99% had at least one year of follow-up. Six donors had donated stem cells at other institutions. At the time of the interview none of the donors had been diagnosed with acute or chronic leukemia.

Conclusion: Although the sample size is small and the follow-up is limited, these data suggest that brief (3-5 days) exposure to filgrastim during PBPC donation is not associated with any obvious or striking long-term (3-4 years) risk for the development of leukemia.

O140
Continuous Ca2+ supplementation during stem cell apheresis: a prospective trial
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Hypocalcaemia related symptoms are common side effects during stem cell apheresis. We performed a prospective, randomized placebo controlled double-blinded study in order to analyze the effect of i.v. Ca2+ supplementation on serum electrolytes and the appearance of adverse reactions related to citrate administration during a standardized large volume (4x whole body blood volume) stem cell apheresis. The study population consisted of 28 patients with various forms of solid or hematological malignancies. Patients received either Ca-glucuronat (40 mMol dissolved in 500 mL NaCl; 15 pts.) or placebo (NaCl, 14 pts.). supplementation was applied continuously at a constant flow rate until completion of the apheresis procedure. Continuous Ca2+ support reduced citrate-induced decrease in ionized serum Ca2+ (iCa; delta iCa before and after pheresis = -9.16 % vs.-22.53% in the placebo group) while slightly increasing the extraction of Mg2+ (delta Mg2+ = -19.27% compared to -10.96% in the control group). Other serum electrolytes (Na+, K+) were not affected by continuous Ca2+ supplementation. Increase in the level of parathyroid hormone was less pronounced in the test group (+13.7% compared to controls (+188.3%). Ca support led to reduced frequency of adverse reactions (p<0.001), and patients receiving Ca support required less additionally intervention compared to controls (p<0.05). In addition, patients receiving Ca support felt less discomfort compared to controls, as assessed by a standardized discomfort scale. Continuous Ca supplementation had no influence on the stem cell product collected. We conclude that i.v. Ca2+ supplementation at a controlled flow rate is an effective means to reduce citrate-related changes in serum iCa and the appearance of adverse symptoms during stem cell apheresis. Continuous Ca supplementation should therefore be recommended as an standard treatment in patients undergoing large volume stem cell apheresis.

Supportive Care

O141
Home-based autologous stem cell transplantation: a single-center experience
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Background: To improve the patient’s quality of life and reduce hospital expenses there is a trend to perform ASCT on an outpatient basis. However, there is the disadvantage of overloading Day Care Unit facilities. For that reason, we have analysed the feasibility of home-based ASCT.

Patients and methods: At home ASCT (since day +1) was offered to all patients with a good performance status, a travelling time to the hospital of less than 45 minutes, and a caregiver available 24 h a day able to understand and to carry out basic caring measures. Inpatients and at home ASCT received the same supportive care in terms of transfusions, G-CSF, dietary recommendations, and prophylactic oral antibiotics. At home patients received prophylactic i.v. ceftriaxone once daily. An experienced nurse visited the patient once or twice daily, checked vital signs and examined him, took blood samples and administered i.v. medications. The responsible physician spoke over the telephone with the patient daily after reviewing the test results. A temperature of 38°C was indication for going immediately to the hospital. When a complete check-up did not show neither focal infection nor data of severe sepsis and first dose of antibiotics were administered the patient could go home despite fever. Blood and platelet transfusions were administered in the day care unit.

Results: During the study period (November 2000-September 2001) the overall number of ASCT performed in our unit was 56. Of those, 14 (25%), median age 48 years (range: 21-64), were managed at home. The remaining were not included into the program due to patient preference (17%), poor PS (39%), distant home (34%) and social problems (10%). All patients developed fever but only three (21%) needed hospital admission (2, 5 and 5 days). Days with fever, time to engraftment, days with G-CSF, need for transfusion and other toxicity were not different in both groups. The median (range) number of visits in the day care unit for fever or transfusions was 2 (2-4). The median (range) hospital stay (since day +1) was 0 (0-5) days for at home and 16 (1-69) for conventional ASCT (p<0.00001). A prospective hospital cost analysis showed a saving of around 6000 Euro per patient.

Conclusions: These results suggest that for selected patients ASCT can be cost-effectively performed at home.

Home Care Unit is supported by a grant from Schering-Plough Spain.
The quality of life of patients who undergo HSCT is deeply affected by frequent and sometimes long periods of hospitalisation for the treatment of various complications. Many of these complications don’t require continuous medical supervision and may be cared for at home. On this basis, in April 2000 a program of Home Care (HC) was activated in the Paediatric Haematology and Oncology Department of G. Gaslini Children Hospital in Genoa in order to improve the quality of life of the pts, to reduce the costs, and to improve the availability of the hospital ward for more critical pts. Patients undergone HSCT in stable non critical conditions needing i.v. therapies, parenteral nutrition administration, transfusional support, blood examinations, and central venous catheter (CVC) management, as well as children needing palliative care were considered eligible to the program. After one year of activity 16 transplanted children, aged 1-14 years (median 6 years) were treated at home. 8 and 5 out of 16 children were transplanted with unrelated or related donor bone marrow transplantation respectively. The remaining 3 children underwent autologous HSCT. All of them needed blood tests and/or i.v. therapies including parenteral nutrition administration or blood transfusions. 5 out 14 children were early discharged and trained to use CVC at home. In 2 cases palliative cares were administered. The overall activity consisted in 419 i.v. therapies, 353 blood tests, 44 blood transfusions, 22 CVC use training, 23 total parenteral nutrition, and 177 hydrations. The median duration of the assistance for each child was 33 days (range 5-172). A median of 4 pts per week were assisted for a total of 741 days. A total of 498 accesses at home replaced 385 and 113 outpatient and in-patient days of hospitalisation. The average cost per patient given HC (74700 Euro, range 750-20,700) resulted significantly lower (p<0.001) when compared with the average cost per patient hospitalised to undergo the same procedures (219,600 Euro, range 1,750-66,300). The staying at home itself represents the incalculable advantage of this service for these children and their families. These children had the possibility to be earlier discharged and looked after at home for all the follow-up period, making the beds of the ward easier available. This report shows that HC is a feasible kind of assistance for children after HSCT, improves the quality of life of the pts and their families, and reduces the costs.
Early post-transplantation vaccination with combination vaccines intended for newborns was safe in all our patients. The higher doses of pneumococcal polysaccharide booster as well as HAV and influenza vaccinations were given. Specific antibody titres were monitored and adverse events as well as serious infections were recorded.

Results: Vaccinations were generally well tolerated. Three febrile and two local reactions were noted, predominantly in older children. It is likely, however, that not all minor adverse events have been reported. There was no activation of GvHD related to vaccination.

Titres prior to vaccination on approximately day +100 usually showed antibody protection from routine immunoglobulin prophylaxis following HSCT. After vaccination, all titres monitored on day +200 and one year after HSCT were in the protective range in all but one patient. We documented one pneumococcal septicaemia 11 weeks after the first pneumococcal conjugate vaccination. The boy recovered after antibiotic treatment without further complications. In three adolescent patients transplanted earlier and not included in this study, we had documented infections with HIB (1 septic coxitis and 2 tracheobronchitis).

Conclusion: Early vaccination with combination vaccines intended for newborns was safe in all our patients. The higher doses of Diphtheria antigen present in these vaccines did not result in increased toxicity. Antibody titres showed good protection one year after HSCT, making the convenience of combination vaccines available to BMT patients.

Topically applied recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) for oropharyngeal mucositis (OM) in stem cell transplantation recipients: a randomized double-blind placebo-control study

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Objective: Vaccination following allogenic haematopoietic stem cell transplantation (HSCT) should start as early as possible to prevent infectious complications but requires sufficient immune reconstitution for a response to conjugate or polysaccharide vaccines. The optimal time to start vaccination remains to be determined. We studied the safety and effectiveness in BMT patients of 5- or 6 antigen combination vaccines intended for primary immunization in newborns.

Study Design: We chose an early vaccination approach starting approximately 4 months following HSCT. In 20 patients, the first vaccination was given between day +123 and +182 (median +140) following allogenic HSCT for malignant (n=15) and non-malignant (n=5) conditions. The vaccine preparations included antigens of Diphtheria, Tetanus, Polio, Pertussis, Haemophilus influenzae type B (conjugated), and Hepatitis B, as recommended by the German vaccination commission (STIKO). Additionally, a pneumococcal conjugate vaccine (3x) followed by one pneumococcal polysaccharide booster as well as HAV and influenza vaccinations were given. Specific antibody titres were monitored and adverse events as well as serious infections were recorded.

Results: Vaccinations were generally well tolerated. Three febrile and two local reactions were noted, predominantly in older children. It is likely, however, that not all minor adverse events have been reported. There was no activation of GvHD related to vaccination.

Titres prior to vaccination on approximately day +100 usually showed antibody protection from routine immunoglobulin prophylaxis following HSCT. After vaccination, all titres monitored on day +200 and one year after HSCT were in the protective range in all but one patient. We documented one pneumococcal septicaemia 11 weeks after the first pneumococcal conjugate vaccination. The boy recovered after antibiotic treatment without further complications. In three adolescent patients transplanted earlier and not included in this study, we had documented infections with HIB (1 septic coxitis and 2 tracheobronchitis).

Conclusion: Early vaccination with combination vaccines intended for newborns was safe in all our patients. The higher doses of Diphtheria antigen present in these vaccines did not result in increased toxicity. Antibody titres showed good protection one year after HSCT, making the convenience of combination vaccines available to BMT patients.

Topically applied recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) for oropharyngeal mucositis (OM) in stem cell transplantation recipients: a randomized double-blind placebo-control study

D. Valcarcel, M. Sanz, A. Sureda, M. Sala, L. Muñoz, M. Subira, A. Clopes, J. Sierra (Barcelona, E)

Objective: Vaccination following allogenic haematopoietic stem cell transplantation (HSCT) should start as early as possible to prevent infectious complications but requires sufficient immune reconstitution for a response to conjugate or polysaccharide vaccines. The optimal time to start vaccination remains to be determined. We studied the safety and effectiveness in BMT patients of 5- or 6 antigen combination vaccines intended for primary immunization in newborns.

Study Design: We chose an early vaccination approach starting approximately 4 months following HSCT. In 20 patients, the first vaccination was given between day +123 and +182 (median +140) following allogenic HSCT for malignant (n=15) and non-malignant (n=5) conditions. The vaccine preparations included antigens of Diphtheria, Tetanus, Polio, Pertussis, Haemophilus influenzae type B (conjugated), and Hepatitis B, as recommended by the German vaccination commission (STIKO). Additionally, a pneumococcal conjugate vaccine (3x) followed by one pneumococcal polysaccharide booster as well as HAV and influenza vaccinations were given. Specific antibody titres were monitored and adverse events as well as serious infections were recorded.

Results: Vaccinations were generally well tolerated. Three febrile and two local reactions were noted, predominantly in older children. It is likely, however, that not all minor adverse events have been reported. There was no activation of GvHD related to vaccination.

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Conclusion: Early vaccination with combination vaccines intended for newborns was safe in all our patients. The higher doses of Diphtheria antigen present in these vaccines did not result in increased toxicity. Antibody titres showed good protection one year after HSCT, making the convenience of combination vaccines available to BMT patients.
Glutamine-enriched parenteral nutrition after autologous peripheral blood stem cell transplantation: effects on immune reconstitution and mucositis


(Rome, I)

Glutamine, a nonessential amino acid, has recently received increasing attention because this amino acid becomes essential during stress and catabolic states, including BMT. In fact glutamine seems to modulate immune function, to promote faster intestinal healing and to have trophic effects on the intestine. We designed two randomized clinical trials to explore the role of glutamine-enriched parenteral nutrition (GEPN) in patients affected by haematological malignancies submitted to high dose chemotherapy and autologous peripheral blood stem cell transplantation (aPBSCT) or immunoselected CD34+ aPBSCT. In the first randomized study, group 1A (12 pts, 7m/5f, median age 37,5y) received Glamin (Fresenius Kabi) 1000 ml/die (a parenteral aminoacidic solution also containing glutamine 20 g) from day +1 after aPBSCT, while group 1B (15 pts, 10m/5f, median age 47y) received a placebo. In the second randomized study, group 2A (12 pts, median age 31,5y) received Dipeptiven (recony) Kabi 100 ml/die (a parenteral solution containing only glutamine 13,46 g) from day +1 after aPBSCT, while group 2B (11 pts, 8m/3f, median age 49y) received a placebo. Both study groups were comparable for age, sex, diagnosis, conditioning regimen and CD34+ kg cell dose reinfused. There were no significant differences in PMN, platelets, Reticulocyte recovery, length of hospitalization and prophylactic antibiotic therapy, fever, sepsis occurrence, transfusional requirements. Statistical analysis showed a difference in lymphocytes recovery: in first study lymphocytes counts >0.5x10^9/L was achieved on day 16,5 (range 10-27) in patients receiving Glamin and on day 29 (range 12-50) in patients receiving placebo, p=0.005; in second study lymphocytes counts >0.5x10^9/L was achieved on day 18 (range 12-22) in patients receiving Dipeptiven and on day 29 (range 12-60) in patients receiving placebo, p=0.009. These differences are maintained when CD4+ subset was analyzed separately. Furthermore all patients undergone GEPN (1A and 2A groups), compared to all patients undergone placebo (1B and 2B groups), showed a significant decrease in mucositis severity peak calculated by DMS (daily mucositis score: sum of sign's and symptoms's daily score) (p=0.025). Thus, in our experience, GEPN is safe and efficacious to improve lymphocytes recovery and, particularly, CD4+ subset, after aPBSCT; further studies are needed to assess clinical benefits of such approach to justify economical impact.

Table 1: Inhibition of HPC-growth derived from different animals by mHA-specific CTLs

<table>
<thead>
<tr>
<th>Target cells</th>
<th>dog 1</th>
<th>dog 2</th>
<th>dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>0%</td>
<td>11%</td>
<td>0%</td>
</tr>
<tr>
<td>DLA-type</td>
<td>2-4/1-13</td>
<td>2-4/1-13</td>
<td>2-4/4</td>
</tr>
<tr>
<td>Other targets</td>
<td>2-4/4</td>
<td>2-5/1-11</td>
<td>2-4/4</td>
</tr>
<tr>
<td>dog 4</td>
<td>3-12-0-66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O148

Characterization of minor histocompatibility antigens on canine hematopoietic precursor cells


(Munich, D)

Minor histocompatibility antigens (mHA) are mainly defined by the following characteristics: they are recognized by T cells, their presentation is restricted to a specific MHC type, they occur with variable phenotype frequencies and they are segregated in a Mendelian fashion. Here we report the in vitro immunization of T cells against mHA in DLA-identical canine littermates and the subsequent characterization of the resulting CTLs. Bone marrow derived dendritic cells were used for the generation of mHA-specific CTLs using co-culture with fresh donor T cells. The ability of immunized CTLs to suppress the growth of hematopoietic precursor cells (HPC) was tested in a Delta-Assay, performed in analogy of the delta culture in the mouse (Muench MO and Moore MA, 1992) using lineage-negative bone marrow as target cells. The target cells were plated in a 96-well plate at decreasing cell numbers ranging from 16.000 to 125 cells per well. The growth of HPC was stimulated by the addition of 20% FCS, 100 ng/ml SCF, 800 U/ml GM-CSF, 2 U/ml Epo, 100 ng/ml IL-3 and 3% allogeneic MLC supernatant. At the start of the Delta Assay irradiated mHA-specific CTLs were added to the wells in a fixed number of 10.000 cells, resulting in a range of effector to target cell ratios from 0.6:1 to 80:1. After 4 days the 3H-thymidine uptake of the target cells was measured and compared to the control.

The results showed a highly specific inhibition of HPC-growth in 53% (8/15) analysed DLA-identical littermate combinations, ranging from a HPC-growth inhibition of 30% to 98% compared to the control. In vitro segregation analysis performed in one selected family showed that mHA-specific CTLs were able to recognize HPC from some but not all littermates of the family apart from the stimulator (table 1) whereas HPC from an unrelated animal were not recognized.

We further observed one way reactivity in two cases in different dog families. One setting between two male littermates proved the involvement of autosomally-encoded mHA. The other case was showing a one-way reaction of female-derived CTLs against male HPC, strongly indicating the involvement of a Y-chromosome encoded mHA.

We have shown an in vitro immunization of CTLs against mHA in DLA-identical canine littermates and shown the heredity of the involved mHA through segregation analysis in one canine family. These mHA in the dog may prove as a valid model for the treatment of leukemia in man by mHA-specific T cells.

O150

Generation of surviving-specific cytoxic T-Lymphocytes for the treatment of hematological malignancies

M. Zeis, S. Siegel, A. Wagner, P. Dregen, N. Schmitz

(Kiel, D)

Survivin is a member of the family of inhibitor of apoptosis proteins and is overexpressed in many types of cancer including hematopoietic malignancies. Recently, Survivin-derived peptides were shown to induce CD8+ cytoxic T lymphocytes (CTL) when presented on dendritic cells (DC). In the current study, we determined whether Survivin-reactive CTL were able to recognize and to kill Survivin-expressing hematological targets. Survivin-specific CTL were generated from leukaphereses products obtained from healthy HLA-A’0201 positive volunteers by incubating T cells with autologous dendritic cells pulsed with DOTAP complexed Survivin-RNA. Survivin-specific CTL lysed HLA-A2+ Survivin + tumor cells including primary lymphoma, acute lymphatic leukemia, multiple myeloma, as well as fresh leukemic blasts from AML patients, indicating that Survivin-derived peptides are naturally processed and presented by tumors. Normal Survivin-negative target cells including CD14+ monocytes and activated B cells were not attacked by Survivin-specific CTL. For the first time, these data show that Survivin-specific CTL are capable of lysing both tumor cell lines and fresh leukemic blasts and thus can be used for specific cellular immunotherapy against several hematological malignancies.
O151
Isolation and expansion of human cytomegalovirus-specific cytotoxic T cells. An IFN-gamma secretion-assay - based technique

Adoptive transfer of donor-derived human cytomegalovirus (HCMV)-specific cytotoxic T-lymphocytes (CTLs) can restore protective immunity after stem cell transplantation (SCT). Different strategies to generate HCMV-specific CTLs have been described, using either HCMV-infected antigen-presenting cells (APCs) or protein-peptid-pulsed APCs with limits in application. In this report we demonstrate a different strategy to isolate HCMV-specific CTLs within hours, using magnetic enrichment of cytokine (IFN-gamma) secreting cells. 1-5x104 HCMV-specific CTLs were isolated from buffy coats (100 ml, 1-1.5x108 PBMCNs) of 5 different healthy, HLA-A*0201 HCMV-seropositive blood donors using the IFN-gamma secretion assay following stimulation with the HLA-A2 restricted HCMVpp65 peptide NLVPVMATV. After 3 and 6 weeks of ex vivo expansion with IL2 and autologous or allogeneic feeder cells >1 x 107 and > 1 x 109 HCMV-specific T cells, respectively could be obtained. 92% (range 54.7 - 99.3%) of these cells isolated and in vitro expanded cells stained positive with MHC/peptide tetramer complexes and 78% (32.2% - 99.5%) showed HCMVpp65 peptide-specific IFN-gamma secretion. 75% (range 55.3 - 88.0%) of these cells produced perforin and >50% specific killing of peptide-pulsed T2-cells as well as HLA-A*0201- positive HCMV-infected fibroblasts was detected (E:T ratio 20:1 and 40:1). Auto- and alloreactivity could be excluded using autologous and allogeneic skin fibroblasts as target cells. Thus, the approach with the IFN-gamma secretion assay allows to isolate HCMV-specific CTLs which can be expanded sufficiently for clinical application after SCT. In upscaling of the technique and using for selection leukapheresis products, the technique will allow to isolate up to > 107 HCMV-specific T cells sufficient for adoptive T cell therapy without further ex vivo expansion.

O152
Definition of the breakpoint fusion peptides for HLA-A3 and HLA-B8 in CML cells. Their use to generate tetramers and to expand and detect peptide specific T cells in patients in vivo
A Madrigal, S. Rusakiewicz, G. Aubert, R. Clark, C. Cresser, P. Bonner, R. Rees, P. Traverson, A Dodi (London, Liverpool, Nottingham, UK)

Chronic Myelogenous Leukaemia (CML), characterised by the presence of Philadelphia chromosome (Ph) is the result of the t(9;22) translocation, which encodes for the bcr-abl fusion oncogene. The b3a2 junctional region peptides therefore represent potential immunogenic antigens. K562 is a Ph+, HLA class I negative cell line, which expresses b3a2 bcr-abl mRNA. These cells were transfected with single HLA-A*0301 or HLA-B*08011 alleles by electroporation. Acid elution of CML specific peptides from HLA-A3 or HLA-B8 K562 transfectants as well as from HLA-A3 (b3a2 +) CML patients, was performed. The resultant peptides were analysed by mass spectrometry with nanospray ionisation. Sequencing results obtained confirmed the presence of the HLA-A*03011 restricted peptide KQSSKALQR on both transfected K562 and primary CML cells. Tetramers of HLA-A3 and HLA-B8 with the corresponding eluted peptides were produced. These are currently being used to detect antigen-specific CTLs from HLA-A3 and HLA-B8 patients at various stages of their disease and treatment. These data demonstrate that (1) CML cells do express HLA-associated leukaemia-specific peptides and that (2) CML patients have circulating CTLs specific for the b3a2 fusion peptides. (3)That these cells can be expanded with specific peptides in vitro. These findings provide encouragement for an eventual immunotherapeutic approach for the treatment of CML by in vitro stimulation of patients T cells against specific leukaemic peptides providing the basis for immunotherapeutic intervention in CML.

O153
Clinical scale immunomagnetic selection of CD14+ monocytes to generate dendritic cells (DC) for vaccination - a phase I trial
J. Babatz, C. Röllig, G. Moeneclay, G. Ethninger, M. Schmitz, M. Bornhäuser (Dresden, Bergisch Gladbach, D)

Objectives: The aim of this study was to test the performance of the CliniMACS platform to select large numbers of CD14+ monocytes. In a second step varying doses of DC generated out of the CD14+ positive fraction were administered either fresh or after thawing in 3 different parenteral routes.

Material and methods: Standard apheresis was performed in 6 patients with metastatic solid carcinoma. The mononuclear cell preparation was stored at 4 °C overnight. Labeling of cells with CD14+ microbeads and loading on the column according to the standard CliniMACS procedure. The positive fraction was then harvested and cultured for 8 days in medium supplemented with 1% pretested AB serum, GMP grade GM-CSF (1000 IU/ml), IL-4 (1000 IU/ml). Immature DC were either cryopreserved in aliquots or differentiated into mature DC by culture with TNF-alpha (1100 IU/ml), prostaglandin E2 (1μg/ml), IL-1 (1000 IU/ml), IL-1 beta (1000 IU/ml), IL-4 (1000 IU/ml) and GM-CSF (1000 IU/ml) in 3 days. For consecutive applications immature DC were thawed and differentiation was induced as described above. The quality of the cells was tested by microscopy, dye exclusion, annexin staining and expression of CD83, HLA-DR and CD80/86 by FACS. The capacity to stimulate autologous and allogeneic T cells was tested using thymidine incorporation. All patients received 1x10e7 DC i.v., 1x10e6 DC subcutaneously (s.c.) and intradermally (i.d.) at the same day on 3 occasions every two weeks (one fresh, two thawed preparations).

Results: The median number of CD14+ monocytes recovered in the positive fraction after immunomagnetic selection was 3.24 x 10e9 (range (r ), 1.9-4.7) with a yield of 87.6 % (r, 63.9-100.0) and a median purity of 97.0 % (r, 94.9-99.0). The median percentage of CD83+ DC after 10 days of culture was 80 % (r, 59.5-88) leading to an overall yield of 9.8 % (r, 6.7-15.7) for the whole procedure. Allo- and autostimulatory capacity could be shown for all DC preparations by thymidine incorporation. DC cell stimulation was significantly reduced in thawed samples. No grade 2-4 toxicities were documented after DC administration.

Conclusion: Clinical scale immunomagnetic CD14+ selection using the CliniMACS procedure leads to a pure preparation of monocytes which can be used to generate DC. Fresh and thawed DC obtained by a standard culture method are functionally active in-vitro and can be safely infused i.v. or injected s.c./i.d.
product and matured in vitro during Id-KLH pulsing for 36 hours. 21 patients have been included in this trial. 11 patients went off study before having received half of the vaccines. Out of the remaining 10 patients 9 have completed the series. 9 out of these 10 patients generated an idiotype-specific proliferative immune response and all 10 patients attained a KLH-specific response. We also measured the cytokines TNF-a, IFN-g, IL-2, IL-4, IL-5, IL-10 secreted by PBMC after stimulation with idotype and KLH and detected mainly TH1 type immune responses. In 2 patients tested we detected idotype specific cytotoxic T cells. The high rate of induced idiotype-specific immune responses of patients convalescing our new vaccination schema seems to favor this approach of vaccination. We conclude that vaccination with Id-KLH prior to and after AHCT can induce id specific cellular responses with a high efficiency.

O155
CMV and EBV-specific CTL expanded with dendritic cells is skewed towards functional effector phenotype
F. Chen, R. Duarte, L. Barber, P. Travers, A. Dodi, A. Madrigal (London, UK)
Severe infective complications including CMV infection and EBV-associated post-transplant lymphoma arise following SCT as a result of deficient T-cell reconstitution. Ex-vivo expansion of viral epitope-specific CD8+ T-cells for adoptive immune immunotherapy may confer long-term antigen–specific immunity whilst avoiding GVHD. However considerable functional and phenotypic heterogeneity exists among antigen-specific cells both in healthy individuals and after SCT and we have characterised this heterogeneity in EBV and CMV-specific CTL generated with dendritic cells. We used peptide-pulsed DC to generate thirteen lines of autologous EBV and CMV-specific CTL's. DC's were established from PBMC in FCS free media with GMCSF and IL-4, matured with TNF-a and Poly-IC and pulsed with HLA-A2 and HLA-B8 restricted peptides from CMV-pp65 (NLV), EBV-EBNA3C (FLR) and EBV-LMP2a (CLG). After co-culturing with positively selected CD8+ T-cells, flow cytometric analysis with HLA-tetramers showed that tetramer positive cells for NLV, FLR and CLG expanded up to 50%, 75% and 80% of CD8+ cells respectively. The tetramer staining CD8+ T-cells were further characterised with anti-CCR7 and anti-CD45RA antibodies which define four sub-populations corresponding to naïve, central or long-standing memory, effector memory and terminally differentiated effector cells. Most of the in-vitro expanded tetramer positive cells were of CCR7-phenotype, corresponding to effector CD8+ T cells with a mean of 60% in the CCR7-/CD45RA- compartment and 40% in the CCR7-/CD45RA+ compartment. This is in contrast to findings in healthy donors and SCT patients where CD45RA- CMV-specific cells account for 30 to 40% of tetramer positive cells. Intracellular studies of our CTL lines demonstrated high levels of function with expression of perforin, granzyme A and interferon gamma. The high proportion of CCR7-/CD45RA- CTL's suggests that despite the high specificity and functionality of in-vitro DC-generated cells, they may not contribute to the memory pool of expandable long-lasting cells with important implications for CTL-immunotherapy trials.

Graft versus Malignancy / Minimal Residual Disease

O156
The frequency of cytotoxic CD8+ effector-cells with specificity against the Wilm's tumor gene encoded transcription factor (WT1) antigen after allogeneic stem cell transplantation for CML: evidence for their involvement in tumor clearance
U. Hilbers, G. Hartung, T. Lange, D. Niedenwieser, L. Uharek (Leipzig, D)
The malignant transformation of immature CD34 progenitor cells in pts with CML is associated with elevated expression of WT1 and it has been demonstrated that WT1 can serve as a target for cytotoxic T lymphocytes (CTL). Therefore, we have investigated the frequency of HLA-A0201-restricted CTLs specific for WT1 in patients with CML before and after allogeneic stem cell transplantation. PBMCs were stimulated with the WT1 peptides RMFPNAPYL (aa 126-134) and SLGEOQYSV (aa 187-195) and the frequency of peptide-specific CTLs was determined by Elispot-analysis of interferon-secreting responder cells. An HIV-specific peptide was used as negative control. In patients with high numbers of WT1 specific cells, the responding cells were purified by the interferon-secretion assay (Miltenyi Biotec) and further characterized with regard to their immunologic phenotype. None of 5 controls and none of 9 pts with CML showed WT1-specific CTLs before transplantation. However, in 4 of 8 pts WT1-specific cells were detected in high frequency (up to 211:100,000) up to 465 days after transplantation. In all positive pts, the CTL-response started very early (ca. day 30) and was directed against both WT-1 peptides, indicating that multiple epitopes were involved. The response was either temporary or long-lasting. In 2 pts who had become bcr/abl negative very rapidly, the CTL-response diminished after day 50. Separation of the responding cells revealed that they were predominantly of CD8+ effector cell type (Cd27-/CD45RA+/CD57+). Despite high numbers of WT1-specific CTLs, all patients showed regular engraftment. Thus our findings support previous in vitro data indicating that colony formation by normal CD34 progenitor cells is unaffected by WT-1 reactive cells. According to our data the tissue specific transcription factor (WT1) antigen after allogeneic stem cell transplantation (PBSCT) donor T cells can induce potent graft-versus-tumor (GVT) effects in hematologic malignancies and possibly solid tumors such as renal cell carcinoma. Two patients (27 and 30 years old) with metastatic melanoma received allogeneic PBSCT from an HLA-identical sibling donor after reduced conditioning with fludarabine, carbustine, and melphalan. One patient showed a delayed mixed response with complete regression of lymph node metastases but persistent liver metastasis at day +60 and +120 consistent with a GVT response. In order to generate donor-derived tumor-reactive cytotoxic T lymphocytes (CTL), peripheral blood mononuclear cells were stimulated with donor monocyte-derived dendritic cells (DC) loaded with host tumor lysate. We have shown previously, that two weekly stimulation cycles with tumor-loaded DC followed by restimulation with irradiated tumor cells alone were optimal for induction of tumor-specific CTL responses in vitro. Using these
culture conditions a marked increase of CD8+ CTL was observed in both donors exhibiting a strong MHC class I-restricted cytotoxic activity against the host tumor without cross-reactivity against nonmalignant host cells. To characterize the expansion of clonal tumor-reactive T-cell subpopulations, length pattern analysis of the complementary determining region 3 (CDR3) of the T-cell receptor (TCR) Vβ chain was performed. Results demonstrate that oligoclonal T cells are expanded in vitro showing a marked overrepresentation of TCRVβ3 (donor 1) and TCRVβ4/Vβ11 (donor 2) subfamilies. Functional (ELISPOT assay) and phenotypic (CDR3 spectratyping) analysis of patients’ T cells at different time points after transplantation demonstrate an expansion of alloreactive T cells with a limited polyclonal TCR Vβ pattern. Clonal T cells detected after in vitro stimulation, could not be identified in vivo after transplant. Altogether, our results provide the first evidence that (A) GVT effects against metastatic melanoma can induce tumor regression and (B) oligoclonal donor-derived CTL specific against host tumor cells can be generated and expanded in vitro that may be used for adoptive T-cell transfer after allogeneic transplantation.

O158

Renal cell carcinoma-reactive CD8+ T cell clones can be generated from peripheral blood lymphocytes of allogeneic HLA class I-matched healthy donors

A. Dörschuck, A. Schmidt, E. Schnürer, T. Gareis, V. Lennerz, A. Lifke, C. Huber, M. Karas, T. Wölfel, W. Herr (Mainz, D)

Nonmyeloablative conditioning therapy followed by transplantation of allogeneic hematopoietic stem cells from HLA-matched healthy donors has recently been suggested as a treatment modality for patients with refractory metastatic renal cell carcinoma (RCC). However, the role of alloreactive T cells directed against differentiation antigens of renal origin is unknown. We studied the effect of leukemic levels on the kinetics of DLI in NOD/scid mice. After inoculation of human ALL cells, blood samples were sequentially taken and analyzed for leukemic cells. DLI was performed by administration of T cells from an unrelated donor at different leukemic engraftment levels: animals received DLI at 15, 50 or 70 days post leukemia inoculation when <0.5%, 5% or 50% leukemic cells were detected in the blood, respectively. After DLI, blood was monitored for ALL cells and T cell counts in 3 mice that received DLI at the 50% engraftment level, T cells emerged from 8 days post DLI and expanded to 3.7 million cells/mL. Leukemic progression temporarily decreased but after stagnation of T cell expansion leukemic cell counts again increased to fatal values. In 4/4 mice that received DLI at the 5% level, T cells emerged 18 days post DLI and reached 1.8 million cells/mL. Average leukemic cell counts decreased by 90% and in one animal remission was observed. In 6/6 animals that received DLI at the <0.5% level, T cells emerged 90 days after DLI and reached 0.5 million cells/mL. In these mice leukemic cell counts decreased during T cell expansion but leukemic levels remained fatal. Logarithmic plotting of T cell counts revealed exponential expansion with similar in vivo doubling times of 7.3±0.8 days, 10.1±1.8 days and 7.9±2.2 days in the 50%, the 5% and the <0.5% cohorts respectively. However, the moment of onset of the T cell expansion differed significantly between the cohorts: T cell counts reached 1 million cells/mL at 29.3±1.3 days, 45.5±1.9 days and 112.1±3.2 days post DLI in the 50%, the 5% and the <0.5% cohorts respectively. The existence of a Graft versus Myeloma (GVM) effect was recently proven by the induction of long term remissions following Donor Lymphocyte Infusions (DLI) given to patients with relapsed Multiple Myeloma after allo-SCT. In a recent follow-up of 38 patients, chemoresponsive disease and the occurrence of acute and chronic GVHD were associated with a response to DLI. The latest observation suggests that minor Histocompatibility antigens (mHa) expressed both on normal patient cells and myeloma plasma cells are involved in GVM. Two patients with clinical GVM following conventional partial T cell depleted Allo-PBSCT (cyclo/anti-TBI, patient 1) and Non Myeloablative Allo-PBSCT (fludarabine/melphalan 70 mg/m2, patient 2 ) were studied. Cytotoxic T cell lines (CTL) were generated using patient PBMC before transplantation (Tx) as primary stimulator cells for donor derived patient PBMC post Tx. Expansion of cytotoxic T cell lines was performed using immortalized EBVpat cells. Following a limiting dilution, 22 and 12 patient specific clones were generated from
lymphatic node metastases. Sensitive detection and quantification of minimal residual disease is essential for making clinical decisions and is relevant to the detection of tumor contamination in autologous peripheral blood stem cells (PBSC) used in these patients as rescue after a high-dose chemotherapy. RT-PCR for the expression of tyrosine hydroxylase (TH) mRNA, a tissue-specific marker of neuroblasts, appears to be the most sensitive test, detecting one malignant cell per million normal BM or peripheral blood (PB) cells (Tchirkov et al., Med. Pediatr. Oncol. 1998, 30: 228-232). We developed a real-time PCR assay on the LightCycler system, allowing the quantification of TH transcripts normalized to the transcription of a reference housekeeping gene, GAPDH (glyceraldehyde-3-phosphate dehydrogenase). A total of 138 samples from 32 patients for this analysis. Primary tumors showed high levels of TH expression, with a mean of 738171 copies (per 10E6 GAPDH transcripts). BM samples obtained at diagnosis revealed the levels of transcripts, which were approximately 10-fold lower. In patients on partial remission after treatment, the level of transcripts in BM decreased 15-fold, but TH mRNA was still detectable in 75% of samples. Importantly, this level was comparable to that found in PBSCs collected from patients. Nevertheless, studies of harvests during CD34+ cell selection showed a reduction of tumor contamination by 2.5-3.0 logs. In addition, the use of CD34+ cell expansion ex vivo with SCF, FL3, G-CSF and MGDG cytokine cocktail showed a similar rate of tumor cell depletion. The control of patients after CD34+ cell transplantation confirmed the disappearance of detectable residual disease. These results demonstrate that the quantitative real-time RT-PCR for TH mRNA may be efficiently used for monitoring patients and controlling autologous PBSC harvests in advanced neuroblastoma.

O163 Kinetics of MRD and chimerism in CML patients receiving allogeneic transplants after myeloablative or reduced conditioning

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It is not known whether reduced conditioning is associated with prolonged period of detectable disease after SCT in patients with CML. We have therefore compared bcr-abl transcript levels in patients receiving a conventional conditioning with those receiving reduced conditioning. We also made chimism studies in both patient groups.

A competitive RT-PCR analysis was performed for quantification of the bcr-abl translocation product. Chimerism status of T-cells and granulocytes was studied with VNTR-PCR. We also considered the effect of DPB1 matching on the incidence of relapse. There was a relapse rate of 36% within the cohort studied. We found a highly significant increase in the incidence of relapse in those pairs which were matched for both DPB1 alleles [15 of 28 (54%) completely matched versus 36 of 115 (31%) mismatched at one or both alleles (p=0.0074)]. As with aGVHD, this effect was maintained in multivariate analysis.

There was no difference found in the incidence of chronic graft versus host disease, nor was there a noticeable effect of DPB1 on the overall survival within this group.

We believe this proves that HLA-DPB1 has biological effects which should be considered both when choosing an unrelated donor, as well as when planning the conditioning and GVHD prophylaxis for each individual patient.

O162 Molecular quantification of residual disease in neuroblastoma

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Approximately 60% of patients with neuroblastoma, the most frequent extracranial solid tumor in children, have advanced stage IV disease at diagnosis with bone marrow (BM), bone and lymphatic node metastases. Sensitive detection and quantification of
Granulocyte mixed chimerism was usually observed in patients with high bcr-abl transcript levels. Conclusion: Despite higher bcr-abl levels during the early post-transplant period and higher incidence of mixed chimerism, nonmyeloablative transplantation for CML patients may induce molecular remission in the majority of the patients.

O164

A dose-finding study of DLI in adult haplo-identical transplants

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Haplo-identical transplant is becoming a procedure of choice for patients in whom an allogeneic transplant is indicated and who lack a compatible donor. However, patients are often referred at very advanced stages and usually heavily pre-treated. This results in very high risk of relapse and infections. We therefore started a DLI dose finding study to improve both relapse rate and immunity. Eleven consecutive patients (5 ALL and 6 AML) were investigated. Nine had progressive disease at the time of transplant (6 early refractory relapses post-ABMT and 3 with primary refractory disease) and two were in CR2. Conditioning consisted of TBI, melphalan, ATG, fludarabine and CSA pre-transplant. In 4 progressive patients, Ara-C 2x1 gr/m2 for 2 days was added. The graft was T and B-cell depleted with a fixed reinfused CD3 dose of 5x10^4/kg. All patients engrafted before day 20. No G-CSF was used and GM-CSF was given in the last 4 patients. Non-relapse related mortality was 0/11 at day 100 and 1/11 at 1 year. Prophylactic DLI started at month 1 (5x10^4 CD3/kg) in the 2 first patients. This resulted in aGVHD in both and in prolonged CSA-prednisone treatment in one, eventually leading to fatal infection. We gave next 1x10^4/kg monthly for 3 consecutive months. This was well tolerated with only one grade 1 GVHD. Overall, 5/9 patients relapsed rapidly (before month 6) and were given therapeutic DLI, starting at 1x10^5 CD3/kg with escalation every 2 weeks if no GVHD. This led to CR in 1/5. We next gave monthly escalated (1, 3 and 10x10^4) doses in bad risk patients. This produced cutaneous grade III aGVHD in one, resolute on prednisone 1 mg/kg that is now tapered. We conclude that prophylactic DLI are safe at a monthly dose of 1x10^4 CD3/kg. They results in faster CD4 recovery and a low rate of infections. We adopted this scheme for patients transplanted in CR2. For progressive refractory diseases, they remains insufficient to induce a protective GVL effect. In this group, escalated doses are feasible but warrants further investigations. Therapeutic DLI can be given at higher doses, depending on the timing: 1x10^5/kg producing GVHD when given during the first two months, while doses up to 5x10^5/kg have been given without GVHD for relapse occurring after day 100. Given the safety of the transplant procedure with monthly DLI, it should be proposed earlier in the course of the disease to give enough time to donor lymphocytes and NK cells to expand and exert a GVL effect.

Infections (viral)

O165

Definition of a previously unknown HCMV IE1 peptide motif by computer-based epitope prediction


HCMV-specific cytotoxic T lymphocytes (CTL) have been shown to provide protective immunity towards human cytomegalovirus (HCMV) infection. The HCMV proteins pp65, pp150 and IE1 have been described as potential CTL targets with the majority of peptides described being derived from the pp65 protein. In an attempt to define previously unknown IE1-derived, HLA A*0201-restricted peptides, a computer-based epitope prediction was performed. The epitope prediction was further improved by taking into account potential cleavage sites of the proteasome. Peptide-specific CTL responses were assessed by IFN-gamma release assay using the intracellular cytokine stain. By this approach, an optimal HLA-A*0201-restricted CTL epitope was identified (residues 316-324: VLEETSVML), with 5 out of 15 healthy HCMV-seropositive individuals demonstrating specific IFN-gamma release after peptide stimulation. The frequency of CTLs in healthy individuals varied between 0.15 to 2.27% of all CD8+ T cells. After peptide stimulation, VLEETSVML-specific CTLs were captured using the Miltenyi IFN-gamma capture assay and expanded in vitro by non-specific stimulation with IL-2 and allogeneic feeder cells. After 15 to 32 days of culture, 8.07 - 86.61% of CD8+ T cells were found to be peptide-specific. These T cell lines were found to specifically lyse peptide-loaded T2 cells as well as HCMV-infected fibroblasts expressing HLA-A*0201. In a limited number of patients after allogeneic stem cell transplantation (n=3), comparable precursor frequencies of VLEETSVML- and NVLTMVATV-(pp65-epitope) specific CTLs were demonstrated in some patients, whereas other clearly demonstrated CTL reconstitution directed against pp65.

In conclusion, this novel epitope prediction algorithm incorporating proteasome cleavage sites very efficiently allows to predict previously unknown CTL epitopes as demonstrated here for the IE1 protein.

O166

Isolation of CMV-specific CD4 and CD8-cells from donors: A possible tool for CMV-prophylaxis?

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In order to improve protection against reactivation of enogenous viruses, such as CMV, we evaluated the immune status of CMV-seropositive patients after allogeneic hematopoietic stem cell transplantation (HSCT), who reactivated CMV. The "cytokine secretion assay" (Miltenyi) allows to screen for CMV-reactive T-cells after stimulation with CMV-antigen, based on the detection of interferon-gamma (IFN-gamma) secretion. Six patients were monitored closely for CMV-reactive T-cells at regular presentation at the institute. From these 6 patients 5 were transplanted from matched donors and one received a transplant from a mismatched family donor. One of the donors was CMV-seronegative. All reactivated CMV between day +30 and 6 month after HSCT. In the peripheral blood of one recipient CMV-reactive T-cells (0.2% CMV-reactive per total T-cells) could be detected 1 year after the CMV-reactivation had occurred. This patient had reactivated CMV only once after HSCT. In the other 5 patients no CMV-reactive T-cells could be detected. One was transplanted from a CMV-seronegative donor and all patients had recurrent CMV-infections. These data confirm the critical role of CMV-specific T-cells to control CMV-reactivation. In order to prevent reactivation in CMV-seropositive recipients with CMV-reactive T-cells from seropositive donors, we applied immunomagnetic selection (MACS) for isolation of these cells. CMV-reactive cells could be detected in blood of 12 healthy CMV-seropositive donors at a frequency between 0.1% to 4.5% per total T-cells, whereas in CMV-seronegative individuals (n=3) IFN-gamma secreting T-cells occurred at frequencies less than 0.01%. We could isolate and enrich CMV-reactive cells from seropositive donors in a range from 20% up to 60% IFN-gamma secreting T-cells. Currently an up scaling for clinical grade production adjusting the assay to the ClinimACS is ongoing. We propose to use isolated, enriched, CMV-specific T-cells for the prevention CMV-disease in order to induce early immune-reconstitution for CMV after allo-HSCT and minimize the risk of GVHD development.
Influence of cytomegalovirus seropositivity on outcome after T-cell depleted bone marrow transplantation - contrasting results between recipients of grafts from related and unrelated donors

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Whether cytomegalovirus (CMV) seropositivity still remains a serious adverse risk factor for survival and transplant-related mortality (TRM) in allogeneic bone marrow transplantation (BMT) is under debate. We therefore analysed the effect of CMV serostatus on overall survival and TRM in 253 consecutively treated patients receiving partial T-cell depleted (TCD) bone marrow from either matched related donors (MRD, n=205) or matched unrelated donors (MUD, n=48). All patients were given leukocyte-depleted blood products. CMV monitoring was performed using the pp65 antigenemia assay. Pre-emptive therapy consisted of short-course (2 weeks) low-dose (2.5 mg/kg intravenously b.d.) ganciclovir treatment as soon as a positive antigenemia assay was obtained (1 or more positive staining granulocytes/150,000 cells). Ganciclovir prophylaxis, identical to pre-emptive therapy, was given to CMV-seropositive patients with acute graft-versus-host disease (aGVHD) grade II-IV who were treated with high-dose corticosteroids. After multivariate analyses, inferior overall survival and increased TRM were predicted by extensive chronic (c) GVHD (p<0.001 and <0.001, respectively), high-risk disease status (p=0.075 and 0.031, respectively) and age (p=0.075 and 0.031, respectively) in MRD recipients. After multivariate analyses in MUD recipients overall survival (OS) and TRM were strongly influenced by patient (but not donor) CMV-seropositivity (p=0.009 and 0.001, respectively) and aGVHD (p=0.059 and 0.008, respectively). These data show that CMV-seropositivity is not an adverse risk factor for overall survival and TRM in MRD recipients of partial TCD-BMT, when an appropriate prevention of CMV disease is used. However, in MUD recipients, patient CMV-seropositivity has a high impact on overall survival and TRM.

Adenovirus infections following allogeneic stem cell transplantation

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Adenovirus infections occur in 5-21% of patients following stem cell transplantation (SCT) with an associated mortality of up to 50%. However, a lack of prospective studies has hampered further developments in the understanding and management of this infection in the post-transplant setting. We prospectively studied the incidence and outcome of adenovirus infection after SCT using pre-emptive screening of stool, urine and throat samples for 180 days and a policy of reduction or withdrawal of immunosuppressive therapy if the virus was isolated. A blood PCR was carried out at the first detection of adenovirus in any surveillance sample. Pre-transplant serum samples from both donor and recipients were tested for presence of adenovirus antibodies. The incidence of adenovirus infection was 19.7% (15/76) and the virus was isolated exclusively in recipients of T cell depleted grafts. All paired sera from infected patients and 10 uninfected patients showed presence of adenovirus antibodies. Patients receiving 50 or 100 mg Campath in-vivo were at the greatest risk of adenovirus infection (45% probability vs 11% in patients receiving Campath in-vitro, p=0.01) irrespective of donor-type and this was related to the slower lymphocyte recovery (both total and CD4+ T cells). Patients with a lymphocyte count <500/ mm3 at 100 days had a 56% probability of having adenovirus infection, compared to 5.4% in patients with lymphocyte count >500/ mm3 (p=0.001). Six (40%) of the 15 adenovirus infected patients developed adenovirus disease. Severe lymphocytopenia (<300/ mm3) at the time of first detection of adenovirus was a major risk factor for development of adenovirus disease; 6/7 patients with lymphocyte <300/ mm3 developed adenovirus disease, compared to none from the 8 patients with a higher count (p=0.001). In addition, failure to reduce immunosuppression (p=0.04) and a positive adenovirus PCR at diagnosis (p=0.01) were both associated with fatal adenovirus disease. Based on this study, we recommend active surveillance for adenovirus infection in T cell depleted SCT and withdrawal or reduction of immunosuppressive treatment if possible, in patients with adenovirus infection. Pre-emptive antiviral therapy is warranted for patients with severe lymphocytopenia, positive blood PCR and in those in whom immunosuppressive therapy cannot be reduced.

Human herpes virus 6 (HHV6) Reactivation on day +14 following allogeneic hemopoietic stem cell transplants (HSCT)

The impact on transplant mortality and graft function in 91 patients

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Ninety-one patients undergoing an allogeneic HSCT from an HLA identical sibling (n=46) or an alternative donor (n=45) were monitored for HHV6 on days +14, +28, +42, by detection of HHV6-DNA in plasma in qualitative PCR. Antiviral prophylaxis was given for HHV6-positive recipients: sibling HSCT received acyclovir 500 mg/m2 q12h i.v., daily from day –7 to day +30, and then 1 gr q12 h p.o. day +31 day +100. Alternative donor HSCT received foscarnet 30 mg/m2/q12h, i.v., daily from day –7 to day +30 and then 90 mg/m2/q24 h, daily 5 days/week, from day +31 to day +100. Twenty-eight patients out of 91 (31%) were PCR-positive for HHV6 infection at least once after BMT. On day +14 HHV6 positive patients were 12/91 (13.2%), on day +28 18/82 (19.5%) and on day +42 9/72 (12.5%). The proportion of HHV6+ patients was higher in alternative donor transplants in the 3 time points (p=0.2, 0.07, 0.06 respectively). Platelet counts were lower in HHV6+ patients as compared to HHV6- patients: on day 21 they were 22 vs 18 x10^9/L (p=0.08) respectively for HHV6- and HHV6+ patients, 92 vs 42x10^9/L (p<0.0001) on day +50 after HSCT and 126 vs 83x10^9/L (p=0.2) on day +100. Transplant mortality for patients with day +14 HHV6 positivity was 33% vs 15% (p=0.1): for HLA identical sibling HSCT the difference in TRM was 50% vs 7% (p=0.008) and for alternative donor HSCT it was 25% vs 24% (p=0.9). When looking at HHV6 positivity on day –28 and +42 there was no clear cut difference in TRM. The different impact of day+14 HHV6 positivity in sibling vs alternative donor transplants, may be due to the difference in anti-viral prophylaxis. We will continue to monitor patients for HHV6 on day+14 and we will start treating HHV6 positive patients with foscarnet.

Post-transplant lymphoproliferative disease (PTLD): high incidence after non-T-cell-depleted allogeneic stem cell transplantation

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The incidence of Epstein Barr virus (EBV) associated PTLD after allogeneic transplantation is reported to be <1%. HLA disparity, T-cell depletion of the graft, and severe GVHD increase the risk to 15-25%. The disease often develops early, is rapidly progressive, and usually fatal. The symptoms and signs are often nonspecific and until recent years this disease entity has not been well recognized. We have studied retrospectively 257 adult patients treated with allogeneic transplantation in the years 1994-1999. In case suggestive of PTLD the clinical data were recorded, post-mortem paraffin blocks were re-examined for morphology, EBV antigen and/or RNA (EBER), and EBV-DNA in archived sera was measured by quantitative real-time PCR (EBV-qPCR). 173 donors
O171

Polyoma viruria following T-cell depleted allogeneic transplants using Campath-1H: high incidence but low morbidity
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Haemorrhagic cystitis (HC) is an important cause of morbidity following stem cell transplantation (SCT) and polyoma virus has been reported to be associated with HC. We studied the incidence and outcome of polyoma virus infection in 58 T cell depleted SCT patients. Urine samples were examined by electron microscopy pre-transplant and fortnightly thereafter for 180 days post-transplant. T cell depletion was carried out using Campath-1H, either 10/20 mg in-vitro (n=33) or 50/100 mg in vivo (n=25). Conventional conditioning (cyclophosphamide/ etoposide + TBI in 41 and Bu-Cy in 3 patients) was used in 44 and nonmyeloablative in 24 patients. 21 patients (36%) had polyoma viruria at a median of 3 months (5-114). The incidence was 30% amongst patients receiving Campath in-vitro and 44% amongst those getting it in vivo. The risk factor for polyoma viruria in Campath in-vitro group was GVHD grade >/= 2 (p = 0.01). Polyoma reactivation was not associated with age, gender, conditioning regimen, donor status, CMV sero-status or reactivation. However, onset of polyoma viruria coincided with CMV reactivation in all 6 patients who reactivated both viruses and polyoma virus was not detected after anti-CMV therapy with ganciclovir, foscarnet or cidofovir. 103 sera of 12 patients taken sequentially between diagnosis and death were available for EBV qPCR. EBV-DNA was detectable in the serum in every PTLD patient, and the copy numbers rose progressively towards death. The viral DNA was first seen 23 days (median, range 4-86) before death. Conclusions: PTLD is a significant problem of allogeneic transplantation. Detection of EBV-DNA from asymptomatic patients may offer a possibility for preemptive treatment.

O172

Immunization of the donor in allogeneic bone marrow transplantation can facilitate subsequent post-transplantation responses of the recipient to diphtheria and inactivated poliovirus vaccines; a randomized study
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The HLA-identical sibling donors of 84 adult allogeneic BMT recipients were randomised to receive or not to receive tетanus-diphtheria (Td) toxoid and inactivated trivalent poliovirus (PV) vaccines 2-12 weeks before marrow harvest. Forty-one recipients received the graft from a vaccinated donor, 43 from an unvaccinated donor. All recipients were vaccinated with the Td and PV vaccines at 3, 6, and 12 months after BMT. Tetanus and diphtheria antibodies were measured by a double antigen ELISA and PV type 1, 2 and 3 antibodies by a standard microneutralisation assay in sera drawn from the patients before and one month after each vaccination. Vaccination of the recipients in the immunised donor group at 3 months induced a significant increase in the geometric mean concentration (GMC) of diphtheria antibodies (from 0.09 to 0.15 IU/ml, p=0.006), and vaccination at 6 months in the geometric mean titres (GMT) of PV1 (from 316 to 466, p=0.014) and PV2 antibodies (from 163 to 274, p=0.006). After vaccination at 6 months an increase was seen in both patient groups in the GMCs of diphtheria, tetanus and PV3 antibodies. After the third vaccine doses all antibody concentrations increased significantly. The GMCs of PV3 antibodies were higher at 3 (452 vs. 234, p=0.025), 7 (863 vs. 357, p=0.011), 12 (481 vs. 180; p=0.006), and 13 months (1299 vs. 519, p=0.04) and the GMC of diphtheria antibodies at 13 months (1.69 vs. 0.46 IU/ml, p=0.047) after BMT in the vaccinated donor group patients compared with the unvaccinated donor group patients. Otherwise the GMCs and GMTs were similar in both groups. After the third vaccine dose protective antibody concentrations (> 0.1 IU/ml) to tetanus were measured in 97% and 90% and to diphtheria in 90% and 61% (p=0.012) of the vaccinated and unvaccinated donor group patients, respectively. Antibody titres to all PV types were protective (>4) in all measurements. In conclusion, immunisation of the bone marrow donors with the Td and polio vaccines before marrow harvest resulted in earlier diphtheria and PV antibody responses to the same vaccines, and higher diphtheria and PV3 antibody concentrations in their recipients compared to patients transplanted from unvaccinated donors.

Hemoglobinopathy / Inborn Errors

O173

Mini unrelated donor transplants for congenital immunodeficiencies

We report our results on 22 consecutive unrelated donor(UD)bone marrow transplants in children for congenital immunodeficiency using non-myeloablative(mini) conditioning. The transplants were performed between October 1998 and May 2001 at Great Ormond Street hospital. Of these,15 were fully matched and 8 were mismatched at one or more loci. 6 children had Severe combined immunodeficiency (SCID) and 16 had non SCID immunodeficiencies(CID 7 ,CD40 ligand deficiency 4, Wiskott-Aldrich 2,LAD 2 ,other 1). The mean age at transplant was 8.3 years (range 1-21 years). The majority of children had significant organ dysfunction prior to transplant. The patients were conditioned using Fluadarabine/Melphalan and ATG(13) or Campath 1H (9). One child died due to RSV pneumonitis on day 10. 7 children had viral reactivation (CMV and/or EBV) and 3
children had EBV disease. One child had acute GVHD >grade 2 and one child has chronic GVHD. The median period of follow up is 19 months (range 3-37 months). 21/22 children survive(95%) which compares to 60% survival in 20 previous children receiving UD BMT with ablative conditioning. All children have achieved T cell engraftment and 17/21(80%) >20% myeloid engraftment. We conclude that in comparison to ablative transplants using unrelated donors in children, mini transplants improve survival and reduce transplant related morbidity and mortality while achieving comparable engraftment and immune reconstitution. There maybe a potential for reduced late effects.

O174
Hematopoietic stem cell transplantation in infantile malignant osteopetrosis - a single center experience
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Infantile osteopetrosis (OP) is an autosomal recessive disorder, characterised by excessive bone density due to defective osteoclast function. We report on the clinical course in 10 OP patients treated at the University of Ulm since 1984. Clinical abnormalities included of hepatosplenomegaly, visual impairment and variable cytopenias; interestingly, 4 patients showed hypocalcemic convulsions in the first weeks of life. Genetic analysis could be performed in 13 patients and revealed mutations in the gene of the a3 subunit of the V-ATPase in 11 cases and a heterozygotous mutation in the gene of the chlorid channel protein CIC-7 in 1 case. Allogeneic stem cell transplantation was performed in 15 patients at a median age of 15 months (range 1 to 27 months), using bone marrow of HLA-identical family donors (n=5) and T-cell depleted bone marrow or peripheral blood stem cells of HLA-haploidentical family donors (n=10). Transplant courses were accompanied by an unusual high rate of toxic complications: 6 patients experienced venous-occlusive disease (VOD of the liver in 4 cases, VOD of the lung in 3 cases) and all these 6 patients developed respiratory failure. Hematopoietic recovery was delayed in most patients. Four patients showed graft failures. Three patients transplanted from HLA-nonidentical donors died, one due to Gi bleeding in conjunction with VOD of liver and lung, and two patients following second transplants after autologous reconstitution or rejection. Twelve of 15 transplanted patients are alive and cured of the disease, including 7 of 10 after HLA-nonidentical transplants, with a follow up of 3 to 206 months (median 64 months). This experience indicates an improved outcome of stem cell transplantation in OP, even in the absence an HLA-matched donor.

O175
Polysaccharide antibody responses post bone marrow transplantation for primary immunodeficiency
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Current European survival post BMT for SCID is 70%. Survival post BMT for other PID has also improved and is 60%. Many survivors can discontinue antibiotic prophylaxis and regular IVIG. We analysed our series. 23 SCID (T-B+, T-B-, ADA) and 12 non-SCID (WAS, CD40L, CGD, XLP, NKdef, CID, OLS, CHH, HIgE) BMT survivors (whole or T-cell depleted marrow) not receiving IVIG were followed > 2 years (SCID 2-14 years, non-SCID 2-8 years). Four additional patients in each group still require IVIG and thus are not included in this study. 18 SCIDs have mixed or donor B cell chimerism. 21 have normal immunisation responses to tetanus and Hib antigen, but only 5/23 have a good response to pneumococcal polysaccharide antigen (>20mg/L and >4x rise in post vaccination titre).

All 12 non-SCID PID BMT survivors have mixed or donor B cell chimerism and all have normal immunisation responses to tetanus and Hib antigen. 8/12 have a good response to pneumococcal polysaccharide antigens (p<0.01).

In recent years quality of life following successful BMT for immunodeficiency is very good. The majority of survivors of BMT for non-SCID PID have good specific responses to polysaccharide antigens. However, this is not the case in our experience for patients with SCID, who have poor responses to polysaccharide antigens.

O176
Matched related bone marrow transplantation in a patient with infantile ceramidase deficiency type 2 (Farber disease)
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Background: Farber disease is a lysosomal storage disorder caused by deficiency of ceramidase. Formation of subcutaneous and periarticular ceramide-containing nodules especially of tendon sheaths lead to painful swelling of the joints, progressive disability and hoarseness. Dependent of the level of residual ceramidase activity the patients have a variable degree of CNS involvement. Even in children w/o CNS disease the prognosis is dismal and the patients usually die of pulmonary complications. So far, bone marrow transplantation has only been reported in 2 patients. In both, the neurodegenerative course was progressive while peripheral symptoms resolved.

Case Report: This 3 year-old female patient presented with the typical clinical features of Farber disease including hoarseness and a severely decreased range of motion of basically all joints. The diagnosis was confirmed by ceramidase deficiency in fibroblast cultures of the skin. She had no hepatosplenomegaly and a thoracic CT scan was normal. As this patient had type 2 ceramidase deficiency w/o signs of CNS-involvement she was thought to be eligible for BMT. After conditioning with BuCy she was transplanted with bone marrow (3.7 x 108 MNC/kg BW; 11.3 x 106 CD34+ cells/kg BW) from her 11 year-old HLA-identical sister. She received standard immunosuppression with CSA/MTX. Only minor complications occurred during the early posttransplantation course including mucositis (CTC grade 2), enteritis with C. diff., staphyloccocal septicaemia and a mild, transient pulmonary edema following general anesthesia. She engrafted on day +14. She was treated for clinically suspected grade II GVHD of the GI tract (histologically non-proven) and was discharged from hospital on day +52. The further posttransplantation course was complicated by a CSA-induced pseudotumor cerebri with papillar edema necessitating change of immunosuppression to FK506. Immunosuppression could be tapered and stopped on day +181 without flare of GVHD. Her graft is stable with a 90% mixed chimerism. On day +210 post transplantation, the subcutaneous and periarticular nodules have nearly completely resolved leading to a dramatic improvement in the range of joint motion and motor activity. Her voice has normalized. She is growing along the 3rd percentile and is doing well.

Conclusions: This positive posttransplantation result confirms that patients with Farber disease w/o CNS involvement may benefit from an allogeneic stem cell transplantation.

O177
Transplantation of bone marrow as compared with peripheral blood stem cells from HLA-identical relatives in patients with Thalassemia major in Iran
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There is limited experience in the use of peripheral blood stem cell (PBSC) for allogeneic transplantation in children with beta-thalassemia major. In the present study we compared engraftment kinetics incidence of acute graft-versus-host disease (GVHD) and outcome of
allogeneic PBSCT vs BMT in 125 children with beta-thalassemia major in a single institution. All children transplanted with a similar conditioning regimen and the same GVHD prophylaxis. Patients underwent PBSCT achieved myeloid and platelet engraftment more rapidly than patients who underwent BMT (PMN to >0.5X10^9/L and PLT to >20X10^9/L occurring at median 12 and 14 days after PBSCT, 21 and 19 after BMT respectively) (P<0.01). Incidence and severity of acute GVHD were similar in both groups (grade 3-4): (20.9% for PBSCT vs 20% for BMT). Hospital stay was shorter for PBSCT than for BMT group (39 days vs 44 days respectively).

In beta-thalassemia major, HLA-matched sibling PBSCT resulted in faster neutrophil and platelet engraftment compared to BMT, with no subsequent differences in GVHD.

O178

New preparative regimen for bone marrow transplantation in Class 3 young and adult thalassemic patients


In April 97 we adopted a new Protocol for the Class 3 patients called Protocol 26. In this protocol we have extended the conditioning regimen starting on day -45 from the transplant with hydroxyurea 30mg/Kg and azathioprine 3mg/Kg given daily until day -11. fludarabine 20 mg/m² given from day -17 to day -11. Both groups of the Class 3 patients, young and adults, received busulphan 14 mg/Kg starting on day -10. Following the busulphan, in Class 3 patients aged less than 17 years, for whom the major problem was not the excess of toxicity, but the increased number of patients returning thalassemic after the transplant, the cyclophosphamide total dose remained 160 mg/Kg. In Class 3 patients older than 16 years, for whom the major problem was the toxicity, but not the return of the disease, the total dose of cyclophosphamide was reduced to 90 mg/Kg. In the group of 29 Class 3 young thalassemic patients, two , 7%, rejected the transplant and returned thalassemic and one died ( 4% ), with 97% of survival and 90% of thalassemia-free survival, 1723 days, near to 5 years, after the transplant. In the group of 14 adult Class 3 thalassemic patients, 4 died , two returned thalassemic, with 70% survival and 57% thalassemia-free survival.

O179

Stem cell transplantation for mucopolysaccharidoses type 1, Hurler's disease - Results at 5 years follow-up from a single center

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The Royal Manchester Children’s Hospital is a supra-regional centre for stem cell transplantation (SCT) of children with inborn errors of metabolism. Between 1990 and 1996 a total of 17 SCT were performed on 13 patients with mucopolysaccharidoses type 1 (MPS-1).

The donor was an HLA-identical family member (FMD) in 7 patients and a matched unrelated donor (MUD) in 6 patients. Autologous reconstitution developed in 8 SCT with a 2nd SCT performed in 7 patients, one death resulted following a 2nd SCT. 5 year overall survival was 69% (33% MUD, 100% FMD).

Conditioning was with either Busulphan-Cyclophosphamide in 14 SCT, with additional lymphodepletion in 7 or total body irradiation in 2 or with Melphelan-Cyclophosphamide-ATG in 3 patients. Acute graft versus host disease (GVHD) greater than grade 2 appeared in one SCT. Chronic GVHD occurred in one patient, whom is currently alive and off therapy. Nine patients are long-term survivors (LTS) at a minimum 5 years from SCT, range 66 to 124 months. The median age of the long-term survivors at time of SCT was 13 months with only one patient over 2 years. Data is currently available in 8 of the 9 LTS. Prior to SCT all had no detectable alpha-L-iduronidase (IDUA). Eight patients had a phenotype of MPS-1H with a characterised, severe genotype in seven and an unidentified genotype in one. 100% donor engraftment has been demonstrated in 7 patients and a stable 70% donor chimerism in one. All of the available LTS are in resourced mainstream schools with a median developmental quotient of 76 (9th centile), range 63 (1st centile) to 115 (84th centile). Two patients have developed gonadal failure following radiotherapy and are receiving growth hormone; currently three LTS are on the 25th centile for height with only one below the 0.4th centile. One child has developed glaucoma and another is showing visual evoked potential latency. Five LTS have mild valvular regurgitation on cardiac echocardiography but none have required intervention. Five have evidence of kyphoscoliosis with no required surgical intervention. 

SCT performed at an early age appears to protect the brain but bone and cartilage are less well corrected. Careful review of long-term survivors following SCT is required to enable counselling and decision-making for newly diagnosed patients with this severe disorder.

O180

Successful allogeneic bone marrow transplantation in a child with bloom syndrome and acute myeloblastic leukemia

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Bloom syndrome is a rare autosomal recessive disorder characterized by a high incidence of cancer and genetic instability. An eleven year old girl with Bloom syndrome was diagnosed with acute myeloblastic leukemia after a 6 months period of myelodysplastic syndrome. Her sister, who suffered also from Bloom syndrome died from disseminated Wilms tumor. At diagnosis her peripheral white cell count (WBC) was 2000/µl with 48% blast cells. Her hemoglobin was 11.2g% and the platelet count was 19,000/µl. Her marrow biopsy was diffusely infiltrated with blast cells. Immunophenotyping revealed CD13+, CD33+, CD34+, DR+, CD7+, CD5+, CD56+. Karyotypic analysis revealed 45XX, -1p-, 5q-, 12p-, Xq+. A matched sibling donor was found who was a carrier for Bloom syndrome. She received cytarabine 200mg/m²/day for 5 days with etoposide 200mg/m²/day for 5 days. Ten days later, her bone marrow aspirate showed severe aplasia, while the biopsy showed fibrosis (grade 4) and hypoplasia. Due to the known toxicity to chemotherapy in this syndrome, no further chemotherapy was added before transplant. She received anti-thymocyte globulin (Fresenius) 5mg/kg/day for 5 days and then fresh bone marrow from her matched sister, which contained CD34- 4.5x106/kg. GVHD prophylaxis included cyclosporine 1.5mg/kgx2 per day and methotrexate- 5mg/kg at days +1, +3, +6. Engraftment of neutrophils occurred on day +15 with last platelet transfusion on day +20. No severe infection, mucositis, VOD or GVHD occurred. She is currently 75 days post transplant with normal blood count and no evidence of disease. To our knowledge, this is the first successful BMT done in a child with Bloom syndrome and AML.

Working Party Chronic Leukemia

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Donor lymphocyte infusion for relapsed chronic myelogenous leukemia - The effect of its method of administration in separate risk groups

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Recent studies of the Chronic Leukemia Working Party in patients with relapsed CML after allogeneic stem cell transplant, showed

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that: 1) both disease phase at transplant (1st chronic phase [CP] vs more advanced) and pattern of relapse (cytogenetic or hematological in CP vs hematological in accelerated or blastic phase) are risk factors for survival (Blood 95:3328, 2000); 2) DLI regimen (DR)[bulk dose (BD) vs escalating doses (ED)] or the initial cell dose (ICD)[ie. mononuclear cells x10E8/kg received in the first instance] may influence the outcome of therapy with DLI. Therefore, we analysed the effects of DR and ICD depending on risk category: standard risk (SR; defined as patients in CP) and high risk (HR; defined as patients with disease phase beyond CP at any one time point). 271 patients (188 SR, 83 HR) with CML transplanted from an HLA-identical sibling donor and treated with DLI until 1998 for a CML relapse at 51 EBMT centers were studied. ICD was classified into 3 classes: A (range, median)(0.002-0.20, 0.1): n=69 (53 SR, 16 HR); B (0.2-2.0, 1): n=85 (56 SR, 29 HR); C (2.1-24, 3.5): n=84 (58 SR, 26 HR). Outcomes: were GVHD after DLI (acute grade II to IV and/or chronic vs acute grade 0-I and no chronic), myelosuppression (MS)[neutropenia and/or thrombocytopenia vs none], response rate (RR)[cytogenetic and/or molecular complete remission vs none], overall survival (OS), failure-free survival (FFS), and DLI-related mortality (DLI-RM). GVHD was 47% in SR, 40% in HR; MS was 18% in SR, 21% in HR (both p<0.10). SR patients had higher RR (80% vs 43%), better OS (% at 3 years)(80 vs 38), better FFS (66 vs 25), and less DLI-RM (12 vs 21) as compared to HR patients (all p<0.001). In both risk categories: 1) % RR was similar for all DR (SR: 80, ED=83; HR: BD=44, ED=50) and ICD groups (SR: A=87, B=86, C=81; HR: A=44, B=52, C=50)(all p>0.10); 2) DR and ICD were associated with % GVHD (SR: ED=29, BD=57; A=33, B=53, C=62)(HR: ED=11, BD=51; A=13, B=42, C=58), % MS (SR: ED=12, BD=21; A=13, B=18, C=24)(HR: ED=10, BD=26; A=6, B=35, C=20), % OS at 3 yrs (SR: ED=87, BD=77; A=80, B=82, C=75)(HR: ED=70, BD=29; A=69, B=38, C=29); % FFS at 3 yrs (SR: ED=72, BD=60; A=76, B=77, C=59)(HR: ED=45, BD=20; A=38, B=31, C=19), and % DLI-RM at 3 yrs (SR: ED=4, BD=16; A=4, B=13, C=19)(HR: ED=0, BD=31; A=0, B=27, C=36). These data form the basis for future trials. Methods for administering DLI should be examined in SR patients; novel treatment strategies in HR.

Safety and efficacy of glivec prior to allografting for CML and Ph-positive ALL: European experience


Background: Glivec is widely used in patients with advanced phase CML and relapsed Ph-positive ALL prior to allografting, an approach that may combine the advantages of transplanting in remission and avoiding the toxicity of intensive chemotherapy. We evaluated the safety and efficacy of Glivec prior to allografting. Patients and methods: A retrospective analysis was carried out within the EBMT. All centers were contacted with a questionnaire that focused on transplant-related data. Results: Fifty-six patients were identified, 81% with CML (47% blast crisis (BC), 38% accelerated phase (AP), 9% chronic phase (CP), phase unknown 6%), and 19% with Ph-positive ALL. Median time on Glivec was 116 (18-405), and median time between the last dose of Glivec to transplantation 10 (3-292) days. Thirteen % of patients received salvage therapy after failing Glivec. At the time of allografting, 41% of CML patients were in CP or cytogenetic remission, 33% in BC and 20% in AP. Of the ALL patients, 45% were in complete remission. In 56% of cases, the state of disease at the time of allografting had improved compared to the time of initiating Glivec. Conditioning was conventional in 55% and reduced in 45%. Donors were MUDs in 55%, identical siblings in 41%, and haploidentical siblings in 4%. A female/male donor/recipient constellation was present in 23% of cases. PBSC were used in 72%, BM in 24%, and both in 4%. Graft failure occurred in 4 patients (8%), 3 of whom had received conventional conditioning. Severe (>1) acute GVHD occurred in 41% and chronic GVHD in 53.3% of evaluable cases. At last reporting, 47% of the patients had died, 31% from TRM, 9% from disease progression, and 5% from unknown causes. Fifty-three % of patients are alive at a median follow-up of 151 (12-522) days. Survival is 70% for patients allografted in remission and 20% for patients allografted with active disease (p=0.039). Seventy-seven % of patients are in remission (31% in molecular remission), while 6 (23%) have active disease. Conclusion: (a) Pre-treatment with Glivec does not appear to have a major negative impact on TRM; (b) Transplants should be done as soon as remission has been achieved; (c) Given the high risk population, the results are encouraging. Prognostic factors are needed to identify those patients who are unlikely to respond to Glivec alone, but may respond to drug combinations prior to allografting.

Intrathecal prophylaxis in allogeneic hematopoietic stem cell transplantation for malignant blood diseases


Intrathecal prophylaxis with cytotoxic drugs is widely used in allogeneic haematopoietic stem cell transplantation for malignant blood diseases, but the indications vary from centre to centre and the efficacy has not been properly documented. In order to obtain information of the present practice of i.t. prophylaxis, the Chronic Leukaemia Working Party of the EBMT undertook during autumn 2001 an HLA-identical donor and patient survey. This survey included 90 centres and 868 patients. The survey was an explorative study and the results are not necessarily representative. Results: The centres were asked about the administration of i.t. prophylaxis before the transplantation, as a part of conditioning, and after the transplantation to patients without a history of CNS disease. Patients with previous CNS manifestations were excluded. One of the 90 centres reported giving i.t. prophylaxis before transplantation to all patients, 58 (64%) to selected groups, and in 31 centres (34%) no pre-transplant i.t. prophylaxis is used. Among the 90 centres, the number of centres giving i.t. prophylaxis: is: ALL 59 (66%), all patients 55, selected groups 4; AML 36 (40%), all patients 16, selected groups (mainly FAB M4,M5) 20; lymphoma 30 (33%), all patients 8, selected groups 22; CML 14 (16%), all patients 2, selected groups (mainly accelerated phase) 12; MDS 8 (9%), all patients 1, selected groups 7; CLL 4 (5%), all patients 3, selected groups 1; MM 2; MM selected groups 1. Approximately two thirds of the prophylaxis is given with methotrexate and one third with methotrexate. Patients with previous CNS manifestations were excluded. One of the 90 centres reported giving i.t. prophylaxis before transplantation to all patients, 58 (64%) to selected groups, and in 31 centres (34%) no pre-transplant i.t. prophylaxis is used. Among the 90 centres, the number of centres giving pre-transplant i.t. prophylaxis: is: ALL 59 (66%), all patients 55, selected groups 4; AML 36 (40%), all patients 16, selected groups (mainly FAB M4,M5) 20; lymphoma 30 (33%), all patients 8, selected groups 22; CML 14 (16%), all patients 2, selected groups (mainly accelerated phase) 12; MDS 8 (9%), all patients 1, selected groups 7; CLL 4 (5%), all patients 3, selected groups 1; MM 2; MM selected groups 1. Approximately two thirds of the prophylaxis is given with methotrexate and one third with cytarabine. Before the transplantation, 8 of 57 centres give folinic acid following i.t. methotrexate administration. Intrathecal prophylaxis with cytarabine in allogeneic haematopoietic stem cell transplantation for malignant blood diseases is greatly variable and not supported by solid evidence.
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Post-transplantation tumor load in the bone marrow
assessed by quantitative ASO-PCR as a prognostic
parameter in multiple myeloma (an EBMT/GMMG joint study)
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High dose chemotherapy (HDT) with autologous hematopoietic
stem cell transplantation (PBSCT) significantly improves survival
in multiple (MM) patients. However, in a subgroup of patients early
relapses occur. In order to investigate whether the amount of
residual tumor cells in the bone marrow (BM) after transplantation
can predict the timing of relapse, a quantitative PCR assay was
used to measure tumor load in BM at 3 and 6 months posttransplant in 68 patients with MM. In 45 patients, HDT comprised
a single cycle of 140 mg/m2 and total body irradiation of 12 Gy
(EBMT phase III trial on CD34-selection), in 22 patients two
sequential cycles of melphalan 200 mg/m2 were administered
(GMMG-1998 trial), and one patient received a single cycle of
melphalan 200 mg. All patients received PBSCT, either CD34+selected or unselected. In 61 patients, the BM tumor load at 3
months was analyzed and in 29 of these the 6 months value was
also determined. In 7 patients, the tumor load was investigated at
6 months only. The myeloma Ig heavy chain variable sequence
was used as a tumor specific target, and quantitation was
performed by limiting dilution. The median level of tumor cells in
the BM was 380 per 10E6 total mononucleated cells (MNC) at 3
months post-PBSCT (range, 1 to 25,900; n=61) , and 279 at 6
months (range, 2 to 70,000; n=36). Previous analysis of a smaller
group of patients had suggested that a threshold value of 200
tumor cells per 10E6 MNC (0.02%) at 3 or 6 months posttransplant might predict outcome. Therefore, we determined
whether this threshold value of (0.02%) tumor cells could again
place patients into a good or bad prognostic group. For patients
with a lower tumor burden (n=23), the progression-free survival
(PFS) was 70% at 2 years vs. 50% for patients with more than
200 tumor cells per 10E6 MNC at either time point (n=45). This
difference was statistically significant (p=0.007).
The kinetics of MRD between 3 to 6 months after PBSCT were
evaluable in 29 patients. PFS at 2 years was 56% in those
patients with no increase in tumor burden (n=14) vs. 34% in those
who showed a rise in tumor burden (p=ns).
In conclusion: quantitative molecular monitoring may help to
discriminate between low and high risk groups of patients 3 to 6
months after transplantation, and thus to identify those patients
who are in need of further treatment after PBSCT.

Working Party Solid Tumors
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High-dose chemotherapy (HDC) with autologous hemopoeitic
support for advanced ovarian cancer in 1st complete
remission: retrospective analysis from Solid Tumor Registry
of the European Group for Blood and Marrow Transplantation
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Solid Tumour Working Party of the EBMT
Ovarian cancer is one of the leading cancer death in women.
Despite the high response rate, patients (pts) with advanced
ovarian cancer have a poor long term survival with a very high
percentage of relapse and only 25-30 % remain alive at 5 yrs.
HDC has a potential benefit in pts in remission and randomized
trials are ongoing. We performed a retrospective analysis on 77
pts with ovarian carcinoma in 1st CR treated with high dose
chemotherapy (HDC) and hemopoietic support from 20 centres of
EBMT group. Median age was 48 years (range 15-64), median

PS 0 (0-2), median number of conventional chemotherapy
courses before HDC was 6 (range 2-9). After surgery, 14 pts had
no residual disease (RD), 24 pts <2 cm, 19 pts >2 cm. After
induction chemotherapy 14 pts (18%) were in pCR, 8 pts (10%)
had microscopic RD, and 40 pts (52%) were in cCR. Interval
debulking surgery was performed on 24 pts. Fifty-one pts received
a single course of HDC; 26 pts received multiple cycles of HDC
(from 2 to 5). Hemopoietic stem cell support was bone marrow in
the 21 % and peripheral blood in 79 % of the pts. With a median
follow up of 4 years (range 0.5-11), median (95% CI) time to
progression (TTP) and overall survival (OS) of all patients were
19.4 mos (10-29) and 46.5 mos (37-55.7) respectively. According
to RD after surgery, median (95% CI) TTP and OS for pts with no
RD have not yet been reached: at 8 years, PFS and OS are 52%
and 73% respectively. Median TTP (95 % CI) for pts with RD <2
cm and >2 cm were 13,5 mos (7,6-19,4) and 24,7 mos (8,5-41)
respectively; median OS (95 % CI) was 46,1 mos (23-69,4) and
41.2 mos (21,5-61) respectively. (p:NS). According to the disease
status before HDC, median TTP and OS (95 % CI) were 26.8 mos
(11.3-42.4) and 45.8 (20.5-71) for pts in pCR, 24 mos (3.4-45) and
35 mos (30-41) for pts with microscopic disease, 16 mos (10-22)
and 49 mos (25-73) for pts in cCR (p: NS). Median TTP (95 % CI)
for pts receiving single vs multiple cycle of HDC was 19 mos (9,429,4) and 27 mos (5,2-48,5) respectively (p:NS); median OS (95
% CI) was 46,4 mos (39,4-53,3) and 45,8 mos respectively
(p:NS). Tumor grade, source of stem cells and type of cytokine
(G-CSF vs. GM-CSF) had no impact on TTP and/or OS. In
conclusion these data show interesting long-term progression-free
and overall survival rates after high-dose consolidation therapy in
advanced ovarian cancer in complete response (clinical or
pathological) after induction chemotherapy. Due to the limited
number of pts no prognostic factor has shown a significant impact
on survival.

Working Party Immunobiology
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Human cytomegalovirus induces a direct inhibitory effect on
antigen presentation by monocyte-derived immature
dendritic cells
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Antigen-presenting cells (APCs) are targets of human
cytomegalovirus (HCMV) infection. Infection of these cells might
either result in presentation of viral antigens to immune effector
cells or in virus-induced inhibition of antigen presentation. But,
HCMV infection is known to induce a state of immunodeficiency.
We tested the hypothesis that productive HCMV infection of
monocyte-derived immature dendritic cells (DCs) is associated
with decreased immunostimulatory capacity. DCs generated from
PBMNCs of healthy HCMV-seronegative donors were infected
with 60 -80 % efficiency by the endothelotropic HCMV strain
TB40/E. Infected versus uninfected cells were analyzed by FACS
and by immunocytochemistry for surface expression of the
immediate early antigen (IEA), MHC and costimulatory molecules
(CD80, CD86 and CD40) as well as cytokine secretion (IL-1, IL-6,
IL-10, IL-15, IL-8 and TNF-alpha) during 3 days after infection.
The immunostimulatory capacity of these cells was measured by
mixed leukocyte reaction. In spite of the fact that HCMV infection
of DCs induced an increased release of TNF-alpha and a
decreased IL-10 production, expression of MHC class I and II, as
well as CD40 and CD80 molecules were downregulated on
infected DCs. The mixed leukocyte reaction showed significantly
reduced immunostimulatory capacity of infected DC cultures. In
contrast, expression of CD1a and CD83 was not significantly
different between non-infected and infected DCs. Simultaneous
detection of MHC antigens and virus antigens by double
immunofluorescence revealed, that downregulation occurred only

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in infected cells, but not on uninfected bystander cells. These findings demonstrate on a single cell level, together with the marked down-regulation of MHC and costimulatory molecules in the presence of high TNF-alpha and low IL-10 levels, a direct viral inhibitory effect on antigen presentation by immature DCs independent of soluble mediators. The inhibition of antigen presentation by direct infection of DCs might be one mechanism of HCMV-induced immunosuppression.

**Chronic Leukemia**

**O237**

Outcomes of 379 consecutive patients with chronic myelogenous leukemia (CML) with an UD search activated between 1989 and 1996 (BJH 102, 544-552, 1998). The overall probability of transplant before and after December 1991 was 16% and 49%, respectively (p=0.001). The 4-year survival was 41.5%. We have now asked two questions: (a) of the original 379 patients, how many have been grafted and (b) what is the outcome of the transplants as compared to the non transplants. Thirteen out of 379 patients were excluded because the underlying disease was not a Ph+ CML; 99 other patients were excluded since they underwent autologous (27), aplidiental (16), or cord blood (1) stem cell transplant due to the absence of an UD or because data lacking (55). Two hundred sixty-seven patients were available for analysis, with a median age of 30 years (3-46); 118 have been transplanted from an UD, whereas 149 received interferon, hydroxyurea and more recently STI. Survival was calculated from the day of UD search. The overall 10 year survival for the entire patient population (n=263) was 26%; it was 40% vs 17% for transplant vs non-transplant (p=0.01) patients. The difference was significant for patients under the age of 30 (48% vs 16%, p=0.01), but not above the age of 30 years (26% vs 17%, p=0.8). This study indicates that a significant advantage could be shown for UD transplants over non UD transplant procedures in CML patients under the age of 30 with donor search activated in the period 1989-1996; for patients above the age of 30 UD transplants were still beneficial but not significantly.

**O238**

**Validation of the EBMT Risk Score for recipients of allogeneic hematopoietic stem cell transplants for chronic myeloid leukemia (CML)**


The EBMT CML Risk Score uses a limited number of variables (donor type, disease stage, recipient age, donor-recipient sex combination and interval from diagnosis to transplant) to predict survival after allogeneic transplants for CML (1). The first objective of this study was to validate this score by applying it to an independent population. We studied 3,211 CML transplant recipients between 1989 and 1997, reported to the International Bone Marrow Transplant Registry, Medical College of Wisconsin, Milwaukee, USA.

The EBMT CML Risk Score has been shown to be safe and may be an attractive alternative to BMT and T cell add-back for patients with CML in 1st chronic phase seems to be safe and may be an attractive alternative to BMT and PBSCT.

The estimated probability for chronic GVHD were 73% for PBSCT, 67% for BMT, but only 34% for CD34+-PBSCT (p<0.05). Molecular relapse, defined by two consecutive positive PCR assays for bcr-abl within a 4-week interval, occurred in 86% patients after CD34+-PBSCT, in 53% after BMT and 37% after PBSC (n.s.). The estimated probability for a cytogenetic relapse was 64% for CD34+-PBSCT, 41% for BMT and 22% for PBSCT (p<0.08). 22 of 30 patients after CD34+-PBSCT received T-cell add-back. Donor leukocyte infusions (DLI) were given at a median of four times (range 1-7) with a median T cell dose of 1x 10^6 x kg/body weight of recipient (range 1x10^4-3x10^6).

The estimated probability of disease free survival after T cell add-back at 1000 days after transplant were 85% in the CD34+-PBSCT group, 65% in the PBSCT group, and 60% in the BMT group (p<0.03 for CD34+-PBSCT versus BMT and p<0.08 for CD34+-PBSCT versus PBSCT). Transplant of CD34+-PBSCT with T cell add-back for patients with CML in 1st chronic phase seems to be safe and may be an attractive alternative to BMT and PBSCT.
O240

STI571 (glivec) in the treatment of patients with chronic myeloid leukemia (CML) relapsing after allogeneic stem cell transplantation (allo SCT)


Objectives: Treatment options for relapsing CML after allo SCT are termination of immunosuppression, DLI or retransplantation. However, treatment outcome for CML recurring in advanced stage is poor. STI571 (imatinib mesylate, Glivec) shows promising single agent activity in Ph+ CML in phase I and II studies. We therefore examined the clinical effects of STI571 administered once daily at a dose of 400-600 mg in 21 pts. with Ph+ CML who relapsed after allo SCT and were enrolled in successive multicenter, phase II trials.

Patients: Interval between allo SCT and STI571-therapy was a median of 10 (1-51) mo.. Disease stage at study entry was: blast crisis (BC) (n=12) (lyBC n=2, myBC n=10), accelerated phase (AP) (n=5) and chronic phase (CP) (n=4). 3 pts. with BC had previously received STI571 prior to allo SCT. Response was assessed by bone marrow morphology, cytogenetic and FISH analysis, donor chimerism and Taqman PCR.

Results: Median treatment duration is 4.6 (range 1-26) mo.. 13 pts. (62%) (5/12 in BC, 4/5 in AP and 4/4 in CP) achieved complete hematologic response within a median time of 1 (0.5-3) mo., 7/13 (54%) evaluable pts. with AP and BC achieved complete cytogenetic response, data on cytogenetic response in CP are not yet available. 6/17 pts. with advanced CML (3 BC, 3 AP) are in ongoing hematologic and cytogenetic remission with a median duration of 18 (9-26) mo., 4/6 pts. achieved a complete molecular response. Progressive disease occurred in 8/12 pts. with BC, 1/5 pts. in AP and in no CP pts.. Median time to progression in non-responders and relapsing pts. was 34 (10-191) days. PFS is 100% in CP with a median follow-up of 7 (3-14) mo., 60% in AP and 25% in BC with a median follow-up of 4 (1-26) mo.. Responding pts. showed an increase of donor chimerism in PB from median 36% at study entry to > 99% within 3 mo.. GvHD developed in 3/21 pts.. The most frequent treatment-related side effects were mild to moderate gastrointestinal discomfort and edema. Neutropenic colitis occurred in one pt., 3 pts. developed subcutural hematoma/hygroma associated with thrombocytopenia. Grade III/IV neutropenia and thrombocytopenia were seen in 12/21 (57%) and 11/21 (52%) pts. respectively.

Conclusion: STI571-therapy is a new, promising approach even in advanced-stage relapse of Ph+ CML after allo SCT. Further investigations in prospective clinical trials are warranted, some of which are ongoing.

O241

Simultaneous in vitro exposure to STI-571, Apo2L/TRAIL and interferon-alpha, with or without prior Ara-C treatment, Apo2L/TRAIL-induced cytotoxicity and apoptosis in BCRABL+ leukemic blasts


The BCR/ABL tyrosine kinase is implicated in the pathogenesis of chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL). STI-571 (GLIVEC, Novartis) is a novel anticancer agent that selectively inhibits the BCR/ABL tyrosine kinase. After binding with its signaling death receptors (DR4 and DR5), the tumor-necrosis-factor alpha-related apoptosis-inducing ligand (Apo2L/TRAIL, Genentech and Immunex) triggers the intrinsic “mitochondrial” pathway of apoptosis more efficiently in neoplastic than in normal cells. We studied in vitro the effects on cytotoxicity and apoptosis in the M01K and M02K Ph+ leukemic cell lines in vitro with STI-571 or simultaneous-exposure to Ara-C, Apo2L/TRAIL and/or STI-571 and/or Interferon-alpha (IFN). The cytotoxic effect, of 48-hour single-agent exposure were: 12% for Ara-C; 27% for STI-571; 24% for Apo2L/TRAIL. After simultaneous exposures, the respective effects were: 44% for STI-571 plus Apo2L/TRAIL; and 63% for STI-571 plus Apo2L/TRAIL plus IFN. The apoptotic single-agent exposure were: 5% for Ara-C; 4% for STI-571; 17% for Apo2L/TRAIL. After simultaneous exposures, the apoptotic respective effects were: 30% for STI-571 plus Apo2L/TRAIL; 31% for STI-571 plus Apo2L/TRAIL plus IFN. These data provide evidence that single-drug resistance may be overcome by administering a combination of agents directed against different targets, such as STI-571 plus Apo2L/TRAIL plus IFN.

The treatment of human leukemic cells with Ara-C or etoposide or doxorubicin has been reported to up-regulate DR5 levels and sensitize cells to TRAIL-induced apoptosis. As an internal control, 48-h exposure to Ara-C was found to produce cytotoxicity and apoptosis levels of 18% and 5%, respectively. By comparison, 24-h Ara-C treatment followed by 24-h single-agent exposure produced cytotoxic and apoptotic effects, respectively, of: 44% for Ara-C; 41% for STI-571; 41% and 18% for Apo2L/TRAIL; and 13% and 4% for IFN. After 24-h combined exposures (following 24-h Ara-C treatment), the respective effects were: 48% and 30% for STI-571 plus Apo2L/TRAIL; and 39% and 31% for STI-571 plus Apo2L/TRAIL plus IFN.

Furthermore, these data provide evidence that, priming with a DR5-inducing drug like Ara-C followed by treatment with STI-571, Apo2L/TRAIL or combined alone or in combination with Apo2L/TRAIL, could be an effective strategy to better expose BCR/ABL+ leukemic blasts to apoptosis.

O242

Unmutated VH gene status predicts for inferior relapse-free survival after autologous stem cell transplantation (SCT) for chronic lymphocytic leukemia (CLL)

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In conventionally treated CLL, the Ig VH mutational status has a major prognostic impact. Unmutated VH genes are associated with a dismal prognosis, whereas patients with mutated VH genes are 21% less likely to experience disease progression. We investigated the influence of mutational status on prognosis after SCT in 75 patients with CLL who had undergone myeloablative radiochemotherapy with autotransplantation of immunomagnetically purged stem cells. We used a multiplex FR1 PCR with 6 family specific VH primers in combination with one consensus JH primer. Clonal rearranged VH genes where directly sequenced in duplicates after pooled 5 fold PCR reaction to minimise false positive results due to polymerase misreading. Sequences where compared to known germlines by DNAplot software and VBASE database. Unmutated and mutated cases where defined as more or less than 98% homology to closest defined germline, respectively.

Results: Mutated VH configurations were found in 23 of 75 (31%) patients, and 9% vs. 41%, p=0.001, respectively. However, overall survival
of autografted patients appeared to be excellent even in the unmutated cohort with a 4-year probability of 96% (95%CI 89-100%) calculated from diagnosis.

Conclusions: In patients with CLL, an unmutated VH gene status predicts for an inferior outcome after SCT. Autologous SCT does not appear to be a curative treatment for this subgroup of patients. Nevertheless, with hitherto only two deaths in the unmutated cohort, survival data suggest a substantial beneficial effect of SCT for this high-risk population.

O243
Safety and tolerability of STI-571 in patients with Ph+ chronic myeloid leukemia in accelerated phase (CML-AP) previously autotransplanted
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Introduction: STI-571, a bcr-abl tyrosine kinase inhibitor, has shown significant activity with low toxicity in CML patients in different disease phases. The most frequent side effect is mild to moderate nausea, vomiting, muscle cramps, edema, diarrhea and headache. The aim of this study was to determine if there were differences in the response rate, side effects and hematological toxicity in CML-AP patients treated with STI571 which had previously undergone an autologous stem cell transplant (ASCT) while compared with de novo-transplanted patients.

Patients and treatment: Between September 2000 and November 2001, 23 patients with CML-AP were treated in a clinical trial with STI-571 at the Institut Català d’Oncologia. Eight patients had received an ASCT previously while in chronic phase. No differences between age (median 55, ranges 26-71), sex distribution (56% males, 44% females), Sokal Index distribution and the different criteria for AP were detected between both groups. Interval between diagnosis and AP was 69.4 months, and the median follow after starting STI-571 was 6 months. STI-571 was administered orally, at a initial dose of 600 mg on an outpatient basis and the doses were adjusted as recommended according to toxicity.

Results: Complete hematological response rate at 3 and 6 months was assessed in 17 and 15 patients respectively: ASCT 71% at 3 months, 57% at 6 months; non-ASCT 70% and 75%. Edema, nausea, vomiting, muscle cramps and skin rash were similar in both groups and never attained grade 3-4. Previously ASCT patients had more frequent grade 1-2 hepatic toxicity. No statistical differences were detected regarding anemia and neutropenia between the two groups. Rate of thrombocytopenia was similarly noted in both groups but grade 3-4 thrombocytopenia was seen in 83 % ASCT vs 33 % in non-ASCT patients. STI-571 was to be discontinued because of hematological toxicity in 62% of ASCT vs 40% of non-ASCT patients.

Conclusions: Rate of hematological response to STI-571 and tolerability was similar in the two groups. Thrombocytopenia grade 3-4 was more frequently noted in patients who previously underwent ASCT.

O244
Safety and efficacy of STI571 in patients with CML relapsed after autografting (ASCT) + IFN-a
A.M. Carella, G. Beltrami, E. Rossi, M. Miglino, R. Varaldo, M. Gobbi, M. Spriano, F. Frassoni, M.T. Corsetti (San Giovanni Rontodo, Genoa, I)

The bcr-abl tyrosine kinase, generated by Ph-chromosome translocation, has been demonstrated to be the causative agent of CML. Phase II studies with STI571 (imatinib mesylate), a selective inhibitor of the bcr-abl tyrosine kinase, showed promising results in patients with different phases Ph-positive CML. We have evaluated the safety and the efficacy of this drug in patients with chronic CML progressed after ASCT and IFN-a.

Sixteen patients (CP: 15 pts, AP: 1 pts) entered this pilot study. The median age was 48 years. All patients were treated with mobilizing therapy (ICE or mini-ICE) and ASCT followed by IFN-a. Median time from diagnosis to STI571 was 48 months (range: 20-165). After median of 41 months from ASCT (range: 9-98) these patients started STI treatment because of progression in 15 patients, or intolerance, 1 patient. Treatment was started at 400 mg/daily, CP patients, or 600 mg/daily in the AP patient. Median follow-up from the start of STI571 was 15 months (range: 1-15). Grade >3 non-hematological toxicity (congestive heart failure) was observed in the AP patient; grade >3 hematological toxicity occurred in two patients, who restarted STI571 at 300mg/daily.

Eleven major cytogenetic remission were achieved, with 8 complete (50%) and 3 major (18%) responses. Other 4 patients achieved complete hematological response. This study indicate that STI571 is a safe and effective therapeutic option in patients who progressed after ASCT and IFN-a. The high rate of cytogenetic responses and the safety of the treatment in these late CP patients suggests a possible synergistic effect of STI and ASCT in restoring Ph-negative hemopoiesis in CML patients.

Infections (bacterial / fungal)

O245
What are the infectious causes of deaths in 2000 in allogeneic HLA-identical sibling stem cell transplant recipients?
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With the development of effective prophylactic and pre-emptive strategies for CMV, the morbidity and mortality attributable to CMV has dramatically decreased. Despite this considerable progress, recipients of allogeneic SCT still die from infections. In order to identify the more lethal infections in the setting of HLA-id. sibling transplant, we prospectively collected the data on infectious complications in a cohort of 200 patients included in a randomized, dose-effect, placebo-controlled, prospective trial on the prophylactic use of IVIG in allogeneic HLA-id sibling recipients between 1998-2000. The overall results of this IVIG study were previously presented (C. Cordonnier et al, ASH Meeting 2001): there was no influence of IVIG at any dose on the occurrence of infectious complications. The causes of infectious deaths were analysed in this cohort of patients. The median age was 40 y (23-49). The patients were mostly transplanted for leukemia (CML: 57%; AML: 57%; ALL: 40). Seventy four per cent were good-risk patients, 69% received TBI in the conditioning regimen, 48% experienced acute grade > 2 GVHD. All the infectious complications were prospectively collected. All patients were housed in laminar air-flow rooms during the neutropenic phase. All patients except the CMV-/-, were weekly screened for CMV by antigenemia or PCR from transplant to day 100, and were pre-emptively treated in case of CMV infection.

Twelve (6%) patients, with no relapse at time of death, died from infection as the primary or contributive cause of death: 1 from CMV pneumonia, 2 from probable (n=1) or possible (n=1) toxoplasmosis, 1 from Acenromium septicemia, 1 from P. aeruginosa, and 7 from aspergillosis.. Among the 7 cases of lethal aspergillosis, 1 was associated with P. aeruginosa, 1 to CMV and P. carinii infection, and 1 to severe bronchiolitis obliterans. The whole number of aspergillosis in the series was 12. In contrast, three cases of candidemia were observed in the series, none was lethal.

Fungal infections, and especially aspergillosis, is now the main cause of infectious death after allogeneic SCT, even in good-risk, HLA-identical sibling transplant recipients. Until to be able to control the occurrence of severe GVHD which is highly linked to aspergillosis, the transplant community should focus much effort in order to prevent the occurrence of aspergillosis whose mortality
remains extremely high despite the availability of new antifungal drugs.

O246  
Infections as the cause of late mortality after allogeneic hematopoietic stem cell transplantation (HSCT)  
Infections are a major cause of mortality early after allogeneic HSCT. Patients with chronic GVHD have an increased risk for severe infections. The aim of this retrospective analysis was to study the impact of infections on deaths occurring later than 6 months after an allogeneic HSCT. All patients transplanted at Huddinge University Hospital from 1975 until May 30, 2001 were included in the study. Patients were excluded if they died after hematological relapse and had received cytotoxic chemotherapy. However, patients receiving donor lymphocyte infusions were included in the analysis. 637 (75.5%) of 843 patients transplanted during the study period survived for at least 6 months and were included in the analysis. The results are shown in Table 1. Although the overall risk for death decreased over time, the relative risk for dying from infections compared to death from other causes remained unchanged. Chronic GVHD was an important risk factor for death caused by infections. In patients dying due to infection between 6 months and 2 years after HSCT, 23/35 (65%) had chronic GVHD. The corresponding numbers for patients dying later than 2 years after HSCT were 7/9 (78%). Pneumonia was the most common infectious cause of death (29/44) followed by septicemia (8/44) and encephalitis (4/44). Fatal pneumonias occurring between 6 months and 2 years after HSCT were caused by fungal infections (n=1), bacterial infections (n=3) P.carinii (n=2), and adenovirus (n=1). However, in 10 patients the cause was unknown. In contrast, all 6 fatal pneumonias occurring later than 2 years after HSCT were caused by bacterial infections. We conclude that infections remain a significant cause of death for many years after allogeneic HSCT and the relative proportion of deaths caused by infection compared to other causes does not change over time. Chronic GVHD is an important risk factor for late infectious disease mortality.

<table>
<thead>
<tr>
<th>Survival &gt; 6 months</th>
<th>Survival &gt; 1 year</th>
<th>Survival &gt; 2 years</th>
<th>Survival &gt; 5 years</th>
<th>Survival &gt; 10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of surviving patients</td>
<td>636(80.2%)</td>
<td>529(70.0%)</td>
<td>413(55.3%)</td>
<td>296(39.1%)</td>
</tr>
<tr>
<td>No. of death:</td>
<td>201(25%)</td>
<td>130(29%)</td>
<td>66(16%)</td>
<td>24(8%)</td>
</tr>
<tr>
<td>Percentage of deaths due to infection</td>
<td>44(1.1%)</td>
<td>16(1.3%)</td>
<td>6(1.6%)</td>
<td>2(0.7%)</td>
</tr>
</tbody>
</table>

O247  
Effect of lenograstim on infections after high-dose chemotherapy and autologous peripheral stem cell transplantation  
R. Marcus, W Linkeshe, S. Solano, A. Alegre, P. Ljungman, B. Simonsson, T. Fischer, R. Soufi-Mahjoubi, N. Schmitz (Cambridge, UK; Graz, A; Valencia, Madrid, E; Huddinge, S; Mainz, D; Paris, F; Kiel, D)  
This randomised double blind study compared lenograstim (glycosylated rHuG-CSF) 150 μg/m²/day with placebo on the incidence of fever and proven infections after autologous PBSCT. 193 patients with NHL and HD, multiple myeloma, breast cancer and other solid tumours were randomised to receive Lenograstim (98 pts) or placebo (95 pts) maximum duration of treatment was 28 days. Stem cells were mobilized by chemotherapy followed by Lenograstim (150 μg/m²d s.c.) to collect at least 2 x 106 CD34+ cells/kg. Patients in the placebo group were younger(47 years vs 50 years). Other patient characteristics were similar. Median CD34+ cells reinfused : 4.4 x 10⁶/kg in the Lenograstim group (LG) and 4.1 in placebo group (PG). Duration of study drug was 10 d in LG and 14 d in PG (p=0.0001). One patient in PG was not evaluable.

O248  
Influence of myeloperoxidase gene polymorphism on incidence of severe bacterial infections after HLA identical bone marrow transplantation for patients with leukemia  
Myeloperoxidase (MPO) is a lysosomal enzyme found in neutrophils with antimicrobial activities. A single base substitution G to A in the promoter region of the MPO gene (position –463) markedly reduce transcription. Patients with deficiency of MPO have a delayed bactericidal ability. We hypothesized that the incidence of infections after HLA identical BMT could be influenced by the MPO gene polymorphism. Patients and Methods: Genotyping for the MPO was performed in 108 donor/recipient DNA pairs, by PCR amplification. Severe bacterial, viral or invasive fungal infections were studied retrospectively during 180 days after BMT. Univariate and multivariate proportional hazards regression models were performed in a competing risk setting to identify independent risk factors of death and infections. BMT were performed in Saint Louis Hospital from 07/93 to 08/99: 68 patients had chronic and 40 AL. Median age was 35 y (3-56). Conditioning regimen and GVHD prophylaxis consisted mainly in BU+ CY(54%) and CsA+Mtx (90%). Median follow up time was 4.4 years. Results MPO G/G genotype was detected in 59 recipients (55%) and in 64 donors (60%). Neutrophils recovery was 96%. Acute GVHD (II-IV) was observed in 45 (42%) and chronic in 50/98 patients at risk. Estimate survival at 5 years was 54%. First episode of severe bacterial infections was diagnosed in 30 patients, viral infections in 44 and invasive fungal infections in 13. In univariate analysis, for bacterial infections, donor genotype (G/G) influenced the incidence of severe bacterial infections. In fact the cumulative incidence of bacterial infections was 20 % in patients transplanted with a G/G MPO BM donor and 39,5% in patients transplanted with a A/G or A/A MPO donor (p=0.03). In a multivariate analysis the following factors increased the risk of bacterial infections: presence of TBI (HR=2.12; p=0.04), major ABO incompatibility (HR=2.4; P=0.02), and AG and AA MPO
(donor) (HR=2.16  P=0.03). Viral and fungal infections were not influenced by MPO gene polymorphism.

In conclusion, the presence of a single (A/G) or double (A/A) base substitution at the position +453 in MPO gene from a BM donor increases the incidence of bacterial infections in BMT recipients, probably reflecting the decreased bactericidal activity of MPO present in engrafted neutrophils. This finding can define better bacterial surveillance and prophylaxis for BMT recipients from donors with A/G or A/A MPO genotype.

O249

Survival cultures for Clostridium difficile in hematopoietic stem cell transplantation

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Clostridium difficile (C. difficile)is a leading cause of nosocomial diarrhea. We performed a study to know whether cultures and early therapy could avoid toxicities associated with C. difficile in hematopoietic stem cell transplantation (HSCT). We also collected data on the prevalence and relapse of C. difficile in these patients. Since 1988, biweekly surveillance stool cultures for C. difficile were performed in the patients admitted to the hematology wards. 468 patients have had a HSCT. Data from stool cultures for C. difficile and detection of toxin A and/or B (tox+) were obtained in 426 (178 alloHSCT, 251 autoHSCT). The treatment policy was to prescribe Metronidazole (500 mg tid for 10 days) as soon as a positive culture result was transmitted. In 170 patients (40%) one or more episodes with a positive culture for C. difficile were recorded (before HSCT: 58 patients, at HSCT: 68, before and at HSCT: 29, after HSCT: 15). In 62, a positive culture was associated with toxin A or B (tox+). C. difficile was found at conditioning in 25 patients (6%). In 97 patients (23%), C. difficile (tox+, n=38; tox-, n=59) was cultured during hospitalisation for HSCT. Tox+ C. difficile infection was more frequent in auto- than in alloHSCT (26/55 vs 12/42, respectively, p=0.05). Relapse of C. difficile occurred in 56 of the 170 positive patients (33%). The incidence of relapse of C. difficile did not differ between tox+ and tox-, and was similar in allo- and autoHSCT. In 20/56 a relapse of C. difficile occurred at HSCT; in the other patients the relapse was not related to HSCT. A positive culture for C. difficile (either tox+ or tox-) did not affect the toxic mortality in either allo- or autoHSCT. In alloHSCT, C. difficile (either tox+ or tox-) was not associated with any increase in graft-versus-host disease.

Conclusions 1/ The prevalence of C. difficile infections in HSCT recipients is high 2/ Despite therapy with Metronidazole, a third of the patients who had had C. difficile relapsed. HSCT does not increase the risk of relapse of C. difficile 3/ In HSCT, surveillance cultures and early therapy may avoid non-relapse complications that might be caused by C. difficile infections.

O250

Analysis of T-cell responses to aspergillus fumigatus antigens in healthy individuals and patients with hematological malignancies - final analysis

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Invasive Aspergillosis has become a major cause of infection-related mortality in non-neutropenic patients after allogeneic stem cell transplantation. To assess the potential role of Aspergillus-specific T-cell responses for the successful control of invasive aspergillosis, lymphoproliferative responses to A. fumigatus antigens were studied in healthy individuals and leukemia patients with evidence of invasive aspergillosis.

In 14 of 16 healthy individuals, a positive lymphoproliferative response was documented to cellular extracts of A. fumigatus, in 4 of 16 to the 88 kDa dipeptidylpeptidase, and in 8 of 11 to the 90 kDa catalase. In 13 of 17 healthy individuals a predominant release of IFN-g in culture supernatants upon stimulation with A. fumigatus antigens was demonstrated, indicating a TH1 response. In patients with clinical evidence of invasive aspergillosis, a low A. fumigatus-specific lymphoproliferation was found to be associated with low leukocyte counts (p=0.033), steroid treatment (p=0.037), and a low release of IFN-g in culture supernatants (p=0.017). A dominant release of IFN-g compared to IL-10 in culture supernatants was documented in 7 patients with favourable outcome (median ratio IFN-g/IL-10=1.0; range 0.09-24.8), whereas 10 patients with progressive (n=6) or stable (n=4) disease showed a dominant release of IL-10 (median ratio IFN-g/IL-10=0.1; range 0.05 - 1.0) (p=0.04). In addition, phosphoantigen-reactive Vg9Vd2 T-cell clones were found to produce significant amounts of TNF in response to Aspergillus fumigatus antigens.

In conclusion, theses results further support the hypothesis, that alpha/beta and gamma/delta T-cells contribute to the host defense against Aspergillus fumigatus.

O251

Systemic fusarium infection in bone marrow transplant recipients


Systemic infection by Fusarium sp. has been reported in bone marrow transplant (BMT) recipients, with 39 cases published until 2001, and a death rate of 90%. The objective of this study was to evaluate the epidemiology, clinical findings, therapeutic practices and outcomes. We performed a study to know whether surveillance and prophylaxis for BMT recipients from related donors (13 cases). The median time of diagnosis was day +25 (range day +6 to day +1017). Neutropenia was present in 13 patients, and it occurred in all 11 patients with fusariosis before day +30, 2/5 patients diagnosed between day +30 and day +100, and in none of 2 diagnosed after day +100 (P=0.002). Graft versus host disease was present in 6 patients, and 8 patients were receiving steroids. Fever (91%), skin lesions (66%), pulmonary involvement (47%) and sinusitis (19%) were the most frequent manifestations. Standard amphotericin B was the treatment of choice in all but 2 patients, who received liposomal amphotericin B, and 16 patients received cytokines (G-CSF or GM-CSF). Nineteen patients (40%) responded to therapy, but the median survival was only 23 days, and the death rate 60 days after the diagnosis of fusariosis was 70%. Patients with disseminated skin lesions had a higher death rate (86%) than patients with localized lesions (17%, P=0.03). The incidence of fusariosis in BMT recipients seems to be low, but the infection is associated with a very high death rate. The presence of disseminated skin lesions is a poor prognostic factor.

Keywords: Fusarium, prognosis, bone marrow transplant
O252
Assessment of maximum tolerated dose (MTD) and pharmacokinetics (PK) of FK463 used for anti-fungal prophylaxis in neutropenic patients after hematopoietic stem cell transplantation
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FK463 is a novel i/v echinocandin antifungal agent. In vitro studies indicate that FK463 has potent broad-spectrum cidal activity against Candida spp, including azole-resistant C. albicans and static activity against Aspergillus spp. In an earlier US study, MTD was not reached in doses up to 200 mg/d (Co Report 2000000097). This MTD study starts with an equivalent dose of 200mg/d. Sequential groups of 10, 10, 8 and 8 patients undergoing stem cell transplantation (median age 47.5 y; range 19-62; 23M, 13F; 23 autologous and 13 allogeneic transplantation) received 3, 4, 6 and 8 mg/kg/d FK463, respectively starting from day -2 or -3 of the transplant as antifungal prophylaxis. Median doses were 245, 294, 420, 578mg/d and median treatment period was 16.5, 18.5, 22.5 and 20 d for patients who received 3, 4, 6 and 8 mg/kg/d FK463, respectively. The highest dose given was 900mg/d PK data for all 4 dose groups is being analyzed. Treatment (by 1 h infusion) was for a minimum of 7 d and could be stopped following recovery of neutrophils to 0.5x10⁹/L. Maximum treatment period allowed was 28 d. Adverse events (AE) were graded, from 1-4 according to SWOG Toxicity Criteria. MTD was defined as highest dose of FK463 that did not cause the same treatment-related Grade 3 or 4 AE in at least 3 different patients. There was no Grade 3 or 4 AE in at least 3 different patients. We conclude that FK463 is safe, effective and well tolerated at doses tested, and that the MTD is FK463 that did not cause the same treatment-related Grade 3 or 4 AE in at least 3 different patients. There was no Grade 3 or 4 AE in at least 3 different patients. We conclude that FK463 is safe, effective and well tolerated at doses tested, and that the MTD is defined as highest dose of FK463 that did not cause the same treatment-related Grade 3 or 4 AE in at least 3 different patients.

O254
Chronic graft versus host disease after allogeneic blood stem cell transplantation - Long term results of a randomized study from the Société Française de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC)

The transplantation of peripheral blood stem cells (PBSC) is rapidly growing in the allogeneic setting as an alternative to bone marrow (BM). However, the issue of the relative incidence and severity of chronic GVHD (cGVHD) remains unresolved. We recently reported a higher incidence of cGVHD associated with PBSC transplantation in a comparative randomized trial. In the present report, we undertook a prospective analysis of clinical and biological features of cGVHD over a long follow-up period in the patients (n=101) enrolled on this study. We found that extensive cGVHD was more frequent with PBSC (P = .004), and its prevalence was always higher whatever the time point. Ocular involvement was more frequent in PBSC recipients (P = .002), while cutaneous and liver involvement were not different. In addition, this cGVHD required multiple highly immunosuppressive treatments in addition to cyclosporin and corticosteroids during a longer period (P = .03). All together, this translated into longer periods of hospitalization post-transplantation in the PBSC group (P = .03). These observations strongly support more frequent and more aggressive cGVHD after PBSC transplantation impairing the quality of survival. Finally, we also confirm that cGVHD after PBSC transplantation is associated with an antileukemic effect which is at least as potent as after BM transplantation. However, to date, this had not translated into an overall survival difference in this population of early stage leukemic patients. For these reasons, we conclude that caution should be applied in using allogeneic PBSC transplantation in early stage leukemic patients.

Graft versus Host Disease

O253
In-vivo T-cell depletion with pretransplant anti-thymocyte globulin (ATG) reduces GvHD without increasing relapse in good risk myeloid leukemia patients after stem cell transplantation from matched related donors

We investigated the effect of anti-thymocyte globulin (ATG) as part of the conditioning regimen in HLA-related allogeneic stem cell transplantation on toxicity, engraftment, graft-versus host disease and relapse rate. 45 patients with good risk myeloid leukemia (CML 1. chronic phase or AML 1. CR) were included in the study and compared with the results of a historical group consisting of 57 patients with the same diagnosis but conditioned without ATG. No graft failure was observed in the ATG group, while one graft failure was seen in the Non-ATG group. The median time to leukocyte engraftment (>1x 10⁹/L) was 16 (range 12-33) in the ATG group and 17 days (range 11-29) in the Non-ATG group (n.s.). The platelet engraftment (>20 x 10⁹/L) was reached for the ATG group after a median of 24 days (range 14-277) and for the Non-ATG group after 19 days (range 11-34) (p=0.002). Acute GvHD grade II-IV was observed in 47% of the Non-ATG and in 20% of the ATG group (p=0.004). Severe grade III/IV GvHD occured in 7% of the ATG and in 32% of the Non-ATG group (p=0.002). Overall chronic GvHD was seen in 36% of the ATG and in 67% of the Non-ATG group (p=0.005). Extensive cGVHD was more frequently observed in patients conditioned without ATG (33% vs 17%, p=0.08). After a median follow-up of 26 months (range 7-75) in the ATG and in 65 months (range 1-126) in the Non-ATG group, the 5 years estimated overall survival is 66% (95% CI: 51-81%) for the ATG group and 59% (95% CI: 46-72%) for the Non-ATG group (n.s.). The 5 years estimated disease free survival is 64% (95% CI: 50-78%) for ATG and 55% (95% CI: 43-67%) for the Non-ATG regimen (n.s). During follow-up 6 relapse were observed. Median time to relapse after transplantation was 15 months (range 5-64). The 5 years probability of relapse was 5% in the ATG- and 15% in the Non-ATG group (n.s.). We conclude that ATG (anti-rabbit, ATG-Fresenius) as part of the conditioning regimen leads to a significant reduction of severe acute GvHD and of chronic GvHD without increase of relapse in patients with AML 1. CR or CML chronic phase after stem cell transplantation from HLA-related donors.
The presence of mild acute and chronic graft versus host disease in a T-cell depleted unrelated donor transplant setting improves overall survival


We have analysed the transplant outcome in 152 unrelated donor/patient pairs provided by the Anthony Nolan Trust. All these pairs had tissue typing performed to allele level at HLA – A, -B, -C, -DRB1, -DQB1 and -DPB1. All transplants were T cell depleted, either by campath/ATG in vivo only (100 of 152, 66%), campath "in the bag" only (5 of 152, 3%) or both (47 of 152, 31%). The procedures were carried out between September 1996 and May 2001, with a median follow up of 277 days (mean = 400). Of the patients studied 35% had CML, 23% AML, 22% ALL and 20% other disease groups. The overall survival is 49% at a median of 621 days (mean = 641).

101 of the pairs were completely matched at allele level for HLA – A, -B, -C, -DRB1 and -DQB1. This group had a significant survival advantage when compared to the group of 51 patients with either single or multiple mismatches at any of these loci (p=0.0009). The number of single or multiple mismatches at either class I or class II were too small to give any meaningful results in this analysis. The incidence of acute graft versus host disease (aGVHD) was 62%, with only 9% of this representing clinically severe grade 3 and 4 aGVHD. The presence of grade 1 or 2 aGVHD translated into a significant survival advantage. Survival was 60% in the mild GVHD cohort and 39% in the cohort with no GVHD at day 1400 (p=0.0001). Of the deaths 4% (6 of 152) were attributable to aGVHD with or without other factors (infection/ veno-occlusive disease).

The incidence of chronic graft versus host disease (cGVHD) was 33%. The presence of cGVHD was significantly associated with a survival advantage (p=0.0004). 3% of deaths were due to cGVHD. The rate of relapse was 38%, however this did not affect overall survival as many of the patients were rescued by donor lymphocyte infusions. The factors affecting relapse were, patient age, donor sex and patient CMV status.

The independent factors improving overall survival in multivariate analysis were found to be HLA matching, patient CMV negativity prior to transplantation and the presence of mild acute and/or chronic graft versus host disease. Although these results have previously been published in a T cell replete setting, we believe this is the first report of comparable results in a cohort of patients all receiving T cell depleted transplants.

Risk factors for acute graft-versus-host disease in patients transplanted with CD34+ selected blood cells from HLA-identical siblings


The incidence and severity of acute graft-versus-host disease (aGVHD) decreases when the inoculum is T cell depleted, but a still significant proportion of patients, between 10-40%, develops aGVHD II-IV. Risk factors for aGVHD in T-cell depleted transplantation are unknown. A study was therefore performed on 315 consecutive adult patients, receiving T-cell depleted grafts by means of CD34+ selection from peripheral blood from HLA-identical siblings, to determine what characteristics of the donor, recipient, and cell graft content, are predictive of aGVHD. The cumulative incidence (marginal probability) of aGVHD increased progressively as the number of CD34+ cells and CD3+ cells in the graft increased. Thus, recipients of a dose of CD34+ cells (x10^6/kg) of <2, >2-4, and >4 had a cumulative incidence of aGVHD of 21%, 35%, and 43%, respectively (log-rank p=0.01); similarly, recipients of a dose of CD3+ cells (x10^6/kg) of <0.05, >0.05-0.1, and >0.1 had a cumulative incidence of aGVHD of 18%, 35%, and 44%, respectively (log-rank p=0.007). Using a Cox regression model, four independent factors for aGVHD were identified: increased CD34+ cell dose (p=0.02), increased CD3+ cell dose (p=0.02), female patients (p=0.01), and higher patient age (>42 years) (p=0.007). In this model, the CD34+ and CD3+ cells doses most strongly associated with the development of aGVHD were >4x10^6/kg (p=0.007) and >0.1x10^6/kg (p=0.006), respectively. This study shows, for the first time in T cell depleted transplants, a positive correlation between the number of CD34+ cells and aGVHD rate, and that the number of CD3+ cells necessary to initiate aGVHD is lower than previously reported.

Mesenchymal stem cells (MSC) suppress T-cell activation and proliferation in vitro: a model for graft versus host disease prophylaxis

M. Vallee, M. Podestà, G. Piaggio, A. Pitta, F. Vassallo, S. Lucchetti, F. Frassoni, A. Bacigalupo (Genoa, I)

We have tested the effect of human mesenchymal stem cells (h-MSC) on T cell activation and proliferation. Human MSC were isolated from bone marrow stromal cells (BMSC) of healthy donors by bone marrow mononuclear cell cultures in Petri dishes and expanded for several generations in presence of McCoy’s Medium plus 10% FCS. A murine stromal cell line (M210-B4) was also used as source of murine mesenchymal cells (m-MSC). T-cell activation was studied using as target cells 100x10^3 responder cells and 50x10^3 stimulator cells. Proliferation was detected by 3H thymidine incorporation at 72 hours-5 days and expressed as mean counts per minute (cpm) of triplicate cultures. PHA: After 72 hours exposure to PHA, 1x10^5 normal PBMC had 75x10^3 cpm; in the presence of PHA mismatched h-MSC 6x10^3, 12x10^3, 25x10^3, 50x10^3 and 100x10^3 cpm were 28, 26, 20, 6 and 0.2x10^3 (average of 4 exp). Suppression could not be achieved. Exposure to PHA, 1x10^5 norma PBMC had 75x10^3 cpm; in the presence of PHA mismatched h-MSC 6x10^3, 12x10^3, 25x10^3, 50x10^3 and 100x10^3 cpm were 28, 26, 20, 6 and 0.2x10^3 (average of 4 exp). Suppression was seen also with m-MSC and PHA. The latter was at a lower level. The addition of Interleukin-2 (30U/ml) in the cultures decreased suppression (80% to 50%), but the complete correction of suppression could not be achieved. Activation expression markers such as CD38 were down regulated by h-MSC on PHA blasts (6% expression vs 41% of cultures without h-MSC).

In conclusion: MSC suppress activation and proliferation of T-cells exposed to either PHA or allo-antigens: the suppression is not allo-specific and can be shown also across species. It seems to involve a soluble mediator and down regulation of CD38. The immunosuppressive activity of MSC in vitro may be exploited in vivo in the transplantation setting, as suggested by a recent clinical trial.

Human bone marrow stromal cells suppress T-cell proliferation induced by allogeneic lymphocytes or dendritic cells

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The distinct immunophenotype profile of human bone marrow stromal cells (BMSCs) suggests a role of BMSCs in modulating T-
lymphocyte alloreactivity. Therefore, the present study was planned to investigate the ability of BMSCs in inhibiting T-lymphocyte proliferation. BMSCs generated in a Dexter-type culture system lacked co-stimulatory molecules and class II HLA expression. Immunoslected CD2+ T-lymphocytes obtained from either the donor of BMSCs or a third party, were cultured in mixed lymphocyte reactions (MLRs) with either allogeneic dendritic cells (DCs) or peripheral blood lymphocytes (PBLs). When autologous or allogeneic BMSCs were added-back to T cells stimulated by DCs or PBLs a statistically significant and dose-dependent reduction of T cell proliferation, as measured by a 18-hours pulse with 3H-Thd was evident. Similarly, addition of BMSCs to T-cells stimulated by aspecific polyclonal activators such as PHA or IL-2 resulted in a significant suppression of proliferation. BMSC-induced suppression of T-cell proliferation was still evident if BMSCs were added in culture as late as 5 days after starting of MLR. BMSC-inhibited T-lymphocytes were not apoptotic and efficiently proliferated when restimulated with cellular or aspecific polyclonal activators in the absence of BMSC. CD4+ and CD8+ T cells were equally inhibited in MLR by BMSCs. When cell-cell contact between BMSCs and effector cells was prevented, T-lymphocyte proliferation was substantially inhibited suggesting that soluble factors might be involved in the mechanism of T-cell suppression. Transforming growth factor beta1 and hepatocyte growth factor, but not interleukin-6 or interleukin-11 were identified by means of neutralizing monoclonal antibodies as cytokines mediating BMSC effects. In conclusion, BMSCs were equally effective in preventing rejection following pre-transplant Campath which may be important in preserving post-transplant immune reconstitution.

O260

Impact of high resolution HLA typing on severe acute GVHD in chronic phase CML patients treated with unrelated donor bone marrow transplantation

With the advent of high resolution HLA typing it became evident that HLA mismatching at DNA level increases the incidence of severe acute graft versus host disease (aGVHD; grade III-IV) in patients treated with unrelated donor bone marrow transplantation (UDBMT) and adversely affects the overall outcome. However, it is controversial whether this effect is primarily due to mismatching at HLA class I or class II loci. We studied the effect of pre-transplant Campath 1G on the incidence of severe aGVHD in patients with chronic phase CML treated with UDBMT. Between July 1996 and August 2001, 45 patients (33 males and 12 females; range 6-52yr, median 32.6yr) were transplanted at the Hammersmith Hospital using as conditioning Cy 120mg/kg, TBI 14.4 Gray and Campath 1G or 1H (10mg for 10 days until 8/98, 10mg for 5 days for CMV+ patients from 8/98 to 5/99; and 10mg for 5 days for all patients since 5/99). Cyclosporine and accelerated myeloid recovery induced by aspecific polyclonal activators such as PHA or IL-2 were used for aGVHD prophylaxis. In this cohort of patients, we studied the effect of allele mismatch (as determined by high resolution DNA typing) at the HLA class I (A and B) and class II (DRB1 and DPB1) loci on the incidence of severe aGVHD. 41.7% of patients were mismatched at least at one class I allele and 47.9% of patients were mismatched at least at one class II allele. The incidence of severe aGVHD between patients mismatched at class II and patients fully matched at class II was not statistically different; similarly, there was no difference in the incidence of severe aGVHD when patients mismatched at both class I and class II were compared with patients fully matched at both class I and class II. In contrast, there was a statistically significant higher incidence of severe aGVHD in patients with class I mismatch as compared to patients fully matched at class I (15% v 0%; p=0.04). However, the overall incidence of severe aGVHD (grade III-IV) in the total cohort of the patients was only 6.6%, much lower compared to protocols using no or different type of T cell depletion, other than Campath. Engraftment, relapse rate, and overall survival were not statistically different between patients mismatched at class I or II loci and patients fully matched at both loci. We conclude that mismatching at class I increases the risk of severe aGVHD in patients receiving UDBMT but this risk is much lower when intense in vivo T cell depletion using Campath is applied.

Cytokines / Gene Therapy

O261

Clinical and financial benefits of granulocyte colony-stimulating factor therapy after bone marrow transplant in children: results of a prospective randomized trial

Hematopoietic stem cell transplantation (HSCT) is associated with profound neutropenia, which can result in significant morbidity and mortality. To evaluate the safety and efficacy of recombinant human granulocyte-colony stimulating factor (rhHuG-CSF) in accelerating myeloid recovery and its influence on infectious, supportive therapy, and transplant related mortality we carried out a randomized study in pediatric patients given HSCT.
Two-hundred twenty one children, recipients of allogeneic or autologous bone marrow (BM) or peripheral blood progenitor cell (PBPC) transplant, entered the study. Patients were randomized to either receive rhHuG-CSF at a dose of 10 ng/Kg (n=110) or not (n=111). Children given rhHuG-CSF were treated from day +5 to +30 or until the absolute neutrophil count was >1x10^9/L. Myeloid engraftment was faster in the treated arm (12 vs 16 days, p=.0001). Analysis according to type of stem cell source employed confirmed the accelerated neutrophil recovery both in PBPC (p=.0005) and BM subgroups (allogeneic and autologous, p=.002). Median time to the last platelet transfusion and to discharge was favourably influenced by rhHuG-CSF in PBPC recipients only in the treated patients given allogeneic/autologous BMT (p=.02 and p=.04, respectively). In the group of patients transplanted with BM progenitors and treated with rhHuG-CSF the cost of the cytokine was offset by the shorter hospitalization length with a median saving of 11,000 US$ per patient. We conclude that BM recipients receiving rhHuG-CSF have faster neutrophil recovery and this led to clinical and financial benefits, while, in the PBPC subgroup, the accelerated myeloid recovery did not translate into any other clinical or financial advantages.

O262
MMP-9 (gelatinase-B) release in IL-8-induced hematopoietic progenitor cell mobilization in mice
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Proteases play a major role in cytokine-induced hematopoietic progenitor cell (HPC) mobilization, since they are able to release hematopoietic progenitor cells from the bone marrow (BM) microenvironment by interrupting essential cell-cell and BM stroma-cell interactions. The matrix metalloproteinase MMP-9/gelatinase-B is able to degrade certain types of collagen present in the BM and plays an essential role in interleukin-8 (IL-8) induced HPC mobilization in primates. In mice, other proteases such as neutrophil elastase and cathepsin G, were shown to be involved in cytokine-induced HPC mobilization through cleavage of VCAM-1 and SDF-1-alfa (Levesque et al., ASH 2001). However, the role of MMP-9 in HPC mobilization in mice remains unclear. In this study we investigated the relation between MMP-9 and IL-8-induced HPC mobilization. In Balb/c mice, within 15 minutes after a 30 µg i.p. bolus injection of IL-8 was given, a threefold increase in MMP-9 levels was seen, followed by a return to baseline level at 30 minutes. MMP-9 induction coincided with HPC mobilization (CFU-GM peripheral blood at t=0 22/ml, at t=20 minutes 223/ml). In Balb/c mice, rendered neutropenic by injection of an anti-neutrophil antibody (anti-Gr-1, 250 µg i.p. 24 hours before IL-8 mobilization), IL-8 injection did not induce HPC mobilization or MMP-9 release. Tail-vein infusion of 7x10^6 neutrophils, collected from the peripheral blood of mice treated with cyclophosphamide and G-CSF, in the neutrophenic mice collected IL-8-induced HPC mobilization with a simultaneous fourfold increase of serum MMP-9 levels. To further determine the role of MMP-9 in IL-8 induced HPC mobilization, MMP-9 deficient mice were mobilized with IL-8. Peripheral blood CFU-GM in MMP-9 deficient mice increased from 42/ml to 332/ml (SD 132/ml) at 20 minutes after IL-8 injection, as compared to an increase from 98/ml to 535/ml (SD 244/ml) in wild-type mice (p=0.15). The number of bone marrow CFU-GM was similar in wild-type and MMP-9 deficient mice. In summary we showed that 1) the kinetics of MMP-9 release by neutrophils coincides with mobilization of HPCs, 2) neutrophils are indispensable for IL-8-induced HPC mobilization, 3) IL-8-induced mobilization is not reduced in MMP-9-deficient mice. Our data indicate a different role for MMP-9 in mice as compared to primates and show that induction of MMP-9 reflects activation of neutrophils, but is not responsible for IL-8-induced mobilization in mice.

O263
A role for G-CSF in the generation of human T regulatory 1 cells
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Granulocyte-CSF (G-CSF) may affect T-cell homeostasis by multiple mechanisms, inducing polarization of cytokine secretion, function of T-cell proliferation and enhancement of T-cell apoptosis. We analyzed the production of IL-10 and TGF-beta1 by T cells from healthy volunteer donors treated with s.c. recombinant human G-CSF. Highly purified CD4+ T cells obtained prior to (preG) and after G-CSF administration (postG) were activated using the allogeneic MLR. PostG CD4+ T cells produced high levels of IL-10 but undetectable levels of IL-2 and IL-4, whereas the level of TGF-beta1 release was comparable with that of preG CD4+ T cells. Notably, postG CD4+ T cells proliferated poorly in response to allo-Ags as well as to recall Ags and suppressed the proliferation of autologous CD4+ T cells in cell contact-independent and Ag nonspecific manner. TGF-beta1 and IL-10 were not dispensable for postG CD4+ T cells to mediate suppression, as shown by neutralization studies. Compared with preG CD4+ T cells, allo-Ag activated postG CD4+ T cells expressed predominantly the markers associated with memory T cells, in conjunction with reduced levels of CD28 and CD62L. Collectively, these data demonstrate that CD4+ T cells exposed to G-CSF in vivo acquire the properties of T regulatory (Tr) cells once triggered in vitro through the TCR, including 1) peculiar cytokine production profile (IL-10+++TGF-beta1+IL-2low/-IL-4low/-), 2) intrinsic low proliferative capacity and 3) contact-independent suppression of Ag-driven proliferation. Tr cells generated ex vivo after exposure to G-CSF might be clinically relevant for transplantation medicine and for the treatment of human immunemediated diseases.

O264
G-CSF receptor gene expression in class I- and II-restricted T-cells identified by single cell RT-PCR: implications for transplantation and autoimmune diseases
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Objectives: Results from experimental models, in vitro studies, and clinical data indicate that G-CSF stimulation alters T-cell function and induces Th2 immune-responses. The immunomodulatory effect of G-CSF on T-cells results in an unexpected low incidence and severity of acute GvHD in PBSCT despite a 10-fold higher number of mature T-cells in the graft compared to BMT. But it is still under discussion whether T-cells do express the G-CSF receptor and thereby enable direct effects of G-CSF on T-cells bypassing other effectors like monocytes and dendritic cells.

Methods & Results: Therefore, we performed a single-cell RT-PCR on T-cells and could demonstrate the induction of G-CSF receptor (G-CSFR) gene expression in mature class I- and II-restricted T-cells upon G-CSF both in vivo and in vitro. CD4+ and CD8+ T-cell subpopulations were enriched by microbead technology before single cell picking and could be identified as T cells due to the coamplification of the T-cell receptor (TCR)-alpha chain. The efficiency of the single cell RT-PCR for the G-CSFR and TCR-alpha chain was higher than 90%. After in vivo treatment of stem cell donors with G-CSF, the G-CSFR gene amplification might be amplified in 44 of 127 single donor T-cells (34%) without a significant difference in CD4+ and CD8+ T-cells. Co-culture experiments and kinetic studies of more than 200 single T-cells show that G-CSF directly induces its receptor in a time-dependent manner and thereby indicating the functional activity of G-CSF receptor expression in T-cells. In order to study the altered T-cell response in greater detail, we performed an expression profiling of T-cells from G-CSF treated peripheral stem cell donors and uncovered a considerable gene regulation upon G-CSF in vivo at different levels of activation (e.g., upregulation of CD69), proliferation (e.g.,
downregulation of CD5), functional differentiation (e.g. downregulation of LFA-1a, upregulation of GATA-3).

Conclusions: Our data provide new mechanistic insights into the effector function of G-CSF in the T-cell immunosystem: G-CSF acts as strong immunomodulator in T-cells and can directly modulate T-cell immune responses via its receptor on T-cells. Thereby, G-CSF qualifies as interesting therapeutic tool for a specific immunomodulation in transplantation, especially acute GvHD and other conditions associated with Th1/Th2 imbalance such as bone marrow failure syndromes and autoimmune diseases. Supported by the DFG, SFB 265.

O265

Highly purified CD3+ T-Cells for suicide gene transduction: Feasibility for immunotherapy with large number of donor lymphocytes after allogeneic transplantation

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Objective: Application of donor lymphocytes (DL) after allogeneic transplantation is a treatment option in pediatric patients with leukemia in case of increasing mixed chimerism caused by graft rejection or relapse. However, DL can also lead to a life-threatening graft-versus-host disease (GvHD). In order to eliminate alloreactive T-cells mediating GvHD after DL we established a new suicide gene strategy with retroviral constructs using two independent killing pathways and two different surface markers for selection.

Materials and Methods: 13 negative fractions of CD34 selected allogeneic apheresis products were incubated with magnetic bead conjugated CD3 antibodies. CD3+ cells were selected using the Miltenyi Clnimacs device. The purified T-cells were transduced by a vector containing a ganciclovir-hypersensitive mutant of HSV-TK (TK39) fused in frame to a truncated version of the CD34 surface marker gene (CD34). In a second construct the low affinity nerve growth factor (dLNGFR) was ligated to 2 copies of a dimerization domain (FK506 binding protein FKBP) and the Death Effector Domain (DED) of FADD (Fas associated protein with death domain). Transduced CD3+ cells were purified by CD34- and anti-dLNGFR selection. Different concentrations of Ganciclovir (GCV) and AP20187, a chemical inducer of dimerization (CID), were used to evaluate the elimination rate in normal and transduced T-cells. Additionally, the cytotoxic activity of these T-cells against leukemic blasts were investigated using four-coloured flow cytometric analysis on an EPICS XL-MCL system.

Results: Mean purity of selected CD34-cells was 96.3 ± 3.4 and enrichment of transduced T-cells resulted in populations with a purity of 95 - 98.5 %. Clinically achievable low concentrations of GCV (1 µM) as a single application as well as incubation with AP20187 (10 nM) led to more than 95 % elimination of transduced T-cells. CD3+ cells containing suicide genes showed a median to high killing activity against different leukemic blasts. So far, no marked difference in the killing ability was detected between normal and transduced CD3+ T-cells.

Conclusion: Successful elimination of alloreactive T-cells transduced with either of these vectors is feasible and will enable further optimization of T-cell gene therapy protocols.

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O266

Clinical scale production of HSV-tk/LNGFR transduced allogeneic T-lymphocytes for treatment of leukemic relapse after allogeneic stem cell transplantation


Donor Lymphocyte Infusion (DLI) can induce remission in CML patients who relapse after T-cell depleted allogeneic transplant. Although the occurrence GvHD is closely linked to a beneficial graft-GvL effect it may cause significant morbidity and mortality. Other strategies are required to control DLI induced GvHD and improve the outcome of allo-SCT. One strategy provides a tool to kill dividing T-cells when required and thus 'switch-off' GvHD. Transfer of the Herpes Simplex Virus type 1 encoded thymidine kinase (HSV-tk) 'suicide' gene into T-lymphocytes renders dividing cells sensitive to Ganciclovir (GCV). Co-insertion of a gene coding for the low affinity nerve growth factor receptor (LNGFR), allows transduced cells to be purified by immunoselection. For clinical purposes the procedure must provide an effective T-cell dose and ensure the safety of the product by compliance with Good Manufacturing Practice (GMP).

As part of a multi-centre study we have developed a clinical scale transduction procedure for the insertion of HSV-tk/LNGFR genes into T-lymphocytes. Three clinical scale procedures have been carried out to evaluate safety and overall performance. Following donor T-lymphocyte activation, using PHA/IL2 followed by IL2 alone, lymphocytes are transduced in a single pass for 24hrs in the presence of GMP produced SFcMM-3 viral supernatant (Molmed). Following expansion culture in the presence of IL2 the transduced cells are immunoselected. A double label method is employed using GMP grade biotinylated mouse anti-human anti-LNGFR followed by goat anti-mouse anti-biotin labelled microbeads, immunoselection is carried out using the Miltenyi CliniMACS Plus device using the enrichment 1.1 program. The transduction and immunoselection data (Table1) are comparable between the three procedures, all showing excellent post selection cell recovery and purities. The validation procedures have demonstrated the feasibility of producing sufficient, enriched and potentially functional HSV-tk transduced lymphocytes. To ensure that the products are safe to use clinically production has been carried within a quality system encompassing numerous quality checkpoints. All stages are monitored for sterility, cell numbers, viability and immunophenotype, the immunoselected transduced cells are assayed for GCV sensitivity. The protocol has received approval for HSV-tk transduced lymphocytes to be used within a clinical trial.

O267

Homing and survival of thymidine kinase transduced human T-cells in NOD/SCID mice

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Allogeneic bone marrow transplantation is used to cure almost all malignant haematological disease and the graft vs leukemia (GvL) effect has emerged as one of the most powerful aspects of this strategy. However, the inextricable graft vs host disease (GvHD) remains the major cause of transplant related morbidity and mortality. The ultimate aim in attempts to optimize outcome in allogeneic transplant is to modify T cell alloreactivity so as to potentiate the GvL effect to the maximum while at the same time eliminating GvHD and preventing the other related complications. The Herpes Simplex virus thymidine kinase (HSV-tk) gene confers a ganciclovir (gcv)-specific sensitivity to transduced cells, and might help provide a controlled GvL/GvHD. 80×106 human T lymphocytes after transduction with an LSN-tk retroviral vector encoding tk and neomycin resistance (NeoR) genes were injected intraperitoneally in NOD/SCID mice. Engraftment was assessed at day +5 by human CD45+/CD3+ cytometric analysis and NeoR-positive PCR on peripheral blood, bone marrow, liver, thymus and spleen. After 14 days gcv (10 mg/kg/daily) assessment was repeated at day +19. Immunohistological studies with an anti-CD3 MoAb followed by APAAP staining were also performed on spleen and liver on day +5 and on day +19. Cytometric analysis showed human CD45+/CD3+ cells had engrafted and...
immunohistological staining detected human CD3+ cells in the murine spleen and liver. Lymphocytes “home” to the white pulp T cell area and to the red pulp; localization in the liver is prevalently at perportal area. PCR confirmed cytfluorimetric and immunohistological results. After gcv treatment (day +19) cytfluorimetric analysis showed very few CD3+ cells. Scattered human CD3+ cells were detected by immunohistology. PCR identified the transgene in 22% of all tissue samples (only thymic and splenic samples were positive). GvHD did not occur in any animal. These data clearly demonstrated: 1) elevated doses of human CD3+ cells engraft in NOD/SCID mice; 2) after gcv treatment, only few CD3+ cells are detected by cytfluorimetric analysis, immunohistological staining and PCR; 3) these rare lymphocytes, which escape treatment, can be detected in the thymus and in the spleen at day +19 in 22% of tissues samples. Lack of full response to gcv, as demonstrated in this study, may account for cases of GvHD in patients receiving tk-transduced T lymphocytes.

O268

Influence of ex vivo expansion and retrovirus-mediated gene transfer on primary T-lymphocyte phenotype and functions

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Alloreactive T cells present in an hematopoietic stem cell (HSC) graft have both beneficial properties (anti-viral, anti-leukemic and engraftment-facilitating effects) and negative ones, such as the induction of graft-versus-host disease (GvHD). In order to modulate the alloreactivity after HSC transplantation, we have initiated a clinical study aimed at infusing suicide gene-expressing donor T cells and/or cells depleted by a pharmacologic T-Cell-depleting cocktail (Co) cells that had been cultured in parallel with GMC, but non transduced and non selected, as well as with PBMC. Our data show that phenotypical modifications are similar in Co cells and GMC, demonstrating that alterations results from the 12-day culture rather than from the transduction and/or selection process itself. Such modifications include a reversal of CD4/CD8 ratio, activated phenotype (increased expression of CD45RO, CD95 and HLA-DR) and acquisition or increased expression of costimulatory molecules (CD80, CD80 and CD40). This increased expression was associated with an enhanced allostimulating potential of GMC, as compared with resting T cells, when GMC were used as stimulating cells in mixed lymphocyte reactions (MLR). Conversely, when using them as responder cells in MLR, GMC exhibited a strong loss of alloreactivity that was a culture-related event, since it occurred as soon as three days after PBMC activation. The impaired alloreactivity of GMC was not due to alloantigen-mediated, activation-induced cell death upon MLR initiation. Indeed, the percentage of CD3+ apoptotic cells did not increase after activation by irradiated stimulating cells and proliferative response of GMC in MLR was not improved by addition of exogenous blocking CD95 antibody. In conclusion, the retroviral-mediated gene transfer is associated with major phenotypical and functional alterations that result mainly from the ex vivo culture. Thus, future T cell expansion protocols should not only try to improve cell proliferation or gene transfer efficiency, but also try to preserve T-cell functions.

O269

High-dose chemotherapy with hematopoietic stem cell support for germ cell tumors: the Italian experience


Objectives: In the last years, high-dose chemotherapy (HDCT) with hematopoietic stem cell support (HSCT) has become a standard option for germ cell tumors (GCTs) in over 300 patients treated in Europe each year. We report the experience at the Department of Oncology and Hematology in Ravenna with HDCT in patients with GCT.

Patients and methods: Between 1986 and 2000, 99 patients with GCT (91 M, 8 F), median age 29 years, were treated with 126 courses of HDCT with HSCS. HDCT consisted of CE (carboplatin, etoposide) regimen in 28 courses, ICE (ifosfamide, carboplatin, etoposide) in 57 courses, and CarbopEC in 41 courses. Totally, 91 courses was supported by bone marrow transplantation, 33 by peripheral blood stem cells, and 2 by both of them. Eighty-four patients received HDCT as salvage treatment and were stratified as good, intermediate, and poor risk categories according to a validated prognostic index (Beyer et al 1996). Fifteen poor prognosis patients at the diagnosis (ICCCCG staging system) were treated as late intensification of first-line chemotherapy.

Results: Eleven (73%) poor prognosis patients who received HDCT in first-line setting and 28 (33%) patients treated with HDCT as salvage therapy are continuously disease-free. In the good risk group, 24 patients (69%) are continuously disease-free, compared with 4 (13%) in the intermediate risk group (P < .001), and no one in poor risk group (P < .001). Treatment-related mortality occurred in 2 patients in the poor risk (n = 6), and intermediate risk groups (n = 4). Conclusions: In our experience, HDCT induced impressive long-term remissions either in first-line setting or as salvage treatment in good risk patients. Moreover, use of validate prognostic classifications may contribute to better define the role of HDCT besides improving the outcome of patients with GCT. The definitive classification on the possible role of HDCT in GCT will derive from the ongoing phase III randomized studies.

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O270

High-dose BCNU followed by autologous hematopoietic stem cell transplantation in supratentorial high-grade malignant gliomas: a retrospective analysis of 114 patients

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Conventional treatments of high-grade gliomas include maximal neurosurgery resection followed by external radiation therapy. Despite these treatments, the prognosis for patients is poor. Survival of more than one year is exceptional. Using high-doses of chemotherapy might be another way to increase the response rate and the median survival. Increasing doses of BCNU might be more effective but also provokes unacceptable myelotoxicity. This dose-limiting toxicity can be circumvented by using autologous marrow or peripheral blood stem cell rescue. We here report our experience of high-dose BCNU followed by transplantation of autologous hematopoietic stem cells from marrow or peripheral blood in 114 patients with high-grade gliomas.

Of the 114 gliomas, 78 were glioblastoma multiforme (GM) (68%), 24 anaplas astrocytomas (21%), and 12 malignant oligodendrogliomas (OD) (11%). Complete neurosurgical resection was performed for 22 patients (18 GM and 4 AA). The mean age was 44 years. Eighty-four patients received autologous hematopoietic stem cell from bone marrow harvest while 30
patients underwent granulocyte colony stimulating factor followed by apheresis and received peripheral blood progenitor cells (PBPC). High-doses of BCNU (800mg/m²) were given at least one month after neurosurgery. Bone marrow or PBPC were harvested 48 to 72 hours after chemotherapy. Radiotherapy was started approximately 40 days after transplantation. A total of 60 Gy were delivered.

The overall survival (OS) median and time to treatment failure (TTF) median were respectively 15 months and 9 months (p=0.01). The histology type appears to be the main discriminating factor, with the worse prognosis with GM. More specifically in the GM population, the age, the completeness of surgery, and the response rate appear to be important prognosis factors. Seventy-one patients started chemotherapy with a measurable tumour (44 GB, 19 AA, and 8 OD). After a mean of 71 days after radiotherapy, we observed 9 complete responses and 14 partial responses, while the others were stable or in progression. OD tumors had the highest response rates, with seven of eight patients responding.

Thus, this appears to be feasible and without major risks to the patients. The OS and TTF seem to be better than that described for other treatments. However, a larger and randomized study should be performed in order to confirm these results.

O271

Autologous peripheral blood stem cell rescue permits administration of high activities of rhenium-186 HEDP in progressive hormone refractory prostate cancer metastatic to bone


Background: Autologous peripheral blood stem cell rescue was used in patients with progressive hormone refractory prostate cancer metastatic to bone to test the feasibility and toxicity of high activities of a bone seeking radionuclide, Rhenium-186 Hydroxyethylidene Diphosphonate (HEDP), in an a phase I trial. Thrombocytopenia has been identified as the dose limiting toxicity of Rhenium-186 in previous studies. We hypothesised that by increasing the administered activity using peripheral blood stem cell rescue to prevent marrow failure, it would be possible to increase the ionising radiation dose to individual metastases, which might ablate small lesions and reduce new lesion formation.

Methods and Materials: Peripheral blood stem cells were harvested at least 2 weeks before radio-isotope treatment following mobilisation by G-CSF. Stem cells were returned 14 days after Rhenium-186 HEDP. Unacceptable toxicity was defined as grade III haematological toxicity, lasting at least 7 days, or grade IV haematological toxicity of any duration or any serious unexpected toxicity. Twenty five patients with progressive hormone refractory prostate cancer metastatic to bone were treated in a phase I study.

Results: Two patients out of an expanded group of 6 who received 5000MBq experienced grade III thrombocytopenia, which recovered within 10 days in both cases. No unexpected serious toxicity occurred. The platelet nadir occurred at a median of 21 days. An activity limiting toxicity at 5000MBq was defined.

Conclusions: Activities of 5000MBq of Rhenium-186 HEDP are feasible using autologous peripheral blood stem cell rescue in patients with progressive hormone refractory prostate cancer metastatic to bone. The main toxicity is thrombocytopenia, which is short lasting. The potential exists for the delivery of high doses of ionising radiation to individual bone metastases. We have now commenced a Phase II trial to further evaluate response rates.

O272

High-dose chemotherapy in children with nephroblastoma

From April 1992 to December 1998, 27 children with renal tumors received high dose chemotherapy (HDC) with autologous stem cell rescue in Germany, 15 of whom had nephroblastoma and were evaluable. Thirteen boys and 12 girls had a median age of 64 months (11-210) at diagnosis and of 78 months (26-260) at HDC. At diagnosis, 14/25 had stage IV and 15 stage I-III. Histology was standard in 14, high-grade in 5, completely necrotic in 4 and nephroblastoma without further specification in 2 patients. Reasons for HDC were: relapse/progression with initial stage IV (10), second or subsequent relapse (8), bone or brain metastases (2), relapse in irradiated region, extraregional lymph node involvement, unfavourable histology, early relapse and clear cell sarcoma in first remission (1 patient each). At time of HDC, the disease was in CR in 15 and in PR in 10 children. The creatinine clearance at this time was 102 ml/min/1.73m² (mean; SE 43). Conditioning included etoposide 1000 mg/m², melphalan 180 mg/m² and carboplatin in an AUC-taco 1989, 7;1740, in 20 patients. Four children had other melphalan-based regimens without carboplatin, one child was treated with etoposide, thiopeta and cyclophosphamide. There were no treatment related deaths. Progression or relapse occurred in 12 children after three months (median; range 5-75). Overall survival was 58% and EFS 51%. Intermittent hemodialysis was needed for 1 patient. Reversible abdominal serum creatinine was noted in 6, tubular dysfunction in 5 of 21 evaluable patients.

High dose chemotherapy with carboplatin, etoposide and melphalan appears to be a valuable treatment option for patients with high-risk, mainly recurrent nephroblastoma. With adjustment of the carboplatin dose to glomerular function, the risk of kidney damage is limited.

O273

Nonmyeloablative hematopoietic stem cell transplantation for metastatic solid tumors


Objective: Does allogeneic hematopoietic stem cell transplantation (ASCT) have an anticancer effect in patients with solid tumors?

Methods: In total 18 patients, six with colorectal (C1-6), ten with renal (R1-10), one with breast (B1) adenocarcinoma and one with cholangiocarcinoma in liver hilum (L1), and metastases in all, underwent ASCT. Conditioning included Fludarabine 30 mg/m² for 3 days, using sibling donors (C1-6, R3, 7-10 and L1), and 5 repeats. One patient each. The disease was in first remission (1 patient each). At time of HDC, the disease was in CR in 15 and in PR in 10 children. The creatinine clearance at this time was 102 ml/min/1.73m² (mean; SE 43). Conditioning included etoposide 1000 mg/m², melphalan 180 mg/m² and carboplatin in an AUC-taco 1989, 7;1740, in 20 patients. Four children had other melphalan-based regimens without carboplatin, one child was treated with etoposide, thiopeta and cyclophosphamide. There were no treatment related deaths. Progression or relapse occurred in 12 children after three months (median; range 5-75). Overall survival was 58% and EFS 51%. Intermittent hemodialysis was needed for 1 patient. Reversible abdominal serum creatinine was noted in 6, tubular dysfunction in 5 of 21 evaluable patients.

High-dose chemotherapy in children with nephroblastoma

From April 1992 to December 1998, 27 children with renal tumors received high dose chemotherapy (HDC) with autologous stem cell rescue in Germany, 15 of whom had nephroblastoma and were evaluable. Thirteen boys and 12 girls had a median age of 64 months (11-210) at diagnosis and of 78 months (26-260) at HDC. At diagnosis, 14/25 had stage IV and 15 stage I-III. Histology was standard in 14, high-grade in 5, completely necrotic in 4 and nephroblastoma without further specification in 2 patients. Reasons for HDC were: relapse/progression with initial stage IV (10), second or subsequent relapse (8), bone or brain metastases (2), relapse in irradiated region, extraregional lymph node involvement, unfavourable histology, early relapse and clear cell sarcoma in first remission (1 patient each). At time of HDC, the disease was in CR in 15 and in PR in 10 children. The creatinine clearance at this time was 102 ml/min/1.73m² (mean; SE 43). Conditioning included etoposide 1000 mg/m², melphalan 180 mg/m² and carboplatin in an AUC-taco 1989, 7;1740, in 20 patients. Four children had other melphalan-based regimens without carboplatin, one child was treated with etoposide, thiopeta and cyclophosphamide. There were no treatment related deaths. Progression or relapse occurred in 12 children after three months (median; range 5-75). Overall survival was 58% and EFS 51%. Intermittent hemodialysis was needed for 1 patient. Reversible abdominal serum creatinine was noted in 6, tubular dysfunction in 5 of 21 evaluable patients.

High-dose chemotherapy with carboplatin, etoposide and melphalan appears to be a valuable treatment option for patients with high-risk, mainly recurrent nephroblastoma. With adjustment of the carboplatin dose to glomerular function, the risk of kidney damage is limited.
respectively. The cumulative incidence of grades II-IV acute GVHD was 57%. Tumor regression of all metastases was seen in one patient with colon carcinoma. Another patient with colon and 2 with renal adenocarcinoma had regression of lung metastases, but progression of metastases in the liver and/or bone. One patient with renal cell carcinoma, who died of severe aGVHD, showed necrosis of metastases in the lung. Ten patients died; 4 of transplant-related complications, 1 of head trauma and 5 of progressive disease. Eight patients are alive between 3.5 and 13 months.

Conclusion: Following ASCT with reduced conditioning and GVHD, total of 36 metastases were seen in 2 patient with colon and 3 patients with renal adenocarcinoma. The regression of some metastases associated with GVHD provides suggestive clinical evidence that the graft-versus-tumor effect may occur in colon and renal adenocarcinoma.

O274

Allogeneic immunotherapy for solid tumors (ST) : a feasibility trial


Despite recent advances in chemotherapy and other forms of immunotherapy, a large number of pts suffering from ST always die from disease progression. In hematol, allo stem cell transplantation (ASCT) has shown for decades its potential to eradicate malignancies. Due to its high level of TRM, it has however been restricted to some indications. Recent modalities based on chemotherapy reduced intensity regimen prompted us to assess the relevance for ST. Primary goal of this trial was to demonstrate an acceptable toxicity in this field and to optimize procedure. On October 2001, 35 patients with very advanced and heavily pretreated diseases have been included in 8 centers : Renal cell (13); Breast (8); Melanoma (4); Ovarian (4); Colo-rectal (2); Others (5). Median age : 44 (28-60) and M/F : 17/18. 3 pts progressed and died prior to starting conditioning. 32 pts were grafted from an HLA identical sibling (BM :9; PBSC :26). Preparation consisted of Fludarabine (30 mg/m²x6), Busulfan (100mg/kgx8) and ATG (Thymoglobuline : 2.5 mg/kg/d) : Dose of ATG was progressively decreased to lower infections and increase alloreactivity (4 days : 2; 3 days : 6; 1 day : 24). Early course was uneventful. After documented engraftment, and depending upon tumoral evaluation, most of the patients had reduction of post graft CSA with possible subsequent DLI. Overall grade 2-4 GVHD was 31 %. TRM occurred in one patient (GVHD after CSA withdrawal). At time of abstract, response evaluation is still under investigation but at least 3 of the first 14 pts presented a response (renal and ovarian carcinoma (2)). Complete evaluation of transplant course (hematological and immune recovery; chimerism evolution; GVHD evaluation), immunomodulation (CSA variation and DLI) and response are under analysis and will be presented for patients with a minimal follow-up of 6 months. However first conclusions can be drawn: 1) TRM is minimal 2) Pts with very active disease are very unlikely to be controlled by this approach: pre graft tumor kinetic must be slowed down 3) Conditioning immunosuppression can be still lowered and we are presently decreasing the dose of Fludarabine 4) Early post graft immunomodulation is crucial (notably for CSA diminution) to reach early and good level of antitumor alloreactivity. 5) Not all diseases seem to have the same level of sensibility to this approach (high : Renal, ovarian and colorectal Ca; low : melanoma).

O275

A validated, reproducible and sensitive quantitative assay for the detection of residual B Cells after purging of stem cell collections using the ELIGIX(tm) B cell -SC cell separation system

S. Martin, R. Schmittling, K. Pennline, R. Monroy (Brentwood, Bedford, USA)

Cell separation technologies have been developed to purge B cells from autologous stem cell (SC) products to rescue non-Hodgkin’s lymphoma (NHL) patients after high dose chemotherapy. A PCR assay for bcl-2/IgH, t(14;18) rearrangement is frequently used to detect residual malignant cells after purging; however, not all SC products of NHL patients are evaluable by PCR. Furthermore, in existing validated PCR assays, the limit of detection ranges between 1 and 10 tumor cells per 106 cells. A predominant BCell surface marker presentation NHL tumor cells is CD20. Therefore, an immunocytochemistry (ICC) assay directed against CD20 was developed using the ChromaVision Automated Cellular Imaging System (ACIS) which provides quantitative analysis of residual B cells in SC products after purging with the Eligix (superscript: TM) BCcell-SC Cell Separation System. Using SC products, pre and post B cell purging, large format cytospin slides were prepared using 1.2 x 105 cells each, where a mean of 6.1 x 105, 1.2 x 104 cells (N=147) were counted. Staining was optimized to discriminate individual cell morphology, proper chromagen intensity, and minimal background stain. The total number of cells counted on a slide was validated based upon ACIS and estimated manual cell counts. The analyses were shown to be reproducible within multiple re-counts and over time. Slides were prepared containing a titration of residual B cells and analyzed. We demonstrated a linearity of detection for residual B cells over the range of 200 to 0.3 B cells per 106 total cells, establishing the limit of detection at 0.3 B cells /106 cells. To insure reproducibility and sensitivity, multiple slides (7-10), collectively containing > 3 x 106 cells, were prepared for each sample. Since the assay was developed for rare event detection, the accuracy is resolved at higher B cell frequencies >0.5%. The ICC assay has been successfully used to detect residual B cells after purging of SC products from normal volunteers and patients demonstrating 3-6 log B cell depletions. An important valued asset of the assay is its ability to quantitate B cells in patient SC products prior to purging, where the B cell frequency is below the detection limits of flow cytometry. This enables quantitation of the efficiency of B cell purging. We have demonstrated a validated ICC assay that can quantitatively detect as few as 0.3 B cells per 106 cells and can be readily applied to the analysis of residual B cells for all NHL patient stem cell products purged of B cells.

O276

Selective allodepletion by immunomagnetic sorting can reduce alloreactivity in vitro, can be done at a clinical scale and preserves in vitro activity against CMV and EBV.


Graft versus host disease (GvHD) is a cause of significant morbidity and mortality in haematopoietic stem cell transplantation. In the HLA matched setting GvHD can be effectively prevented by pan T cell depletion, but the profound level of T cell depletion required to do so in non-HLA matched transplantation leads to an unacceptable delay in immune reconstitution and an increase in post transplant infections. We have developed a method of selective depletion of alloreactive cells based on the coculture of irradiated recipient mononuclear cells and donor cells in a modified mixed lymphocyte reaction (mMLR). Alloreactive donor cells are identified by their increased expression of the early activation antigen CD69 and these cells
are depleted by immunomagnetic sorting. Five unrelated donor-recipient pairs (3 HLA identical, 2 with a C-locus mismatch) were cocultured in a mMLR. Four pairs exhibited a clearly positive mMLR (Activity >5%, Bishara.) Efficient depletion of alloreactive cells was achieved with Dynal beads and CD69 antibody and a consistent reduction in mMLR activity in 3 of these 4 pairs (Table 1). The efficacy of this system at a clinical scale was demonstrated using HLA non-identical single donor buffy coats as donor-recipient pairs, processing 200x106 cells. Depletion efficiency of alloreactive T cells using CD69 antibody and Dynal beads on the Isolife 300/v2.5 was greater than 90% with a yield of non-alloreactive T cells in excess of 75%. Retention of anti-viral activity in the non-alloreactive fraction has been demonstrated by the use of tetrameric HLA-peptide complexes and ELISPOT assays. The CD69 alloreduction strategy retained over 80% of the specific anti-CMV and all of the anti-EBV reactivity, suggesting that grafts manipulated in this way might protect from CMV reactivation and EBV associated post transplant lymphoproliferative disease. The non-alloreactive fraction might be expected to cause less GVHD but retain broad immune reactivity and thus enhance reconstitution of immunity to a variety of pathogens in the post-transplant period. We believe that this strategy is an effective way of prevention of GVHD post transplantation and a Phase 1 clinical trial is currently underway, preliminary results of which will be presented at this meeting.

### Table 1

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<tr>
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<td>2.7</td>
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**O277**

**Campath 1-H 'in the bag' for T cell depletion in allogeneic peripheral blood progenitor cell transplantation from matched family and unrelated donors reduces both acute and chronic GVHD and limits transplant-related mortality**  
S. Chakrabarti, C. Fegan, D. Milligan (Birmingham, UK)

There is increased concern about an increase in chronic graft-versus-host disease after allogeneic PBPC transplants, which could limit its use despite potential advantages like faster engraftment and lower relapse risk. We evaluated the outcome of 32 allogeneic PBPC transplants (AML 12, ALL 9, CML 10, MM 1) from matched family donors [MFD] (n=24) or unrelated donors [MUD] (n=8) following T cell depletion. Campath 1-H was added to the apheresis product at a dose of 10 mg (9 MFD) or 20 mg (15 MFD and 8 MUD) for T cell depletion and incubated for 30 min before infusion. The conditioning treatment consisted of TBI with cyclophosphamide or etoposide, except one patient who received Bu-Cy. There was no difference between the MFD and MUD groups in terms of age or gender. More patients in the MUD group had advanced disease; 5/8 vs 5/24. The CD34+ cell count was similar in both groups (median 5.1x106/kg). All MUD patients and 23/24 MFD patients achieved durable engraftment. The median time to achieve neutrophil and platelet engraftment were 14 days and 12 days for both groups. Acute GVHD grade 1-2 occurred in 4/24 patients in the MFD group and 3/8 in the MUD group with no grade 3-4 GVHD in either group. Chronic GVHD occurred in only one patient in each group. The incidence of CMV reactivation was low in the MFD group (4/12 at risk in MFD group and 2/2 at risk in the MUD group) with 2 developing CMV disease. The median total lymphocyte count and CD4+ T cell count at 100 days was 1000/mm3 and 287/mm3 for patients receiving 10 mg Campath and 700/mm3 and 180/mm3 for patients receiving 20 mg Campath, with no difference based on the donor type. Life-threatening infections were documented in 4/24 in the MFD group and 2/8 in the MUD group. The probability of 100 day and overall TRM was 25% and 28% for MUD and 5% and 18% for MFD patients. The actuarial survival at 18 months was 63%, with no significant difference between the groups. The risk of molecular/cytogenetic relapse amongst CML patients was 90% in both groups, but 90% of them achieved a remission with donor lymphocyte infusion (DLI). Relapse-risk was 30% for non-CML patients and this was 60% for advanced diseases and 25% for good-risk diseases. Thus T cell depletion with ‘Campath in the bag’ approach was associated with durable engraftment and low incidence of GVHD even in the MUD group. The immune reconstitution was not delayed even in the MUD patients and the TRM was low in the MFD group.

**O278**

**CD34+ cell selection of peripheral blood progenitor cells for allogeneic transplantation using the CliniMACS device: results of a cohort study of 84 patients**  

Graft versus host disease (GVHD) remains the major complication contributing to the mortality and morbidity associated with allogeneic bone marrow or peripheral blood progenitor transplantation. Several strategies have been employed to counteract this problem including the use of new immunosuppressive agents (MMF, FK506) or T cell depletion of the graft. In the present single centre study we investigated the effects of CD34+ cell selection of allogeneic grafts in 84 patients (pts) with a high risk of GVHD (haploidentical, mismatched or MUD grafts, age > 50 y). Half the cohort was at high risk of relapse (n=44, AML >1CR, CML >1cP, lymphoma >1CR). The pts suffered from AML (n=32), CML (n=25), ALL (n=6), multiple myeloma (n=6), MDS, NHL, PNH (n=3), T-PLL (n=3), OMF, SAA and thalassaemia (n=1), the median age of the group was 44.2 y at the time of transplantation. The conditioning regimen consisted of TBI 12 Gy or Busulfan 12.8 mg/kg i.v. plus CTX 120 mg/kg, some patients were treated with additional Thiotepa 10 mg/kg or a radiolabeled anti-CD66 antibody. Pts at high risk of graft rejection received additional ATG. GVHD - prophylaxis consisted of CD34+ selection alone in 56 pts, 28 pts were given additional CyA. The CliniMACS procedure yielded 8.0 x 10^6 CD34+ cells/kg and the number of residual T cells was 1.5 x 10^4/kg (median). The endpoints of the study were engraftment, GVHD, infectious complications and overall survival. Four graft rejections (4.8%) were observed. The incidence of severe acute GVHD was very low with no pt developing more than grade II disease. The actuarial risk of chronic GVHD was 25% for the pts transplanted from a matched related donor as compared with 76% of the pts receiving a graft from a MUD. CMV reactivation occured in 33/52 (67%) of CMV seropositive pts. After a median follow up of 12 months the probability of survival is 62%. Treatment failure was due to TRM in 30% and relapse in 8% of the pts. The main causes of death were infections (51.5%), in particular aspergillus (n=6), CMV pneumonia (n=2) and EBV-LPD (n=2). As a summary, CD34+ selection of peripheral blood progenitor cells by the CliniMACS device is highly effective in preventing acute GVHD with an acceptable risk of graft rejections. Additional immunosuppression appears to be required for pts at high risk of chronic GVHD. The risk for infectious complications in these pts should be minimized by prolonged PCR based monitoring and preventive treatment strategies.
CD34+ yields in bone marrow harvests from 222 volunteer bone marrow donors
R.S. Kaczmarski, P.J. Gravett, R. Jan-Mohamed, M. Monroe, P. Naik (London, UK)
It has been hypothesised that the quality of stem cell product deteriorates with increased volume of marrow collected during bone marrow harvesting. We investigated the stem cell yield in 222 volunteer donors undergoing bone marrow harvests at a single centre. Data refers to 146 males and 76 females of mean age 36.4 years. Marrow was harvested from bilateral iliac crests. The mean values of bag 1 (n=222) were volume 445 mls, WBC 27.6 x 10^9/L, CD34+ 1.08% and CD34+ absolute 1.37 x 10^9. For bag 2 (n=217) the mean values were volume 442mls, WBC 19.0 x 10^9/L, CD34+ 1.05% and CD34+ absolute 0.69 x 10^9. For bag 3 (n=150) the mean values were volume 426mls, mean WBC 16.3 x 10^9/L, CD34+ 1.05% and CD34+ absolute 0.9 x 10^9. On multivariate analysis there was no statistical difference in CD34+ % between bags (p=0.513), however the fall in absolute CD34+ was significant (p=0.047).

We conclude that the proportion of CD34+ cells remains constant throughout the procedure, and the fall in CD34+ yield with increased harvest volume is related to the drop in marrow WBC counts. Nevertheless even the haemodilute product contributes significantly to the stem cell yield.

Leukapheresis tumor cell contamination in neuroblastoma patients is associated with poor bone marrow disease response
L. Faulkner, A. Garaventa, C. Marchi, V. Tintori, L. Lacitignola, F. Bambi, G. Bernini, B. De Bernardi (Florence, Genoa, I)
Gene marking experiments have demonstrated that neuroblastoma (NB) cells reinfused with autologous hematopoietic grafts to replace missing bone marrow (MBM), do not give rise to tumor relapse. However, in our recent series of 30 patients, the presence of detectable tumor cells in leukapheresis (Lkph) was shown. We analyzed LKph from 30 patients with high-risk metastatic NB treated in different centers participating in the Italian neuroblastoma group study NB 97. Cell-sorting enhanced NB-specific immunocytochemistry (VarioMACS, Miltenyi with 3F8 anti-GD2 primary monoclonal antibody; sensitivity 1 in 107-108 leukocytes) was employed to evaluate leukapheresis contamination. End-induction bone marrow disease was evaluated by direct anti-GD2 immunocytoLOGY (sensitivity: 1 in 105-106 leukocytes).

Results: Of 30 leukapheresis in which at least 3x10^7 total cells were evaluated (range 3 to 27 x10^7), 7 (23%) had detectable tumor cells; all patients with positive leukaphereses had also a positive end-induction bone marrow (p=0.001 by Mann-Whitney t test). At a median follow up of 34 months, the PFS in patients with positive or negative leukapheresis was 0% and 29% respectively (logrank p=0.0263, hazard ratio 2.557, 95% CI 1.173-12.83). Multivariate analysis showed a significant association between tumor cell contamination and the diagnosis of end-induction bone marrow disease.

Conclusions: Leukapheresis positivity in neuroblastoma patients seems to be strongly associated with persistent bone marrow disease. This may imply that even if contaminating tumor cells are tumor-negative, their detection in leukapheresis may simply reflect poor treatment response and persistence of substantial amounts of metastatic disease. In the context of current standard treatment strategies for high-risk NB the issue of ex-vivo leukapheresis purging may be far less important than chemotherapy responsiveness and metastatic disease clearance, in fact none of the patients with <10 NB cells per 106 bone marrow mononuclear cells at end-induction had detectable tumor cells in their leukaphereses.

O280
Leukapheresis tumor cell contamination in neuroblastoma patients is associated with poor bone marrow disease response

O281
Haplo-identical peripheral blood stem cell transplantation (Haplo-PBSCT) in children with high-risk hematological disease: the Leiden experience
Objective: to preliminary review the outcome, morbidity and mortality associated with recent paediatric haplo-PBSCT in a single treatment centre in the Netherlands (1998-2000).
Methods: A retrospective analysis of prospectively collected data was undertaken. 11 children (8 m, 3 f) age 1-15 years (median 8y) underwent 14 transplants from parental donors. 4 children received 2 transplants. Indications were: ALL: 5; MDS: 3; ANLL: 1; FEL: 1; SCID: 1. Pre-transplant conditioning varied according to diagnosis. GCSF donor mobilisation and COBE Spectra leukapheresis with CliniMACS separation resulted in highly purified CD34+ stem cells for infusion. No additional GVHD prophylaxis was given.
Results: all 10 male (29-45 years) and 4 female (34-43 years) donors were successfully mobilised / leukapheresed with exchange volumes 14-50 L. (median 23) during a 311-1054 min. (median 364) procedure. 7 required autologous platelet transfusion due to low post-pheresis values: 37-214x10^9/L (median 76). Aiming for high levels of CD34+ cells, 11/14 PBSCT contained more than 15x10^6/kg with 5/14 receiving more than 20x10^6/kg recipient weight (range 10.1-23.1. av. 13.5). Donor T-cells infused varied (CD3+ cell infused: 0.2-5x10^4/kg. av. 1.3) 100% donor engraftment in 9/14 transplants was documented. Mixed chimerism in 3/14, with 2 failures of engraftment and 3 rejection episodes (+23-+50 days). Successfully engrafted patients received an average of 19x10^6/kg CD34+ cells. Haemopoietic reconstitution in those patients was uncomplicated: ANC> 0.5x10^9/L, av. 17.8 (13-26) days: platelets> 100x10^9/L, av. 30.9 (19-50) days. 5/11 (46%) are disease free with stable engraftment (follow-up 15-42 months). Deaths were due to infection (4) and leukemic relapse (2). No acute or chronic GVHD was evident.
Conclusions: 1. In children without a fully matched donor, haplo-PBSCT is feasible with acceptable morbidity and mortality with 46% disease free survival in our cohort.
2. Engraftment failure is the most significant adverse outcome.
3. GVHD did not occur in our patient group.

O282
Lineage specific chimerism in FACS-sorted leukocytes after allogeneic stem cell transplantation in children
S. Matthies-Martin, G. Fritsch, T. Lion, O. Haas, C. Peters, A. Lawitschka, H. Gadner (Vienna, A)
Chimerism of sorted cell populations (CD3+/8+, CD3+/4+, CD3-/56+, CD14+ and CD15+) was monitored prospectively between day +14 and +100 in 100 pediatric patients (pts) undergoing allogeneic stem cell transplantation. Pts with SCID, pts with primary graft failure and pts who died prior to day +50 were excluded from the analysis. A total of 83 transplants in 76 pts was evaluated. Underlying diseases were acute leukemia (n=38), chronic leukemia (n=10), inborn error (n=22) and SAA (n=6). Pre-transplant conditioning was myeloablative in 52 cases whereas 31 pts received reduced intensity conditioning. Donors were MSD (n=21), MUD (n=43) and haploidentical family donors (n=19). T-cell depletion by CD34+ positive selection was performed in 43/83 cases. All cell subsets exceeding 1% of NC were targets for cell sorting; between 1000 and 15000 target cells were sorted for
subsequent chimerism analysis. Dual-colour FISH was performed on specimens from pts transplanted from sex-mismatched donors; in the other pts chimerism was tested by recipient- and donor specific STR-PCR. Leukocytes of recipient genotype in one or more cases occurred in 20/52 (38%) pts after conventional myeloablative conditioning and in 23/31 (74%) pts following reduced intensity conditioning. In pts who had undergone reduced intensity conditioning regimens, mixed chimerism (MC) or recipient chimerism (RC) in CD14+ cells and CD15+ cells was more frequent than in those who had received myeloablative conditioning regimens, but there was no difference in their HLA-DR typing. There was a strong correlation between T-cell depletion and MC/RC in CD3+ and CD56+ cells; MC/RC of CD56+ cells was detected in 9/23 (39%) pts with T-cell depleted grafts vs 4/20 (20%) pts with T-cell replete grafts, and MC/RC of CD3+ cells was present in 21/23 (91%) pts with T-cell depleted grafts vs 4/20 (20%). Mixed chimerism was followed by late rejection in 10/43 pts: 8/36 (22%) pts with MC/RC in CD3+/B cells, in 9/33 (27%) pts with MC/RC in CD3+/A cells and in 8/17 (47%) pts with MC/RC/CD56+ cells. All pts with RC in CD56+ cells between day +14 and +35 rejected their graft. In 9/10 pts who experienced late rejection, CD14+ or CD15+ cells were 100% donor genotype between day +14 and +35. T-cell depletion seems to have a higher impact on T-cell chimerism than conditioning modality. Recipient chimerism of CD56+ cells seems to be highly predictive for late graft rejection.

O283

Marked improvement over time in the outcome for children with acute lymphoblastic leukemia in second remission given hematopoietic stem cell transplantation from unrelated donors

F. Locatelli, M. Zecca, G. Dini, R. Rondelli, C. Messina, N. Sacchi, C. Uderzo, F. Fagioli, V. Conter, C. Favre, G. Giorgiani, F. Bonetti, F. Porta, A. Pession on behalf of the AIEOP-BMT Group

Purpose of this study was to verify if improvement in the outcome of 63 children with acute lymphoblastic leukemia (ALL) in 2nd CR given allogeneic hematopoietic stem cell transplantation (HSCT) from unrelated volunteers has occurred over time. Moreover, we investigated the role of other variables on relapse, transplant-related mortality (TRM) and event-free survival (EFS). We compared the results obtained in 26 children given HSCT before January 1998 with those of patients transplanted beyond that date. We chose 1998, since in that year high-resolution molecular typing for HLA class I and II antigens became available in most centers. In all donor-recipient pairs, compatibility was determined by serology for HLA-A and -B antigens and by high-resolution DNA typing for DRB1 antigen, whereas high-resolution molecular typing of HLA class I antigens was employed in 20 of the 37 children transplanted after January 1998. A preparative regimen containing total body irradiation was employed in 58 patients, whereas the remaining 5 children were given a chemotherapy-based treatment. Cyclosporine (Cs-A) and short-term methotrexate were used for GVHD prophylaxis in 11 children, whereas the remaining patients received the combination of Cs-A, MTX and serotherapy. Probability of both acute and chronic GVHD was comparable in the 2 groups. The 6-month TRM probability was 40% (21-59) and 17% (5-30) for children transplanted before and after January 1998. In multivariate analysis, children transplanted before January 1998, those with T-lineage ALL and those with grade III/IV acute GVHD had a significantly higher relative risk of TRM at 6 months after transplant. Relapse rate was not statistically different between the 2 groups and it was unfavorably affected by a time interval between diagnosis and relapse less than 30 months. Overall, the 3-year cumulative probability of EFS was 40% (25-56) for the whole cohort of patients studied. The 2-year probability of EFS for children transplanted before and after 1998 was 27% (10-44) and 58% (42-75), respectively (p=0.02) and the year of transplantation remained significant in multivariate analysis. Children belonging to S2 group according to the BFM classification for patients with relapsed ALL had a better outcome. We conclude that outcome of unrelated donor HSCT in children with ALL in 2nd CR has remarkably improved in the last few years, mainly due to refinements of HLA-typing, GVHD prophylaxis and supportive care.

O284

Repetitive megatherapy cycles with autologous PBSC support in children with high-risk solid tumors


Aims: Use of repetitive MGT cycles to improve outcome in children and young adults with high-risk solid tumours

Patients and Methods: Between July 1995 and April 2001, 38 patients (pts)/20 males, 18 females underwent a total of 7 MGT courses in 4 Austrian institutions. Diagnosis (Dx) was neuroblastoma (NBL) (18 pts: 17 disseminated at Dx, 1 relapse)); Ewing’s tumour (ET)/(10 pts: 5 metastatic pts at Dx, 3 high risk local disease.2 relapse pts), rhabdomyosarcoma (RMS) (7 pts: 5 metastatic at Dx, 2 relapse pts) and osteosarcoma (OSA) (3 pts at relapse). Median age at first MGT was 7.6 yrs (range, 1.4 to 28yrs). Median time from Dx to 1st MGT was 6 mo in first line pts and 2Gy since Dx in 2nd line pts. Response status prior to 1st MGT was determined in 10 pts (9 CR/2 CR2), very good partial remission in 15 pts. 12 pts in partial remission (7pts PR1/5 pts in PR2), one with resistant relapse. Results: Median number of G-CSF prior to harvest was 9 days , median number of PBSC apheresis cycles was 3; Median number of reinfused CD34 cells /kg body weight per MGT was 6.3 (range, 0.45-20.4). Overall survival (OS) rate at 3years was 62 % in first remission pts and 38% in relapse pts. Bone tumours (ET and OSA) had a 3 yr OS rate of 41% while NBL and RMS reached 65%. Toxic deaths occurred all after a second MGT course in 4/38 pts (11%) or in 4 of 79 MGT courses (5%) related to septicemia followed by multiorgan failure (2 had VOD).

Conclusions: The TANDEM approach was feasible and well tolerated (low VOD rate) reaching a promising overall survival rate in this high risk population, while the TRIPLE approach proved too toxic after intense pediatric chemotherapy regimens prior to MGT.

O285

Prolonged CD3+/CD4+ and CD4+CD45RA+ cell depletion after autologous hematopoietic cell transplantation (AHCT) in children: analysis of factors influencing the immune reconstitution


Data on immune reconstitution after autologous haematopoietic cell transplantation (AHCT) in children is poor and majority of studies are based on low numbers of pts and short observation time. Therefore immune reconstitution was studied prospectively in 49 children with haematologic malignancies (HM; n=30) or solid tumors (ST; n=19), who underwent 54 AHCTs (49 single and 5 tandem transplants). The aim of the study was the prospective analysis of the immune reconstitution with regard to potential factors affecting its speed, incl. age, diagnosis, CD34+ cell dose and the use of interleukin-2 post transplant. Absolute counts of different lymphocyte subsets were determined in PB of pts pre-transplant, on day +16 and at 1, 2, 3, 4, 6, 9, 12, 18 and 24 months post transplant.
We observed: 1. Transient CD4+CD45RO+ peripheral T cell expansion on day +16 resulting in normal CD4+/CD8+ ratio; 2. Prolonged CD3+CD4+ (Th) and CD4+CD45RA+ cell depletion until 12 months post AHCT; 3. Inverted CD4+/CD8+ ratio from day +30 until 2 years post AHCT. 4. Sustained NK-cell and CD3+CD8+ T-cell count normalisation from 1 month post AHCT onwards; 5. B-cell count > 5th percentile of age-matched population at 3 months post AHCT; 6. CD4+CD45RA+/CD4+CD45RO+ ratio normalisation late at 2 years post AHCT; 7. Th-, NK- and B-cell lymphopenia before transplant. Age >10 years correlated with poorer CD4+ (incl. CD45RO+)-cell and total lymphocyte count (TLC) recovery (<0.05 from day +120 onwards). There was no relationship between diagnosis and T- or NK-cell recovery except for trend towards higher B-cell count in pts with HM from 6 to 12 months post AHCT. Higher CD3+ cell count correlated only with higher TLC and B cell count at 3 months post transplant. IL-2 administration resulted in sustained and prolonged higher NK- (but not T- or B-) cell count during therapy. Our results confirm severe prolonged age-related Th- (incl. CD45RA+)-cell recovery impairment in children after AHCT. This phenomenon might be due to cytotoxic effect of pre-transplant chemotheraphy both on prethymic progenitors resulting in poor graft quality and on aging thymus itself. This profound T-cell depletion increases the risk of infections and impairs minimal residual disease control. One solution is PBSC harvest earlier in the course of disease or at diagnosis when possible, another the use of agents, that enhance not only NK-cell (i.e. IL-2) but also T-cell regeneration (i.e. IL-7).

Impact of unrelated donor BMT on outcome for unselected children with acute lymphoblastic leukemia in second remission for whom a search for an unrelated marrow donor was activated


So far all studies on UD-BMT reported the outcome of selected cohorts of patients who actually received an UD-BMT. The purpose of this study was to evaluate the impact of UD-BMT on outcome for the unselected cohort of children with ALL in 2nd CR, for whom the search for an UD was activated. Between June 1989 and April 1998 the search for UD was activated for 167 consecutive children with ALL in 2nd CR in our institution. The cumulative probability of finding an UD was 30%. Nine out of the 70 patients who had an UD did not undergo transplant due to medical reasons; 60 other patients were given UD-BMT during 2nd CR (46) or in a more advanced phase at a median of 6 months from 2nd CR of the disease. Out of the 97 patients who did not find a suitable donor, 39 underwent other types of SCT during 2nd CR (17) or in a more advanced phase (22) at a median time of 6 months from 2nd CR. The remaining 57 children proceeded with second line chemotherapy, most of them experienced a 2nd CR and only 4 (7%) survive. The estimated 3-year DFS from the date of transplant for the 60 children who actually underwent UD BMT was 32.6% (SE 7.4). The 3-year DFS and survival from 2nd CR regardless of treatment received of the unselected series of 167 patients was 15.1% (SE 2.7) and 24.5% (SE 3.3), DFS and overall survival by treatment received in 2nd CR or in subsequent phase, adjusted by the waiting time to transplant, did not show any difference.

We conclude that 1. our results in the selected cohort of patients given an UD BMT in 2nd CR compare favourably with most published reports for children given an UD-BMT before 1998; 2. there is no significant impact of UD BMT outcome for the unselected cohort of children for whom a search for an UD was activated; recurrent disease was the major obstacle to the success of the search.

Absence of acute graft versus host disease after transplantation of purified CD34+ cells with add-back of ten million per kg T-cells

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Five patients (1 CML in second chronic phase, 2 refractory anemia, 1 T-ALL in CR2 and 1 bcr/abl positive ALL in CR2) were transplanted with G-CSF mobilized peripheral blood stem cells from HLA-matched unrelated donors (n = 4) or matched sibling donor (n = 1). Median of age was 13.8 years (7 to 16 years). Conditioning regimens were performed according national therapy protocol guidelines. At the day of transplant patients received a median of 10.6 (4.5 to 22.2 x 10^6) purified CD34+ stem cells and a aliquot of unmanipulated apheresis product containing 10 x 10^6 T-cells per kg. GVHD prophylaxis consisted of cyclosporine A and short course methotrexate (d1, +3, +6). Engraftment was rapid with a median of 16 days in three patients. Two patients failed to engraft at first. However, full donor chimerism and stable engraftment was achieved in both patients after cessation of cyclosporine A treatment and an additional stem cell boost without any reconditioning. None of the five patients developed acute GVHD. The median follow-up was 28 months (8 to 104 months). Four are alive and well. One patient with bcr/abl positive ALL and delayed engraftment died of adenoviral hepatitis and pneumonia. We conclude that add-back of 10 x 10^6 T-cells per kg in combination with CSA and short course MTX appears to be a safe dosage regarding acute GvHD in the setting of alogeneic PBSCT from unrelated or related donors.

Allogeneic bone marrow transplantation for relapsed childhood acute lymphoblastic leukemia: outcome of patients with matched sibling donors compared to patients lacking donors

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Objective: The role of bone marrow transplantation (BMT) for children with acute lymphoblastic leukemia (ALL) following first relapse remains controversial. We evaluated the outcome of 56 consecutive children with relapsed ALL who were treated on uniform intensive initial and relapse protocols in a single institution. We assessed the impact of offering BMT to children relapsing within 3 years to those with a matched family donor (MFD).

Patients and methods: We evaluated the outcome of 56 children relapsing following uniform initial intensive treatment protocols in our institution between 1990 and 1997. The patients were commenced on a single intensive chemotherapy regimen (New York II). They were tissue typed and all patients with a MFD were recommended to receive BMT. Survivors have been followed for a median of 6.8 years. Patients with and without MFDs were compared to determine the effect of BMT on the outcome. Donor versus donor comparison is recognised as a form of analysis that minimises bias in the evaluation of the effect of BMT.

Results: The outcome of patients with a MFD was significantly better than in patients without a donor. The overall survival at 8 years was 60.0% (95% CI 35.7%-77.6%) and 13.5% (95% CI 4.0%-28.6%) for patients with and without a MFD respectively (Logrank chi= 7.50 p=0.0062). The event free survival at 8 years was 55.0% (95% CI 11.1%-31.3%) and 9.2% (95% CI 2.0%-23.3%) for patients with and without MFDs respectively (Logrank chi= 8.87 p=0.0029).

The result of multivariate analysis controlling for potential confounding factors confirmed the survival advantage of BMT. There was no statistically significant difference in survival for patients initially relapsing early (within 3 years of first remission) compared to children relapsing late (beyond 3 years). The advantage of BMT for children relapsing within 3 years was statistically significant. The statistical evaluation of patients relapsing beyond 3 years was limited by small sample size.
Late Effects and Quality of Life

O289
Age impact on mortality and morbidity of autologous PBSC transplantation

Introduction. Age is commonly considered as a factor of failure in peripheral blood stem cells (PBSC) autologous transplantation. However, few data are actually available on peripheral blood stem cells (PBSC) autologous transplantation in old patients.

Patients and Methods. Between January 1993 and December 1999, 125 consecutive patients received chemotherapy followed by autologous PBSC transplantation for multiple myeloma (MM) and for non Hodgkin lymphoma (NHL). According to age, patients characteristics at diagnosis and transplant are shown in the following table. For each patient we assessed severe organ toxicity (OMS grade 3 or 4) and toxic death.

Results. In univariate analysis, age as continuous variable was a significant prognostic factor for transplant-related (TR) morbidity (p=0.02), overall survival (OS) (p=0.05) and there was a trend for TR mortality (p=0.06). However, this effect of age was related to a better outcome of the youngest patients (<45 years). Rates of toxic death, severe organ toxicity and OS were similar for patients 45-60 years and over 60 which displayed similar characteristics at transplantation (Table).

O290
Risk to develop Bronchiolitis obliterans with organizing pneumonia after allogeneic transplantation

The occurrence of bronchiolitis obliterans with organizing pneumonia (BOOP) was prospectively compared in patients with malignancies, hematologic disorders, who were transplanted SCT. Between October 1994 and July 2001 with peripheral blood stem cells (PBSC: n=321) or bone marrow (BMT: n=298) from HLA matched sibling (MRD: n=270), partially matched family (n=98) or matched unrelated donors (MUD: n=251). Grafts were not manipulated, and patients having received dose reduced conditioning regimens were excluded.

With a median follow up of 17 months (range 0 - 86 months), a total of 16 patients developed BOOP median 11 months (range 4.6 - 56 months) post transplant. Four patients had no other adverse event. Fourteen of the 16 patients were male and all patients were grafted from a female donor. Two patients died of complications of the lung disease 27 and 34 months post transplant respectively.

Conclusion: BMT was found to provide a clear survival advantage for children following their first relapse of ALL. We recommend BMT to all children following relapse if a MFD is available.

O291
Growth and endocrine late effects after stem cell transplantation (SCT) in children and adolescents
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Objective: Evaluation and treatment of late effects on growth and endocrine function in long term survivors of SCT.

Patients and Methods: 410 patients treated with SCT from 1996 to 2001 were investigated. The underlying diseases were ALL 21%, AML 13%, CML 7%, MDS 8%, lymphoma 4%, aplastic anaemia 8%, neuroblastoma 11%, solid tumours 15% and others 13%. A standardised follow-up protocol was applied: Height, weight, pubertal development and body proportions were determined. Analysis of TSH, T4/T4, LH/FSH, E2/T, IGF-I/IGFBP-3 and in addition TRH, GnRH and arginine testing was performed. Patients were examined before and 3 and 6 months, 1, 2, 3, 4 and 5 years after SCT.

Results: Height (HT)-SDS before SCT was significantly, p<0.005, reduced at -0.5 SDS (median) (-4.0 to 2.7, range) versus target HT at 0.11 SDS (-2.4 to 2.2) (N=145). HT-SDS significantly decreased further to -0.7 SDS (5.0 to 2.9), p<0.05 (-4.2 to 0.2), p<0.05. Individual changes in HT-SDS within 1 year ranged from -2.0 to +1.9 SDS. Longitudinal data showed that patients with ALL, AML, CML, NB lost HT-SDS, whereas patients with SAA and MDS gained HT-SDS in the follow-up period. Analysis of prepubertal growth revealed 5 growth patterns: regular growth, progressive growth failure, primary/secondary catch-up growth and initial growth failure followed by regular growth. Primary hypothyroidism was seen in 13% (23/172) before and in 19% (44/235) after SCT. Central hypothyroidism was confirmed by TRH tests before SCT in 21% (25/119) of patients with a ‘normal’ TSH and T4/T4 levels in the lowest third of normal. After SCT, 65% (124/191) of patients had low T4 and/or T4 levels and a ‘normal’ TSH, possibly related to hidden central hypothyroidism. Hypergonadotropic hypogonadism was detected in 22% (14/64) female (f) and 18% (26/147) male (m) patients before SCT. GnRH tests revealed a much higher rate of gonadal dysfunction: 50% f and 28% m. Longitudinal data in 28 f and 73 m patients showed normal LH/FSH levels before and elevated levels after SCT in 29% (f) and 18% (m).

Conclusions: Growth failure and endocrine deficiencies are very frequent after SCT. The right diagnostic tools have to be applied to newly developing dysfunction to ensure normal development each patient has to be followed individually. In cooperation of the paediatric haematologist and paediatric endocrinologist investigations must be performed according to a standardised protocol.
Development of an EORTC questionnaire module for assessment of quality of Life during high-dose treatment

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The objective of this project is to develop a treatment specific quality of life (QL) questionnaire module to be used in clinical trials with the EORTC core cancer questionnaire (EORTC - QLC30), for assessing side effects and psycho-social problems of patients undergoing high-dose treatment with haematological stem cell transplantation, including allogeneic bone marrow transplantation (BMT) and autologous BMT or peripheral stem cell transplantation (PSCT). The module is developed according to EORTC QL Group Guidelines, simultaneously in English (UK), German (Germany), Italian (Milano, Italy), French (France), Dutch (Utrecht), Spanish (Madrid, Spain), Hungarian (Budapest) admitted for autologous (n = 101) or allogeneic (n = 15) or peripheral stem cell transplantation (PSCT). Patients and Methods: 185 adult patients (109 from Vienna, 76 from Basel) admitted for autologous (n = 101) or allogeneic (n = 84) SCT were evaluated prospectively by standardized psychometric questionnaires (HADS, POMS, MAC) prior to SCT.

Conclusions: Psychosocial factors are independent risk factors for outcome. Helplessness and hopelessness, depression/dejection and fatigue are associated with transplant-related mortality. This is in line with recent findings from other research groups where hopelessness and helplessness in cancer patients in general and in SCT patients in particular were associated with lower survival. It is time to reconsider the role of psychosocial factors in SCT.

Patterns of extramedullary leukemia after BMT and potential for survival

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The 148 published cases of clinical extramedullary relapse (EMR) after BM/SCT were analyzed. Cases of first EMR (excluding CSF) with adequate data were included. Authors were contacted for follow-up. The database includes 82 AML, 41 ALL, and 25 CML cases. There are 87 male and 61 female pts; 34 are <16 yrs old. Karyotypes were abnormal in 63 of 73 pts studied; no FAB subtype predominated in AML pts. Conditioning was TBI-based in 55% and busulfan-based in 37%. In 23 pts, EMR first occurred after BMT relapse, a median 7 m after treatments which included DLI+/−chemo (13), G- or GM-CSF(3), or second BMT (3). Only one such pt survived >12m, after second SCT. In the other 125 pts, EMR occurred at median 12-16m in AML, ALL, and CML. In ALL, only 7% occurred >2 yrs after BMT, but in AML and CML, 33% of cases occurred 2-10 yr after BMT. Time from BMT to EMR was similar for pts in CR-1 (59), CR-2 (24), and advanced disease (43). Soft tissues were the principal site in AML/ALL. The most commonly reported specific sites were testis in 1/3 of males and breast in 1/3 of females. In AML, 7 of 8 adult men with testis EMR
Cord blood transplantation (CBT) has been successfully used to rescue patients undergoing myeloablative therapy. As the number of Ag-specific mature T lymphocytes transferred with the graft, is significantly lower in CBT as compared with unmanipulated BMT recipients, the number of recipient-specific CD4+ T lymphocytes of both donor and recipient origin may contribute to immune recovery after CBT. Aims of this study were to quantify the frequency of HCVM and CA-specific T cells in PBMC obtained from CBT recipients 2-3 and 12-15 months after transplant and to evaluate the contribution of recipient- and donor-derived T cells to the development of immune response to these antigens. Frequency of HCVM- or CA-specific proliferating T cell precursors (PTCp) was evaluated in 9 children given CBT from either HLA-identical sibling (n=5) or unrelated donor (n=4). Three out of 9 patients received a TBI-containing conditioning regimen. Sizeable or high frequencies of PTCp specific for HCVM were found in 2/5 recipients 2−3 months after transplant and in 1/4 recipients 12−15 months after transplant, suggesting that HCVM-specific T cell precursors (PTCp) were shed in the peripheral blood of CBT recipients. HCVM-specific T cell precursors (PTCp) were detected in 2/5 patients with a nadir of TREC+ cells/10^5 T-cells, mean ± SD. This difference became less prominent after 12 months (11891 ± 10953 vs. 8151 ± 2925 TREC+ cells/10^5 T-cells, mean ± SD). This difference became less prominent after 12 months (11891 ± 10953 vs. 8151 ± 2925 TREC+ cells/10^5 T-cells, mean ± SD). This difference became less prominent after 12 months (11891 ± 10953 vs. 8151 ± 2925 TREC+ cells/10^5 T-cells, mean ± SD). This difference became less prominent after 12 months (11891 ± 10953 vs. 8151 ± 2925 TREC+ cells/10^5 T-cells, mean ± SD). This difference became less prominent after 12 months (11891 ± 10953 vs. 8151 ± 2925 TREC+ cells/10^5 T-cells, mean ± SD). This difference became less prominent after 12 months (11891 ± 10953 vs. 8151 ± 2925 TREC+ cells/10^5 T-cells, mean ± SD).
were constructed for survival, event free survival, transplant related mortality, and relapse incidence. No influence of donor CMV status was found in HLA-identical sibling transplant recipients. Unrelated donor SCT recipients receiving grafts from CMV seropositive donors had improved 5-year survival (35% vs. 27%; HR=0.8), event free survival (30% vs. 22%; HR=0.8), and reduced transplant related mortality (49% vs. 62%; HR=0.7). There was no influence on the relapse incidence. The effects of donor CMV status remained present in multivariate analyses. The effect of donor CMV status were not the same in patients transplanted for different diseases. The effect of donor CMV status was pronounced in patients transplanted for CML and in patients with MDS and SAA, but was absent in patients transplanted for acute leukemia. In patients transplanted for chronic myelogenous leukemia, T-cell depletion abrogated the beneficial effect of donor CMV status suggesting that the beneficial effect is mediated through transfer of donor immunity. We conclude that donor CMV status has a major impact on outcome of unrelated SCT. For a CMV seropositive patient, the data suggests that a CMV seropositive donor is preferable at least in patients with CML.

302 Risk factors for CMV retinitis after allogeneic blood stem cell transplantation


Preliminary results of an EBMT survey on CMV retinitis (CMVR) after blood stem cell transplantation (SCT) was reported at last years annual meeting. The study has been continued, and currently a total of 24 cases have been reported from 14 EBMT centres. Eighteen allogeneically transplanted patients have been possible to include in a statistical analysis on risk factors for development of CMVR. One of these patients was HIV positive. The control group consisted of patients transplanted at the contributing centres during the study period, that had a follow up time of more than one month, which made a total of 2467 patients. The median time from transplantation to diagnosis of CMVR was 160 days (range 31-318) and the frequency was 0.73%. In the patients developing chronic GVHD, CMVR tended to be more common - 2.08% (p=0.06). It tended to be even higher in patients developing extensive chronic GVHD - 2.31% (p=0.053). The overall probability of developing CMVR was 1.07 ± 0.26%. In a multivariate analysis there was no significant increase in risk due to patient or donor age and sex, sex matching or HLA-matching. Chronic GVHD or extensive chronic GVHD did not increase the risk for CMVR in the multivariate setting, nor did acute GVHD. GVHD. The risk factors found or CMVR were: CMV-neg donor to CMV-pos patient, RR=4.30 (95%CI=1.7-10.8, p=0.002). Peripheral blood progenitor cells as graft, RR=3.65 (95% CI=1.37-9.73, p=0.01). Year of transplant later than 1996, RR=3.75 (95% CI=1.23-11.50, p=0.02). T cell depletion of the graft was no significant risk factor, but there was a tendency towards it, with RR=2.82 (95%CI=0.98-8.12, p=0.054). If the donor CMV-status was not considered, T-cell depletion also became a significant risk factor, with RR 4.04, 95% CI 1.37-11.9, p=0.011. In conclusion, the statistical analysis have confirmed that CMV retinitis has become a more common complication to allogeneic SCT. The patients with the largest risk for developing the complication, seems to be CMV positive patients with negative donors, transplanted with T-cell depleted peripheral blood progenitor cells.

303 Outbreaks of infectious disease in stem cell transplant units: a silent cause of death for patients and transplant programmes


Epidemics are a continuous threat for any hospital, even more so for transplant programmes. The IDWP ran a survey so as to identify outbreaks of infection and their consequences for patient management in European stem cell transplant units between 1991-2001. A questionnaire was sent to the centres in June 2001 to collect data retrospectively. An outbreak of an infection was defined as an increase in the incidence of that infection above the expected incidence in the SCT unit with the proviso that there were no coincident changes in the case definition of infection, mode of surveillance, or the laboratory techniques used to identify the infection. Forty one centres returned the questionnaire. Twelve centres reported 19 outbreaks. The pathogens involved were respiratory viruses (4 centres, 6 outbreaks), bacteria, mainly Pseudomonas sp., and Vancomycin resistant enterococci (7 centres, 10 outbreaks), and fungi, Scedosporium (2 centres, 2 outbreaks), Paecilomyces lilacinus (1 centre, 1 outbreak) and Aspergillus sp. (2 centres, 1 outbreak). The centres had to close the ward or whole unit in 9 of these 19 outbreaks, for 2 to 12 weeks. Four centres stopped the transplant programme for the duration of the outbreak to prevent further transmission and to allow cleaning procedures, two additional centres maintained their transplant programme in other wards or hospitals. The deaths of fifty seven transplant patients were attributed to infectious agents involved in the outbreaks. Other non-transplanted patients were also affected. Outbreaks indeed can be a threat to patients and transplant units. Transplant units are encouraged to report outbreaks and to participate in surveillance programmes for infections, in order to either prevent or recognize the start of an outbreak, investigate to find the source, and take appropriate measures to protect patients. Awareness of epidemics should become a topic among transplant teams. In this way patient morbidity and mortality will be avoided, and the transplant programme will not be interrupted.

304 Definitions of Infectious diseases and complications after stem cell transplant


In the past, some confusion and variability in reporting has been noted with significant differences in incidences of infection among centres which may not be accurate. The growing knowledge of infectious complications after stem cell transplant makes it important that investigators agree on common definitions in the reporting of data, to insure that series are comparable. The IDWP of the EBMT has elaborated a list of definitions for infectious complications after stem cell transplant from published guidelines whenever possible, or from the consensus emerging from the members of the IDWP when no satisfactory definition has previously been proposed in the literature. This has been done for two purposes:
- to make the data given to the EBMT Registry, more precise and common to all the centres. This should improve data management, insure the quality of the data in the EBMT centres and facilitate prospective and retrospective studies through the registry.
- to provide official definitions for the Group, so that authors may refer to these definitions for studies or surveys, including those done outside the registry.
The first version of these definitions is available on the web site of the EBMT (ebmt.org) at the IDWP section. The definitions are presented in two parts:

- one for the different clinical entities which are usually observed. Most of these entities are included for entering infectious complications in the EBMT data base.
- the second for definitions regarding specific pathogens: bacteria, fungi, viruses, others. This double entry should help the investigators and data managers to establish the relationship between the clinical presentation, and the role of a pathogen. Although these definitions are not fully exhaustive and will probably evolve in the future according to the progress of investigative procedures, the Infectious Diseases Working Party and the Registry Committee recommend the EBMT members use the present version of these definitions when filling the infection-related complication section in the MED B forms. Suggestions from the EBMT members will be welcome in order to improve the next version.

Working Party Aplastic Anemia

Aplastic Anemia Registry: Report 2002


The EBMT Aplastic Anemia registry contains data of 6086 treatments (as of October 2001). Diagnosis were acquired aplastic anemia (AA) in 85.8%, Fanconi Anemia in 9.7%, other constitutional anemias in 1.2%, pure red cell aplasia in 1% and PNH in 2.3% of cases. In patients with acquired AA etiology was considered idiopathic in 83.3%, post-hepatitis in 8.6%, drug-induced in 6.1%, and toxic in 1.2% of cases. Most patients with acquired AA reported to the registry received allogeneic stem cell transplantation (SCT) (n=2894; 57.2%) or immunosuppressive treatment (IS) (n=2153, 42.8%) with or without hematopoietic growth factors. 81.2% of the patients were grafted from an HLA-identical sibling donor, 2.4% from an identical twin, 3.0% from a matched family donor, 4.4% from a mismatched family donor, 7.3% from a matched unrelated donor and 1.7% from a mismatched unrelated donor. The proportion of SCT from alternative donors increased from 13.5% in the era before 1995 to 23.4% since 1995. Since 1994 the number of allogenic PBSCTS for AA is steadily increasing (33.3% and 34.4% of HLA-ident. sib. SCTs in 1999 and 2000, resp). However, careful evaluation of the impact of stem cell source on outcome after transplantation for AA is required. Probability of survival after both allogeneic SCT and IS increased substantially over time. Probability of survival at 5 years after HLA-identical sibl. transplant was 0.39, 0.60, 0.63, 0.74 and 0.72 in the periods before 1981, 1981-1985, 1986-1990, 1991-1995 and since 1996. In the same time periods probability of 5 year survival after IS was 0.47, 0.57, 0.65, 0.76 and 0.75. Factors leading to improved survival include omission of radiation from conditioning regimens, improved GvHD prophylaxis, introduction of multimodal IS and improved supportive treatment. The role of growth factors as an adjunct to IS for AA is still unclear. Despite the overall improvement in outcome, survival of patients grafted from alternative donors is still significantly lower compared to HLA-identical sib SCT. Survival at 3 years in patients grafted since 1995 is 0.75 for HLA-identical sibling SCT and 0.65, 0.56 and 0.23 for patients grafted from matched related, matched unrelated and mismatched unrelated donors respectively. Thus, new trials in the SCT field should focus on evaluation of new regimens for alternative donor transplantation. For patients not eligible for allogeneic SCT the role of growth factors and new immunosuppressive drugs should be evaluated.

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Fludarabine, cyclophosphamide and ATG for alternative donor transplants in aplastic anemia - A report of the SAA Working Party

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Fifteen patients with severe aplastic anemia underwent an alternative donor transplant following a conditioning regimen without radiation: patients received fludarabine 30 mg/m² + cyclophosphamide 300 mg/m²×4 + antithymocyte globulin (Thymoglobulin) 3.75 mg/kg on days –5-4-3-2 before transplant. The etiology was idiopathic in 8 and constitutional in 7 (Fanconi). The donor was an unrelated donor in 10 or a family member in 5 (1 was a phenotypic matched father, 3 were one antigen mismatched relatives and one was 3 antigens mismatched). The source of stem cells was unmanipulated bone marrow in 14, and CD34+ selected PB cells in one. Graft versus host disease prophylaxis consisted of cyclosporin 1 mg/kg from day –7 to day +20 and methotrexate 10 mg/m² on days +1, +3, +6, +11 after transplant. All patients engrafted with a median level of donor chimerism of 100% within day +100 and 100% beyond day +100. One patient showed graft failure and died after conventional transplant of GvHD. Thirty patients (86%) had grade 0-I acute GvHD, one had grade II and one grade III GvHD. Five patients (33%) developed chronic GvHD. At a median follow up interval of 286 days (range 71-1334) 11/15 (73%) patients survive with graft function. Survival was 75% and 71% respectively in acquired and Fanconi anemia. This study confirms that an immunosuppressive conditioning regimen is sufficient to engraft young patients with aplastic anemia, using unmanipulated bone marrow and CyA+MTX as GvHD prophylaxis.

AML / MDS

O322

Allogeneic stem cell transplantation for adult chronic myelomonocytic leukaemia - A report on behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT)


Chronic myelomonocytic leukaemia (CML) is a rare hematological neoplastic disease, characterised by increased monocytes in the bone marrow and peripheral blood. Because of dysplastic features, CML was classified as a subtype of myelodysplastic syndromes (MDS) by the FAB group in 1982. We report the results of 50 allogeneic transplantations performed between 12/1988 and 1/2000 in 43 European centers in 32 male and 25 female with CML. The median age at transplant was 44 years (range 19-63), and the median time from diagnosis to transplant was 9 months (range 1-57). 18 patients had excess blasts ranging from 5% to 30% at time of transplantation. Cytogenetic data was available in 34 pts, showing abnormal karyotype in 13 cases (mainly monosomy 7). Donors were HLA identical siblings in 38 cases, HLA matched unrelated in 6 cases, HLA mismatched related in 5 cases, and HLA matched other related in one cases. Two graft failures were observed (4%). Neutrophil engraftment (>500/mm³) and platelet engraftment (>50000/mm³) was reached after a median of 17 days (range 11-50) and 29 days (range 11-268), respectively. 26% experienced acute GvHD grade II-IV, while 18% developed severe aGvHD
Cytogenetic characteristics predict outcome of patients with poor risk MDS and secondary AML (sAML) treated with intensive chemotheraphy and stem cell transplantation in a joint study (CRIANT) of the EORTC, EBMT, SAKK, HOVON and GIMEMA Leukaemia Groups


Treatment of MDS with chemotherapy results in few long-term remissions. The CRIANT study tests the value of ASCT versus another additional course of high dose cytarabine. All pts receive remission-induction therapy consisting of idarubicin, cytarabine and etoposide (ICE). Pts who reach CR receive one consolidation course (cons) with intermediate dose of cytarabine and idarubicin. Cytogenetic and FISH analysis is performed at diagnosis in and CR. Between November 1996 and August 2001, 306 pts have been registered in this study, 40% >55 years and 79% had MDS. Cytogenetic data was available for 217 of 259 (84%) evaluable pts. In the good risk group 92 patients had normal metaphases or –Y and 2 pts had a t(8:21). In the poor risk group 72 pts had chrom. 5, 7 or complex abnormalities. In the intermediate risk group 20 pts had +8 or 11q23 abnormalities, and 33 pts other chrom. aberrations. The median follow-up was 2.1 years for the 259 evaluable pts and 151 pts have died. The 2-year survival rate was 31% (SE=12%), 15% (SE=11%) and 20% (SE=6%) resp. (p<0.0001). Age (p=0.8), disease (MDS vs sAML: p=0.81), the FAB classification (p=0.84), the percentage of marrow blasts (p=0.06) and the number of cytopenias (p=0.009) were less predictive for survival. In MDS patients, the overall IPSS predicted survival less well (p=0.02), due to a too high weight allocated to the % of BM blasts in the definition of the IPSS score. The 2-year survival rate was 17% (SE=14%) in the intermed-1 group, 51% (SE=7%) in the intermed-2 group, and 29% (SE=6%) in the poor risk group. In patients who reached CR, 27 achieved a cytogenetic remission and 10 patients had persisting cytogenetic abnormalities The 2-year DFS rates of these 2 groups were 44% (SE=10%) and 15% (13%) resp. (p=0.03). The 2-year cumulative relapse incidence was 39% vs 85% and the 2-year cumulative treatment-related mortality was 18% vs 0%. In conclusion: cytogenetic characteristics predict response to intensive antileukaemic therapy and the chance of remaining in remission.

O324

The choice between bone marrow versus peripheral blood for patients undergoing allogeneic transplant for AML 1CR should be based on the marrow cell dose - A survey of EBMT since 1994


Background: Many studies have addressed the question of which hematopoietic stem cell source [peripheral blood (PB) or bone marrow (BM)] is associated with a better outcome in the allogeneic transplant setting. So far, no conclusive answer has emerged.

Patients and Methods: We have analysed 881 adults patients with acute myeloid leukemia (AML) in first CR, receiving an allogeneic non T-cell depleted and a myeloablative transplant from an HLA id. sibling since 1994 and reported to EBMT registry. Outcomes studied were: Leukemia free survival (LFS), Relapse incidence (RI), Transplant related mortality (TRM), Overall Survival (OS) and acute and chronic GVHD.

Results: peripheral blood (PB) recipients were grafted more recently and were conditioned with less TBI as compared with bone marrow (BM) recipients. The following characteristics were not statistically different between the two groups: Donor/recipients sex, FAB classification, interval diagnosis-CR and diagnosis-transplant, and WBC at diagnosis. Better outcomes (LFS, RI, OS and TRM) were observed among BM recipients receiving a higher bone marrow nucleated cell dose (> 2.7x10^8/kg), however no cell dose effect was identified among PB recipients. Therefore, PB recipients were analyzed as a single group. Therefore, three groups were compared: A (258 pts) = BM < 2.7x10^8/kg; B (257 pts)= BM >2.710^8/Kg ; C (366 pts) = PB. The LFS was 52%,67%,54% respectively (A vs B p= 0.0007; A vs C p= 0.2; B vs C p= 0.04); the RI was 38%, 27%, 24% respectively (A vs B p= 0.02; A vs C p= 0.5; B vs C p= 0.1); the TRM was 29%, 20%, 24% respectively (A vs B p= 0.02; A vs C p= 0.2; B vs C p= 0.2); the OS was 58%,69%,60% respectively (A vs B p= 0.002; A vs C p= 0.1; B vs C p= 0.08). The incidence/severity of acute GVHD was not different in the three groups.

In the multivariate analysis, patients receiving a higher bone marrow cell dose [B] had better outcomes compared to patients transplanted with PB [C], namely OS (RR=0.64, p=0.016), LFS (RR=0.65, p=0.013) and TRM (RR=0.61, p=0.04). However no statistically difference was observed between patients receiving a lower cell dose [A] and the whole group of PB [C]. In conclusion cell dose has a great impact on the outcomes of BMT but not on PB transplants. In comparison of PB transplant recipients, patients transplanted with a high bone marrow cell dose (>2.7x10^8/kg) have better outcomes. These data should not be generalised and they may not apply to transplants for hematopoietic malignancies other than AML in 1CR.

Allogeneic stem cell transplantation for patients with refractory anaemia (RA) with matched related and unrelated donors: results have improved in time

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Transplantation with matched allogeneic donors is the curative treatment of choice for patients with MDS. Early transplantation before transformation to advanced stages of MDS or AML has usually resulted in a 50% EFS, but the high transplant-related mortality has precluded a general application of alloSCT for patients with RA. This analysis evaluated the impact of age, transplant year, early transplantation, cytogenetic characteristics, T-cell depletion, type of donor and early transplantation on the
outcomes of transplantation with matched allogeneic donors. The study population consisted of 135 patients, 108 of whom have been transplanted with an HLA-identical sibling. 44 Patients received T cell depleted grafts. 22 Patients were <20 years, 67 between 20 and 40 years, and 60 >40 years. Transplant periods: 38 pts <1993, 46 pts 1993-1996 and 51 pts >1996. 35 Patients have been transplanted with 6 months after diagnosis, 52 between 6-12 months, and 58 after 12 months. Cytogenetic data were available for 101 pts, 54 of whom had cytogenetic abnormalities. The overall 5-year survival was 51%. Patients transplanted with HLA-identical siblings and other matched donors have a survival of 55% and 42% resp. Younger age was associated with better survival: 67% if <20 years, 44% between 20 and 40 years and 53% >40 years. T-cell depletion did not influence outcome: 48% with and 52% without T-cell depletion resp. Early transplantation within 6 months after diagnosis resulted in 62% survival. The transplantation outcome improved in time. The survival was 39% when transplanted before 1993, but 57% and 52% when transplanted in the more recent periods. Multivariate analysis using hazard Cox model with age, transplant year, type of donor and disease duration prior to transplant as variables showed that the transplant results have improved in time, independently of the other covariables. This data show that alloSCT results in excellent outcome nowadays, even at older age and including matched donors other than matched siblings.

O326
Bone marrow transplantation (BMT) for juvenile myelomonocytic leukemia (JMML): results on 23 patients from a single center
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Autologous BMT is the only curative therapy for JMML. We present single centre data on 23 JMML patients (pts) over a 20 year period (1980-2000).

Patient characteristics: 15 males, 18 females (ratio 1.9), aged 0.3 - 12 years (median 3.9) at diagnosis. Karyotype was abnormal in 11 cases (monosomy-7 in 7). In 20 cases therapy was given before BMT: ANLL therapy (7); 6MP (6). Splenectomy had been performed in 3 cases. An HLA matched sibling donor (ID) was used in 11 cases, another phenotypically related donor (ORD) in 3 cases, a matched unrelated donor (MUD) in 9 cases. Bone marrow was the source of the stem cells in all patients. The conditioning regimen consisted of cyclophosphamide and TBI from 1980 until 1991; thereafter Ara-C was added for matched sibling BMT, as well as T-cell depletion for MUD-BMT. GVHD prophylaxis was cyclosporine A + MTX for ID-BMT and ORD-BMT, CyA alone for MUD-BMT.

Results: Stable engraftment was seen in 20/23 cases (2/23 cases: patients. disease free (1 with a short follow up). Toxicity resulted in 4/12 children, relapse being the major problem leading to death in 8/9 patients.

O327
Stem cell transplantation in patients with high-risk AML in first remission - A low relapse rate and acceptable transplant-related mortality after intensification of the conditioning regimen with Re-188 labelled anti-CD66 monoclonal antibody

Patients (pts) with AML and high - risk cytogenetics or a poor response to primary induction chemotherapy have a poor prognosis even after a stem cell transplant (SCT). Only 20 - 40 % of this subgroup of AML pts receiving an allogeneic SCT become long - term survivors. We have evaluated a strategy of intensifying standard conditioning regimens with a Re - 188 labelled anti-CD66 monoclonal antibody: We have recruited 20 pts with high - risk AML in 1.CR. The patient cohort consisted of 10 males and 10 females with a median age of 43 y (range 19 - 59 y). The indication for an intensified conditioning regimen was the presence of high - risk cytogenetic features (5q-, 7q-, complex karyotype) in 11 pts, nonresponder status after primary induction chemotherapy in 7 pts, a syngeneic donor in 1 pt and secondary AML due to prior MDS in 1 pt. All pts had a favourable dosimetry, and the radiolabelled antibody provided a mean supplemental marrow dose of 18 Gy. The kidney was the normal organ with the highest radiation exposure due to the antibody with a mean of 6, 6 Gy. All pts subsequently received standard - dose conditioning with either TBI 12 Gy (n = 7) or Bu 12,8mg / kg i.v (n = 13) plus Cytoxan 120mg / kg. PBPC were the stem cell source in 19 pts, bone marrow was used in 1 pt. The donors were HLA - identical family donors in 6 cases, matched unrelated donors in 11 cases, and a syngeneic donor in 1 case. Three pts received unmanipulated autologous PBPC. GVHD - prophylaxis consisted of CD34+ selection with the CliniMACS device in 14 pts and Campath 1H in the bag in 2 pts. All pts achieved rapid engraftment, acute graft rejection was subsequently observed in 2 MUD pts, one of whom was successfully regrafted after further conditioning. None of the allogeneic pts have developed - grade II acute GVHD or extensive chronic GVHD. The day +30 mortality was 0%; the day +100 mortality 5 %. Two pts have developed radiation nephropathy, both in the group receiving additional TBI. After a median follow - up period of 12 months (range 2 - 36 mo) the actuarial probability of disease - free survival is 71 %. Three pts have relapsed, 2 of them after autologous PBPC. One pt receiving a MUD transplant died due to graft failure. Thus the actuarial risk of relapse was 24%, the risk of TRM 5%. These preliminary data suggest that the intensification of the conditioning regimen with a radiolabelled antibody may improve the outcome of SCT in high - risk AML grafted in 1.CR.

O328
Full-haplotype mismatched HSCT is a valid option for high-risk acute myeloid leukemia (AML) patients
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Since March 1993 we have used a megadose of T-cell depleted HSCs to transplant 59 patients (median age 26; range: 2 - 62 years) with AML. They were at high-risk for relapse and TRM because of the stages of disease at transplant (7 bad-risk CR I, 16 CR II, 6 CR III, 30 relapse). The 1993-95 conditioning protocol included TBI, thiopeta, ATG and CY for 12 patients who received an inoculum made up of lectin-separated bone marrow and PBPCs. After 1995, fludarabine was substituted for CY in the conditioning of 47 patients who received PBPCs depleted of T-cells by a positive selection of the CD34+ cells with the CellPro (n=21) or CliniMACS (n=26) devices. All graft processing procedures ensured a 10-fold increase in CD34+ cells over bone
marrow alone. Immunoselection of CD34+ cells yielded a median 4.5 log T cell depletion.

Full-donor engraftment was achieved in 55/59 (93%). Without any post-transplant immunosuppressive therapy, acute grade II-IV GvHD occurred in 3 cases which progressed to chronic in 2. TRM, relapse and EFS were respectively 0.65, 0.35 and 0.21 for the 30 patients in relapse at transplant and 0.22, 0.18 and 0.50 respectively for the 29 in CR. TRM and EFS are 0.16 and 0.62, respectively in 15/26 patients transplanted in remission (CR I = 2; CR II-III = 13) since 1999. All received an inoculum processed with the CliniMacs instrument and no post-transplant G-CSF which was abandoned because of its immunosuppressive properties.

The overall 0.28 probability of relapse at 8 years is lower than expected in these 59 high risk recipients of T cell depleted transplants. It is worth nothing there were no relapses when NK alloreactivity was in the Graft-vs-Host direction, confirming a specific Graft-vs-AML effect in the absence of GvHD. These data show a) a megadose of extensively T-cell-depleted HSC after an immuno-myeloablative conditioning regimen ensures sustained engraftment without GvHD; b) besides the conditioning regimen and the stem cell megadose, donor NK-cell alloreactivity facilitates engraftment and prevents relapse; c) stopping post-transplant G-CSF administration to the recipient markedly improves the immunological reconstitution. In conclusion, mismatched transplant can be offered to high-risk AML patients without a matched donor as a viable option in the early stages of the disease.

O329
Dose-reduced conditioning followed by allogeneic stem cell transplantation in patients with MDS or secondary AML


We evaluate in a retrospective study the efficacy of a reduced conditioning regimen followed by allogeneic stem cell transplantation in 42 patients with MDS or sAML, who were transplanted in 8 german transplant-centers. Diagnosis were RA n=9, RAEB n=7, RAEBT n=14, CMML (n=3), MDSCAML (n=9). The median age was 55 years (18-64). 20 pts were transplanted from HLA matched unrelated donors and 20 from HLA-identical sibling. One patient each had a mismatched related and unrelated donor, respectively. Stem cell source was bone marrow (n=12) or peripheral blood stem cells (n=30). In most cases Graft versus Host Disease (GvHD) prophylaxis was carried out with Cyclosporin until day 100 and short course Methotrexate or MMF day 1-28. 29 pts additionally received anti-thymocyte globulin as part of the conditioning regimen. In 36 patients the conditioning regimen consisted of Busulfan (8mg/kg) and Flu darabine (180 mg/m²). 6 patients received a dose-reduced Total body irradiation based conditioning regimen. The median time from diagnosis to stem cell transplantation was 8.5 months (range 1 to 45 ). Cytogenetic analysis was performed in 33 pts. An abnormal karyotype including monosomy 5, 7 and complexe abnormalities was seen in 19 patients. 26 pts received at least one intensive chemotherapy prior to transplantation.

Two patients died prior engraftment. All other pts engrafted with a leukocyte count >1/nl after a median of 15 days (range 8-34). Platelet count > 20/nl was achieved after a median of 15 days (range 8 - 173). The incidence of grade II-IV acute GvHD was 29% and of severe GvHD (III/IV) 6%. Limited chronic GvHD was observed in 9 patients (27%) and extensive cGvHD was seen in 3 patients (9%). During a median follow-up of 17 months 11 pts (26%) died of treatment related causes. 10 patients experienced relaps after a median of 4 months (range 2 –17) and 12 of those died of relapse. The 1 year probability of overall survival is 50% (95% CI: 35 –65%) and of disease free survival 42% (95% CI: 26 –58%).

We conclude that dose reduced conditioning is a feasible approach in patients with MDS/sAML not eligible for standard myeloablative stem cell transplantation.

Minitransplants

O330
Related and unrelated allogeneic hematopoietic stem cell transplants (HSCT) in patients with acute myelocytic leukemia (AML) following minimal conditioning

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Allogeneic HSCT from related and unrelated donors using conditioning with 200 cGy total body irradiation (TBI) continued with mycophenolate mofetil (MMF) and cyclosporine (CSP) were used to treat patients with AML not eligible for conventional HSCT. During the study period 29 in CR and 2 in CR2 were registered and transplanted in remission (CR I = 9, CR II=8, >CR2=1, PR=1, induction failure=3) and unrelated donors (n=29, 2 pts. with 1-Ag-mm; CR1=9, CR2=7, >CR2=6, PR=2, relapse or no response=5). Patients were 18-73 (median 57) years old. Surviving patients have a median follow-up of 413 days (range 29 to 1200).

Four patients (6,6%) rejected their grafts and had autologous recoveries. The 55 remaining evaluable patients showed complete donor engraftment within 2-3 months. Acute GVHD grade II-IV occurred in 37,9% patients after related and in 55,5% patients after unrelated HSCT and was fatal in 16,6%. Chronic GvHD developed in 33,3% of evaluable patients after related and in 61,9% after unrelated HSCT. Of the 31 patients given related HSCT, 12 are alive in complete remission (38,7%), 3 are alive in relapse/CR (9,6%), 12 (38,7%) died of relapse and 4 (15%) died of transplant-related causes. Of the 29 patients given unrelated HSCT, 17 (58,6%) are alive in CR (17,2%) died of relapse, and 7 (24,1%) died of transplant causes.

We conclude that allogeneic HSCT after conditioning with 200 cGy TBI +/- FLU is safe for patients with AML not eligible for conventional transplants. Immobile graft-versus-leukemia effects could be observed, especially after unrelated HSCT in patients with advanced disease at the time of HSCT.

O331
Strategies to reduce transplant-related mortality after allogeneic peripheral blood stem cell transplantation in older patients: comparison of reduced-intensity conditioning vs myeloablative regimens and CD34+cell selection


Allogeneic peripheral blood hematopoietic stem cell transplantation (allo-PBSCT) carries a transplant-related mortality (TRM) higher than 40% in elderly patients. We present the outcome of 47 patients, who were 45 to 66 years of age (median, 52 years), consecutively treated at a single institution with an HLA-identical sibling allo-PBSCT. Twenty-three patients received myeloablative regimens and CD34+ selected cells (CD34+ group) and 24 patients received unmanipulated grafts after reduced-intensity conditioning regimens (RIC group). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine A (CsA) alone or CsA combined with glucocorticoids in the CD34+ group, and CsA plus short-course methotrexate in the RIC group. Twelve patients had acute leukemia, 6 chronic myeloid leukemia, 8

S57
lymphoma, 5 chronic lymphoid leukemia, 12 multiple myeloma, 3 myelodysplasia and 1 myelophthisis. Twenty-three patients were in early phase of their disease and 24 beyond the early phase. Recipient and donor characteristics were well balanced between the two groups. Although patients in the CD34+ group were younger (p=0.05) and a higher proportion of them were in early phase of their disease. Time to neutrophil engraftment was comparable in both groups while platelet recovery was significantly faster (p=0.02) after RIC. One patient in the CD34+ group developed graft failure. The cumulative incidence of moderate-to-severe acute GVHD at 100 days was 35% in the CD34+ group and 58% in the RIC group. The 1-year cumulative incidence of extensive chronic GVHD was 17% vs 34% respectively (n.s.) After a median follow-up of 12 months, 13 patients have died without disease progression, 9 (39%) in the CD34+ group and 4 (17%) in the RIC group. The cumulative incidence of TRM at 1 year was 39% vs 19% (p=0.09). The relapse incidence was similar in both groups, although longer follow-up is necessary to evaluate differences in the risk of relapse. The Kaplan-Meier estimates of progression free survival at 1 year were 43% in the CD34+ group and 64% in the RIC group (p=1) and the overall survival were 48% vs 72% (p=0.06), respectively. The use of CD34+ selection reduced the risk of extensive cGVHD but was associated with a higher TRM than RIC, mainly due to life-threatening infections, while relapses were similar in both groups. Patients in the RIC group had a lower TRM, which translated into a trend for improved survival.

O332
Allografting with fludarabine-based immunosuppressive conditioning regimen
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Non-myeloablative conditioning regimens has been recently experimented in an effort to minimize toxicity while harnessing Graft-versus-Malignancy effects. We designed a strategy to follow an AutoSCT with AlloSCT to take advantage of both the antineoplastic and immunosuppressive effects of AutoSCT to allow allogeneic cell therapy. Eighty-seven patients with a median age of 45 years (range, 16-69), were treated. Follow-up is at a median of 410 days (range, 11-1590). Diagnoses were Lymphomas n=47 (HD=24 NHL=23), Solid Tumors n=15 (Breast=12 Kidney=2 Germ cell=1), Multiple Myeloma (n=13), Leukemias n=7 (CML=6 AML=1) and MDS (n=5). All but nineteen received the combined procedure AutoSCT/Mini-AlloSCT. Seventy-three patients were conditioned with an immunosuppressive regimen consisting of fludarabine 30 mg/m² x 3 days with cyclophosphamide 300 mg/m² x 3 days (Flu-Cy). Seven patients with MM and a patient with CML were conditioned with fludarabine 30 mg/m² x 3 days followed by TBI 200 Gy (Flu-TBI). Six patients with lymphoma, who relapsed after a previous autografting, were conditioned with fludarabine 30 mg/m² x 4 days followed by melphalan 140mg/m² (Flu-Melphalan). All patients received PBSC grafts from HLA-identical sibling donors mobilized with G-CSF. CSP/MTX or CSP/MMF were given post-transplant to control graft rejection and GVHD. Donor lymphocytes were given for persistent mixed chimerism and/or progression of malignancy. In no cases Grade >2 myelosuppression or mucositis were observed after Flu-Cy protocol. Grade 2 mucositis were observed after the Flu-Melphalan protocol in 5 pts, neutropenia in 6 pts and thrombocytopenia in 5 pts. Disease responses were observed in 36/64 patients who had measurable disease pretransplant and had sustained engraftment: 23/41 lymphoma pts, 2/2 CML (both patients achieved molecular remission), 2/2 RAEB, 5/11 breast cancer patients and 4/8 Multiple Myeloma. Acute GVHD occurred in 23 (31%) out of 72 patients and in 11 of them (15%) was of grade >II. Two patients died of GVHD. Twenty other patients died of progressive disease between 15 and 1071 days after allografting (Breast: n=4; HD: n=6; NHL: n=4; blastic phase-CML: n=2; MM: =1; Kidney: =1; MDS: =1; Germ cell: =1). No fatal rejection occurred in 17 patients (RAEB/RAEB-T: 4; CML:4; AML:1; MM: 4; NHL:2; kidney: 2). These data demonstrate that this novel combined approach reduced acute toxicities of conventional allografting even in highly pretreated patients.

O333
Tandem treatment with intensive cytoreduction followed immediately by allogeneic SCT with reduced intensity conditioning for high-risk myeloid leukemias
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Refractory or advanced myeloid leukemias have a poor prognosis. Allogeneic stem cell transplantation (alloSCT) is the only therapeutic option having the potential of inducing long term remissions. AlloSCT however is associated with a 30 - 40% transplant related mortality (TRM), if conventional, myeloablative conditioning regimens are used. Achieving engraftment with fludarabine-based reduced intensity conditioning regimens may decrease toxicity and improve results. We developed a tandem strategy combining a cytoreductive intensive chemotherapy followed by reduced intensity conditioning for alloSCT. Fifty-four patients received fludarabine 4x30mg/m², AraC 4x2g/m², and Amsacrine 4x100mg/m². After a three day rest they received 4Gy TBI followed by Cytoxan 2x40mg/kg or 2x50mg/kg and ATG 3x10mg/kg or 3x20mg/kg, with the higher dose used with unrelated donors. Sixteen patients had progressive MDS or secondary AML after MDS, 8 of them with unfavorable karyotypes, twelve patients had an early or 2nd relapse, 2 of them after prior high-dose chemotherapy, three patients were in delayed 1. CR, four patients in 2nd CR, 2 of them had relapsed after prior high-dose chemotherapy, eleven patients had primarily refractory disease, and eight patients had CML in blast crisis or 2nd CP. Twenty-nine patients received grafts from HLA-matched family members and 33 from matched unrelated donors. Median patient age was 50.0 with a range from 18.5 - 65.8 years. Overall survival was 50% at a median follow-up time of 370 days (range 22 to 784 days). All patients surviving more than 30 days engrafted and all but one patient with CML blast crisis and 2 patients with refractory or relapsed AML achieved a CR. Compared to a historical control, TRM could be reduced from 38% to 24% at day +100. Five patients relapsed at d +48, d +57, d +66, d +70 and d +106 respectively. After discontinuation of immunosuppression one of the patients who did not achieve a CR after SCT and one of the relapsed patients went into lasting CR, while 5 patients died from leukemia. One patient is receiving low-dose AraC in preparation for donor lymphocyte infusions. Causes of death without leukemia were acute GVHD in 3 patients, infection in 11 patients and bleeding in 2 patients. In summary, treatment with HD-AraC, fludarabine and amsacrine followed by alloSCT with reduced intensity conditioning is safe and has the potential of inducing sustained remissions in high risk myeloid leukemias. 

O334
Fludarabine and targeted busulfan as conditioning for patients with high-risk myeloid leukemia and myelodysplastic syndrome transplanted with hematopoietic stem cells from HLA-compatible related or unrelated donors

Objectives: A regimen of cyclophosphamide and busulfan is standard therapy before transplantation of allogeneic hematopoietic stem cells in patients with CML and MDS. We have formulated the hypothesis that fludarabine can replace cyclophosphamide and facilitate donor engraftment with less toxicity. The aim of this prospective study was to test whether a regimen of fludarabine and busulfan can facilitate engraftment
while limiting early non-relapse mortality in patients with high-risk CML or MDS.

Patients and methods: The regimen consisted of intravenous fludarabine 30 mg/m² from day -9 to day -6, and oral busulfan given at 1 mg/kg every 6 h x 16 from day -5 to day -2, with busulfan doses adjusted to target blood levels of 800-1000 ng/ml at steady state. GVHD prophylaxis was with cyclosporine A and standard dose methotrexate. Forty-one patients were enrolled for the treatment of CML blast phase (n=2), CML blast phase/remission (n=2), MDS with high-risk RA (n=6), RAEB (n=13), RAEB-t (n=3), untreated (n=3) or treated AML after MDS (n=3), CMML (n=1), CML (n=33), and CMLM (n=10), the median patient age was 52 (range 12-65) years. Mobilized blood stem cells were infused from HLA-compatible siblings (n=16) and unrelated donors (n=25).

Results: Engraftment to >500 neutrophils/µl and >20,000 platelets/µl was achieved in all patients at a median time of 16 (r, 12-23) and 13 days (r, 11-75) after transplantation, respectively. Chimerism analysis revealed an almost complete myeloid (median 99, range 60-100) and a still mixed T cell compartment (median 87.5, range 50-100) on day 75 after transplantation. The most common extramedullary toxicity was grade 3 stomatitis in 31 patients. Reasons for death were wereplosis (n=4), pneumonitis (n=3), lung hemorrhage (n=1), heart failure (n=1) and bacterial sepsis (n=1). The cumulative incidence of acute GVHD grade II-IV and III-IV was 60 % and 19%, respectively. Extensive chronic GVHD occurred in 44% (12/27) of patients evaluable after day 100. With a median follow-up of 120 (range 9-380) days the probability of 6 month overall-and disease-free survival is 67% and 53%.

Conclusion: These data suggest that fludarabine in combination with targeted busulfan can facilitate engraftment of blood stem cells from matched siblings and unrelated donors. Acute and chronic GVHD remain the primary cause of morbidity. Given the high-risk disease of these elderly patients, the non-relapse mortality of 10% at day 100 is encouraging.

O335
The toxicity and medium term efficacy of donor lymphocyte infusions (DLI) given after reduced intensity conditioning (RIC) allogeneic stem cell transplantation (SCT)

The rationale of RIC SCT is to limit the early TRM and provide a platform for a subsequent GVM effect either through early withdrawal of immunosuppression or DLI. We report the toxicity and efficacy of DLI given to 61 patients (median age 49 years) after reduced intensity conditioning transplants performed by 13 transplant centres in the UK. The major diseases being treated were non-Hodgkin’s lymphoma (21), CML (10), myeloma (9), AML (9) and CLL (6). 31/61 patients had received fludarabine, melphalan and Campath 1H and 20 received BCNU, etoposide, ara-C, melphalan and Campath 1H. 54 were from sibling donors (89%) and 7 (11%) from unrelated donors (6 matched). 12 (19%) had GVHD prior to DLI. The patients received 101 infusions (mean 1.7, range 1-4). The indications for DLI were unsatisfactory response/disease progression (32), mixed chimerism (18) and 9 were pre-emptive and 2 other. 8 had specific antitumoural therapy prior to DLI and 10 were on immunosuppressive agents at the time of the first DLI. The median times of the first, second and third DLI were 127, 220 and 303 days post SCT and the median CD3+ cell doses were 5 x 10^6/kg, 1 x 10^7/kg and 5 x 10^7/kg respectively. Graft hypoplasia was uncommon (8/61=13%) and usually reversible. Grade II-IV GVHD occurred in 17/61 patients (28%) and limited and extensive chronic GVHD in 8/51 and 10/51 evaluable patients (total cGVHD incidence of 29%). The incidence of acute GVHD was not higher in unrelated donors. Giving the first DLI infusion <100 and <180 days had 43% and 37% chance of GVHD (p=NS). Conversion to full donor chimerism occurred in 12/41 evaluable patients (29%) at a median of 37 days after the DLI; partial (>20% increase in donor %) responses were seen in an additional 6 patients (total chimeric response rate of 44%). 11/42 evaluable (26%) patients had a CR (2 molecular). 12/51 evaluable in CR and 5 had a PR (total response rate 38%). The median time to a CR was 144 days. 7/9 evaluable complete responders had full donor chimerism. 4/9 patients with follicular NHL had a CR and 3/9 patients with CML. Forty patients (66%) survive at a median of 416 days post-SCT (range 155-1170 days) with a median KS of 90. Twenty one patients (34%) have died at a median of 244 days post transplant with the major causes being the progressive disease (18%) and GVHD (8%). DLI post RIC SCT have a significant morbidity and mortality and complete clinical and chimeric responses are seen in a quarter of patients.

O336
Influence of ABO-incompatibility on transplant-related morbidity and mortality after allogeneic stem cell transplantation with nonmyeloablative (NMA) conditioning

Allogeneic stem cell transplantation following NMA conditioning regimens is a new approach in pts not qualifying for conventional transplantation because of poor performance status or advanced age. We evaluated 33 pts given NMA transplantation with respect to incidence of GVHD, viral and bacterial infections, rehospitalization and transplant related mortality (TRM). Thirty-three pts underwent NMA conditioning followed by peripheral blood stem cell (n=32) or bone marrow stem cell grafts (n=1) from HLA-id sibling (n=18) or HLA-matched (n=10) or 1 antigen mismatched (n=5) unrelated donors. Diagnosis were acute leukemia (n=8), myelodysplastic syndrome (n=4), CML (n=1), NHL (n=10), MM (n=6) and renal cell cancer (n=4). Conditioning consisted of fludarabin 90 mg/m² and 2 Gy total body irradiation. Cyclosporine A (CsA) and mycophenolate mofetil (MMF) were given for GVHD prophylaxis. Blood groups were identical (id) or major ABO-incompatible in 23 and minor or bidirectional ABO-incompatible in 10 cases.

One patient was excluded from further analysis due to early death on day 15. After a median follow-up of 7 mos (range, 2.5-20), 23 pts are alive. Acute GVHD grades II-IV occurred in 5/32 (16%), whereas chronic GVHD was seen in 12 of 26 (46%) evaluable pts. CMV reactivation was observed in 5 (16%) pts, 9 (28%) showed herpes infections (21 episodes) and 13 (41%) pts had a total of 18 bacterial infectious episodes. Pts in the ABO-id and major ABO-incompatible group had a median KS of 90, whereas pts in the minor and bidirectional ABO-incompatible group had a median KS of 50 (p<0.01). Thrombotic microangiopathy was observed in 4/32 (13%) pts, 3 pts were in the minor or bidirectional ABO incompatible group, had a major ABO-incompatibility (p<0.05), small numbers of patients, in allogeneic stem cell transplantation with NMA conditioning minor or bidirectional ABO-incompatibility between donor and recipient is an important risk factor significantly affecting posttransplant course.
Graft versus Host Disease / Tolerance & Rejection

O337
Inverse correlation between donor CD3 and CD34 cell dose and the risk of graft rejection in CML patients after allogeneic hematopoietic stem cell transplantation (HSCT) with 2 Gy TBI / fludarabine (Flu) conditioning and Cyclosporine A (CyA) / Mycophenol

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Background: T-lymphocytes have been shown to play an important role in conventional HSCT influencing graft rejection and relapse. Moreover, a well-documented negative correlation between the amount of CD34+ stem cells in the graft and the risk of graft rejection has been described. In this study, known risk factors for graft rejection, including CD3+ and CD 34+ cell dose, were analyzed in patients after allogeneic HSCT with immunosuppressive rather than myelosuppressive conditioning.

Patients and Methods: Eighteen CML patients underwent HSCT with 2 Gy TBI / Flu conditioning and CyA / MMF post-grafting. Related donors were used in 5, and unrelated donors in 13 patients, respectively. Graft source were peripheral blood stem cells (PBSC) in 17 patients and bone marrow (BM) in one patient. Disease status prior transplant was chronic phase (CP) in 9, accelerated phase (AP) in 4, and 2 chronic phase (2nd CP) in 4 and accelerated phase (AP) in 5 patients. Parameters studied were graft source, donor/recipient gender constellation, HLA compatibility (based on 12 loci genotyping), disease stage prior to transplant and the amount of CD3+, CD34+ and CD 56+ / CD3- NK cells in the graft counted on a FACscanä flow cytometer. Genomic HLA-typing was performed in unrelated patient-donor combinations, while serological typing for HLA class I and molecular typing for HLA class II was required for related HSCT. Statistical analysis were computed with the Mann-Whitney-test and Fishers exact test.

Results: Fifteen patients showed stable engraftment whereas non-engraftment occurred in 3 patients undergoing unrelated HSCT. The median number of T-cells used in patients with graft rejection was 1,2x10^8/kg BW (range 0,2-2,4) and 4,2x10^8/kgBW (range 1,1-6,0) in patient with stable engraftment (p= 0,015). Median CD34 cell dose for patients with graft rejection was 3,2x10^6/kg BW (range 1,7-4,5) and 6,95x10^6/kg BW (range 3,2-14,2) for patients with engraftment respectively (p= 0,03). No significant correlation was found for any of the other parameters tested.

Conclusion: In this study, low T-cell and CD34 cell doses in the graft were associated with an increased risk of graft rejection in CML patients after HSCT with 2 Gy TBI / Flu conditioning. Our results are consistent with the findings in conventional HSCT and indicate that the T cell and CD 34 cell amount in the graft may be an important tool to avoid graft rejection even in patients undergoing HSCT with immunosuppressive conditioning.

In the present study, mechanisms underlying the maintenance of anergic state were investigated, focusing on the role played by differentiation of antigen presenting cells (APC) during 1°MLC. Production of cytokines (IL-2, IFN-g, IL-4, IL-5, IL-10, IL-12), responsible for T cell proliferation and effectors cell differentiation, together with expression of some of their receptors, were also evaluated. In 1° control MLC (ctrlMLC) we observed a progressive differentiation of dendritic cells (DC, before culture 0,5-1% of PBMC; after 7-day culture 5-8% of recovered cells), mainly of DC1 phenotype, with complete down-regulation of CD14 antigen, a strong up-regulation of HLA class I and II (DR), increased expression of CD80 (20-40%), CD86 (50-70%) and CD40 (35-60%) antigens. In anergic MLC (anMLC), DC were undetectable, expression of CD14 antigen was maintained, HLA class I and II were only weakly up-regulated and, if compared with ctrlMLC, CD80, CD86 and CD40 molecules were expressed on a lower % of cultured cells (5-15%, 20-55%, 5-20%, respectively). In a second set of 4 experiments, CD14+ cells were depleted from anMLC recovered after 7-day culture and CD14-depleted cells were tested in a 2° MLC with original stimulators in the absence of CTLA4-Ig and CsA. Results demonstrated that CD14+ cell depletion completely reversed the state of anergy. Indeed, proliferation of CD14-depleted anMLC cells, detected after a 7-day 2° MLC, was comparable to or higher than that observed in 1° ctrlMLC. Evaluation of cytokine production suggests a down-regulation in the capacity to secrete TH1 cytokines of anMLC in comparison to ctrlMLC, with a decrease of number of cells secreting IL-10, IL-4 and IL-5是比较 much higher in anMLC than in ctrlMLC. The most relevant results of cytokine receptor evaluation show that anMLC are strongly impaired, if compared with ctrlMLC, in the capacity to express CD25 molecule and IL-12R, while IL-10R expression was higher in anMLC than in ctrlMLC. Altogether these data demonstrate that APC differentiating in anMLC play a key role in the maintenance of the state of anergy.

O339
Alleviation of graft versus host disease (GVHD) by oral tolerance while sparing graft versus leukemia effect

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Graft versus leukemia (GVL) effect is the backbone of modern nonmyeloablative allogeneic stem cell transplantation (alloSCT). However, it correlates with GVHD. We previously reported that low doses of orally administered Ags induce tolerance and alleviate autoimmune-like chronic GVHD across minor histocompatibility antigens (Blood 95:3613, 2000). We now asked whether it might be possible to alleviate GVHD by oral tolerance in a semi-allogeneic model (C57BL/6-F1), and (2) whether the induction of oral tolerance would spare the GVL effect. In the first set of experiments GVHD was generated by transplanting 2x10^7 splenocytes from B10.D2 donor mice into F1 (C57BL/6 x Balb/c) mice which received 70Gy 60Co total body irradiation (TBI) prior to transplantation. In the second set of experiments 3x10^7 splenocytes from B10.D2 donor mice were transplanted into Balb/c mice following 7,5Gy TBI. At day of transplantation the Balb/c mice were inoculated with 2x103 BCL1 leukemia cells I.V. Tolerance was induced (1) by feeding the F1 recipient mice with 5 oral doses of proteins (50mg/mouse) extracted from C57BL/6 splenocytes on alternate days following transplantation, or (2) by feeding the B10.D2 mice with proteins extracted from Balb/c splenocytes prior to B10.D2-Balb/c transplantation. Study parameters included in vitro mixed lymphocyte reaction (MLR) and chimerism, as well as clinical, histological and molecular parameters of GVHD, including Th1 vs. Th2 cytokine profile. Development of leukemia was assessed by peripheral blood counts, morphology and spleen size. As for the results, in both sets of experiments induction of tolerance was documented by a significant reduction in the MLR response of tolerated vs. non-tolerated splenocytes, and a shift from a Th1 pro-inflammatory to a Th2 anti-inflammatory cytokine profile. Similarly, in both models a significant alleviation of the clinical and

O338
Role of antigen presenting cells in the maintenance of alloantigen-specific anergy induced in vitro by a combination of CTLA4-Ig and cyclosporin A (CsA)


In previous studies, we demonstrated that addition of CTLA4-Ig and CsA to a primary (1°) mixed lymphocyte culture (MLC) induces a state of anergy of alloantigen-specific lymphocytes. Notwithstanding the state of anergy to stimulus alloantigen, treated lymphocytes maintain immunocompetence towards antigens expressed by widespread pathogens and leukemia cells.
pathological manifestations of GVHD was observed. Induction of oral tolerance neither jeopardized engraftment nor resulted in the development of leukemia. In conclusion, our experiments showed that induction of oral tolerance by administration of GVHD-associated antigens was able to alleviate GVHD whilst sparing the GVL effect, which may indicate split tolerance. These findings may constitute a step towards reducing the frequency of GVHD, which remains the major obstacle to successful outcome of both low intensity and nonmyeloablative alloSCT.

O340

GvHD of the gastro-intestinal (GI) tract in humans - Fas & TNF-mediated apoptosis and its potent prognostic value


Rationale: While TNF is the main mediator of apoptosis of GvHD of the GI tract in rodents, little is known about the respective role of Fas and that of TNF in GvHD of the GI tract in humans.

Patients and methods: From 03/1996 to 12/1999, 258 patients (pts) were transplanted and 94 had an upper GI biopsy. Among these 94 pts (median age 34 y), 63% were male, 88% had malignant disease (early stage; 57%), and 70% were grafted from a sibling donor. TBI was used in 47%, 77% received CSA+MTX (all CSA), and in 17% T-cell depleted graft was infused. Semi-quantitative pathological (Path) estimates on each sample included: density and cell type of the infiltrate (CI), intensity of edema on of the inflammatory reaction (Inf.R), extent of apoptosis by TUNEL [CI and epithelial cells (EC)], Fas-, TNF-, TNF-Rs (p55 & p65) expression.

Results: Apoptosis of EC & within the CI was found in 75% and in 51%, respectively. Fas was expressed in 55% of the cases in the CI (1% on EC), both TNF-Rs in 80%, and TNF in 87.5% of the cases. Path GvHD was found in 82% (50% had Path Grade 2 or more). Inf.R intensity, degree of edema & eosinophils density correlated with clinical GvHD grade II-IV (p<.05). In pts with grade II-IV acute GvHD, extensive apoptosis of EC and that of the CI was found in 30% and 52% of the pts, respectively (p=0.01 as compared to grade 0-1), as was Fas- (p=0.03), TNF- (p<0.01) and TNF-Rs (p<0.01). Sensitivity and specificity analyses of Path. markers in pts with Path grade II or more was highest (p=0.01) for TNF expression, apoptosis within the CI, and presence of eosinophils. Finally analyses of day-100 transplant related mortality by multivariate analysis, including clinical & Path. factors revealed that extent of apoptosis within the CI (RR=12.1) & stage >or = 2 of GvHD of the liver (RR=4.7) were the only defavorable predictors of outcome.

O341

In vitro assessment of calcineurin activity as a therapeutic index of immunosuppression in acute GVH disease


The in vitro assessment of calcineurin activity is a way to measure the functional effect of ciclosporin, and other immunosuppressive drugs with similar mechanism of action, such as FK506. It has been previously shown that CA may not always correlate with ciclosporin blood levels which are more correlated with toxicity than with efficacy. We have previously shown that mononuclear cells derived from patients with resistant GVHD express very high calcineurin activity (CA) compared to stable renal transplant patients, suggesting that in vitro assessment of CA may be a useful index to estimate the degree of immunosuppression afforded by ciclosporine. The goal of this pilot study was to assess the CA during the first 2 months following allogeneic SCT and to correlate its evolution with the occurrence of acute GVHD.

Seven allogeneic SCT recipients were enrolled during a 9-month period. All received GVHD prophylaxis with ciclosporin (2mg/kg/d) and methotrexate (day 1, 3, 6). Five patients developed grade > 2 acute GVHD, at a mean time of day 24+/−8 and were treated with steroids. CA was assessed before transplant, and then once weekly, for at least 2 months. Blood was sampled before the morning dose of ciclosporin when the patient received it orally.

The CA was determined in mononuclear cells isolated from whole blood using multiplex STR-PCR with fluorescence detection. Protein kinase and expressed in µg/ml RII / 25 µ proteins / 30 min.

Conclusions: Between days 14 and 42, at the time of occurrence of acute GVHD, CA was significantly higher in patients with GVHD than in patients w/o GVHD, suggesting that a poor CA under ciclosporin is associated with the occurrence of acute GVHD. In vitro assessment of CA appears as a promising therapeutic index to estimate the degree of immunosuppression afforded by ciclosporin and thus prevent the onset of acute GVHD.

O342

Differential chimerism analysis in CD8+ and CD4+ T-cells: kinetics of engraftment and impact on the development of acute and chronic GVHD


The development and kinetics of T-cell chimerism after allogeneic blood stem cell transplantation (SCT) have been linked to the onset and severity of acute and chronic Graft-versus-Host Disease (GVHD). However, published data on this issue are controversial, with some reports showing a close correlation between these variables and others failing to do so. However, most studies on this issue focussed on the entire population of CD3 positive cells, thus not delineating the potential differential biological behavior of effector (CD8+) and regulatory (CD4+) T cells. We wanted to explore the kinetics of the T-cell populations in patients undergoing SCT. Methods: 80 patients undergoing allogeneic SCT (53 myeloablative, 27 dose modified) for the treatment of hematological malignancies (40 CML, 22 AML, 6 ALL, 6 MDS, 6 various) were investigated. Chimerism analysis was performed on sorted T-cell populations from the peripheral blood using multiplex STR-PCR with fluorescence detection. Results: In the majority of patients, T-cell chimerism was rapid in both subsets (median time to CD4+ complete donor chimerism (CC): 42 days, range: 11-749 days; median CD8+: 41.5; range: 7-344). However, matched pair analysis in 69 patients with full engraftment in both subsets revealed that the CD4+ cells needed significantly longer to achieve full donor chimerism than the CD8 cells (p = .0007; Wilcoxon signed rank test). Thirteen patients remained mixed chimeras in the CD4+ T cells for the entire follow up (64 - 343 days) after SCT and 8 patients retained host cells in the CD8+ T-cells (64 - 749 days). The conditioning regimen had no significant impact on the kinetics of T-cell engraftment. No influence of T-cell chimerism was seen on the development of acute GVHD, but the development of chronic GVHD was associated with complete T-cell chimerism. Interestingly, 5 patients developed extensive chronic GVHD late after transplantation associated with simultaneous development of CC in the CD4+ T-cells. Conclusions: Kinetics of T-cell chimerism after allogeneic stem cell transplantation are complex and are not per se associated with the development of GVHD. CD4+ T-cells need longer to achieve full donor chimerism. Development of CC in CD4+ T-cells may be associated with cGVHD, which might argue for the relevance of inhibitory host T-cells, i.e. CD4+CD25+CD45RO, in controlling alloreactivity. The relevance of this subset has to be analyzed in future investigations.
In haploidentical transplants, T cell depletion is essential to prevent GvHD, but delays immune recovery. It therefore is crucial to find ways to influence T cells without causing GVHD to mice after only mild host immune suppression, infusing donor-vs-recipient alloreactive NK cells efficiently ablates host immune and myeloid cells, such as to allow T cell-depleted BMT across MHC barriers without causing any GVHD (submitted). Here, we determined whether alloreactive NK cells also ablate host APCs and, thus, prevent donor T cells from causing GvHD. Lethally-irradiated H-2b mice transplanted with H-2d bone marrow containing 1 million T cells died from GVHD in 2-4 wks. After conditioning with TBI + alloreactive NK cells, even given as many as 20 million T cells 100% of mice survived until sacrifice (120 days) with no signs of GvHD. If protection was indeed mediated by alloreactive NK cells attacking recipient APCs initiating GvHD (Shlomchik et al., Science 1999), mice with APCs that are resistant to alloreactive NK killing should not protected from GvHD. We made B6×BALB/c into B6 bone marrow chimeras to replace the alloreactive NK cell sensitive H-2b mouse APCs with H-2d/b cells that would be resistant to NK cell killing. While the H-2d allele protects against alloreactive NK cells, the H-2b molecules can still present antigen to donor H-2d T cells, thus priming GvH reactions. When analyzed 4 months post-transplant, > 90% dendritic cells in these chimeras were of H-2d/b origin. When these chimeras were re-conditioned with TBI plus alloreactive NK cells and reconstituted with H-2d BMT containing 1 million T cells, 100% died from GVHD. Control H-2b into H-2b chimeras given 20 million T cells survived with no signs of GVHD. We also found that alloreactive NK cells accelerated the loss of APCs, as compared to mice conditioned with either TBI or TBI plus non-alloreactive NK cells. These data indicate that alloreactive NK cells prevent GvHD via elimination of recipient type APCs. In clinical haploidential transplants without donor-vs-recipient NK cell alloreactivity (i.e., in the presence of KIR ligand incompatibility is in the GvH direction), GvHD is 0% (P<0.01) (Ruggeri et al, this meeting). In the future, an alloreactive NK cell-based conditioning might allow more T cells to be transferred with the graft and protect against infection-related mortality.

Graft-versus-infection and graft-versus-leukemia effects after suicide gene therapy of graft-versus-host disease

The main complications of allogeneic hematopoietic stem cell transplantation (HSCT) include graft-versus-host disease (GVHD) and poor immune reconstitution leading to severe infections and leukemia relapse. Mature donor T cells present in the transplant facilitate T-cell reconstitution but also induce GVHD, which itself impairs immune reconstitution. Based on differences of division rate between alloreactive and non-alloreactive T-cells after transfer in lethally irradiated recipients (1), we developed a strategy of alloreactive T-cell depletion, using T-cells expressing the Herpes simplex thymidine kinase (TK) suicide gene combined with a ganciclovir (GCV) treatment. This system permits the selective elimination of dividing TK+ T cells in vivo. We demonstrate that a preventive treatment with GCV administered close to the time of HSCT can control GVHD in mice grafted with TK+ T-cells (2). This treatment can be combined with cyclosporin A, the standard preventive treatment of GVHD, without altering GVHD prophylaxis (3). We evaluated immune reconstitution and the recovery of functional immune responses in mice protected from GVHD. A 7-day GCV treatment initiated at the time of a semi-allogeneic HSCT supplemented with TK+ T-cells allowed efficient prevention of GVHD, while sparing a pool of non-dividing donor TK+ T cells. These cells later expanded and contributed to the reconstitution of the recipient immune system (4). When these mice were challenged with lymphocytic choriomeningitis virus, they could mount an efficient antiviral response leading to virus elimination (5). In opposite, when mice were challenged intravenously with P815 tumor cells at the time of HSCT, no antileukemic effect could be observed. However, delaying GCV administration to day 6 led to a protection against leukemia. This delay in GCV administration was not associated with the appearance of clinical signs of GVHD (6). Thus, control of GVHD provided by a short course of GCV is compatible with the maintenance of a “graft-versus-infection” effect. Furthermore, by a time-optimized scheduling, this therapeutic approach can also be tuned to efficiently treat hematological malignancies. These observations should help to design improved suicide gene therapy trials in the field of allogeneic HSCT.

Expression of heat shock protein 60 and 70 in a human in vitro skin explant model of acute graft versus host disease - Quantitative assessment using scanning laser confocal microscopy
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The human in vitro skin explant model has been successfully used for predicting acute graft versus host disease (aGVHD) in HLA-matched sibling bone marrow transplants (BMT). The model has also been utilised to investigate possible mechanisms involved in the pathogenesis of aGVHD. In this present study we have demonstrated the expression of heat shock protein 60 (hsp60) and 70 (hsp70) in skin biopsies generated from the human skin explant model by both standard immunhistochemistry and quantitative analysis by confocal microscopy. 91 retrospective skin biopsies taken from 14 HLA-matched patient and donor pairs prior to BMT were assessed for the expression of hsp60 and hsp70 using standard immunohistochemistry, according to an in-house grading system (+, +++, +++) and quantified using integrating density units (IDUs). Two independent assessors carried out the assessment and biopsies were then grouped accordingly. Skin biopsies included the mixed lymphocyte culture, autologous and medium alone controls and varied in severity of graft versus host reaction (GVHR) from grade 0 to IV. Results showed that the level of hsp60 expression was higher in the lower grades of GVHR. High expression of hsp60 (+++) was observed in 86/189 (27%) of biopsies demonstrating a negative skin explant result of grade 0-I compared to only 1/25 (4%) in a positive explant group grade II-IV (P=0.015). Conversely the expression of hsp70 using an antibody recognising both constitutive and inducible forms of the protein increased dramatically with an increase in the severity of GVHR. Higher expression of hsp70 (++ or ++++) was observed in 39/44 (89%) of biopsies demonstrating a skin explant result of II-IV compared with only 14/40 (35%) in a negative explant group grade 0-I (P<0.001). Quantitative assessment of hsp70 expression using the confocal microscope confirmed this increase with intensity of staining consistently higher in GVHR grades III-IV (P<0.001). Results were comparable to that of standard immunohistochemistry and highlighted the confocal microscope as a possible solution to eradicate some of the subjectivity when assessing stained paraffin sections manually. Recent experiments using a specific antibody strongly suggest that the increased expression of hsp70 is due to the inducible form of the protein. These results have demonstrated for the first time the expression of hsp7 in a human model of aGVHD and suggest a possible involvement in the pathogenesis of the disease.
Aplastic Anemia

O346

Long-term outcome of patients with severe aplastic anemia treated with antithymocyte globulin, cyclosporin A with or without glycosylated rHuG-CSF (lenograstim): comparable remission rate, survival and risk of developing myelodysplastic syndrome

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To determine the long-term risk such as clonal hematologic complications and benefit of the immunosuppressive therapy such as increased as survival, a long-term follow-up of SAA patients was performed.

Between september 93 and july 96, 102 patients with newly diagnosed severe aplastic anemia were randomized to receive ATG and CyA with lenograstim at the dose of 5 µg/kg/day s/c from day 1 to day 98 (53 pts) or ATG and CyA without growth factor (49 pts). Median age was 23 years (1-82) and median ANC at baseline was 0.22x10^9/l (0-0.8).

At day 112, the ANC response rate was 87% in the lenograstim group and 75.5% in the control group (p=0.012) with 83% and 45% complete ANC response respectively (p<0.001). There was no difference in the incidence of infections between the 2 groups; however less severe infections were observed in the lenograstim group (22 vs 30).

Failures were considered as follows: drop of ANC<0.5x10^9/l, death, second course of ATG, transplantation, G-CSF treatment, for the non G-CSF group, treatment of relapse, consent withdrawn. The actuarial 6 month probability of failure free survival was 77% for the lenograstim and 57% for the control patients. Median time to treatment failure was not reached in both groups. Relapse (ANC value <0.5x10^9/l) was observed in 20% of responders patients in the lenograstim group and 38% of responders patients in the control group (p=0.02). A total of 12 patients died before days 180: 5 in the lenograstim group and 7 in the control group.

The actuarial 5-year survival (median follow-up 57 months) was 75% in the lenograstim group and 73% in the control group. At 5 years, there is no difference between the 2 groups in terms of CR (51% vs 52%), PR (20% vs 25%) and relapse (9% vs 7%). One patient in each group developed Myelodysplastic Syndrom during the follow-up period.

Conclusion: These long-term results with median follow-up of 5 years, show that there was no difference between both groups in terms of response and survival. In addition there is no increased risk of developing myelodysplasia for the lenograstim patients.

O347

Aplastic anemia: outcome of immunosuppressive and transplant treatment in children and adults


Children suffer fewer transplant complications than adults. In contrast it has been reported that they face worse than adults after immunosuppressive treatment of acquired aplastic anemia. It was the goal of this study to compare outcome in patients younger or older than 16 years of age receiving either primary treatment of severe aplastic anemia using an HLA-identical sibling transplant (SCT) or immunosuppression using ATG (IS) with or without other drugs after 1990 and reported to the EBMT (EBMT Aplastic Anaemia Working Party database as of Oct.2001). 674 adult patients (age > 16 years) received SCT from an HLA-identical sibling and 386 received ATG-based IS. 383 pediatric patients (<= 16 years) received SCT from an HLA-identical sibling and 106 received ATG-based IS. 24 pediatric patients and 112 adult patients were grafted with peripheral blood stem cells. Median age of the pediatric patients was 10.0 years (0-16) in the IS group and 16 years (0 - 16) in the IS group. Median age of the adult patients age was 25.1 (16-63.4) for SCT and 35.5 (16-78.2) for IS. Median interval between diagnosis and treatment of the pediatric SCT patients was 76.5 days (1-2453) and 31 days (1-11351) in IS group. Probability of survival at 5 years after SCT was 86% (82% - 90%) in children compared with 71% (67% - 74%) in adults (p<0.01). After IS survival at 5 years was 78% (69% - 88%) in children and 83% (79% - 86%) in adults (p=0.32). The probability of survival in children was better after HLA-identical sibling transplants as compared to adults but survival probability after IS treatment was similar. There is no evidence that children respond differently to ATG treatment than adults.

O348

Allogeneic mesenchymal stem cells engraft and improve the microenvironment of severe aplastic anemia human bone marrow


Bone marrow mesenchymal stem cells (MSCs) originating from the microenvironment (ME) have a multilineage potential, support hematopoiesis and possess immunosuppressive properties. When infused intravenously MSCs can engraft in the bone marrow of animal models; however there is no report of MSCs engraftment in human bone marrow. Although severe aplastic anemias (SAA) are mostly autoimmune induced, some reports indicate alteration of the bone marrow microenvironment. A 68 year old female patient with criterias of SAA unresponsive to conventional treatments received two infusions of allogeneic MSCs (male donor) at a dose of 2x10^6/kg and 6x10^6/kg in order to stimulate residual hematopoietic stem cells, to replace a defective ME and induce immunosuppression. Following MSCs infusion bone marrow changes and chimerism were demonstrated. Donor cells in the recipient bone marrow were detected at a low level by real time PCR analysis showing the presence of SRY gene in the DNA extracted from bone marrow biopsies. Recipient ME considerably improved as shown by biopsies and stromal cell cultures. In vitro assays showed that no Colony Forming Unit-Fibroblast (CFU-F) was detectable before MSCs infusion but became detectable after. Following MSCs infusion, by bone marrow histology interstitial hemorrhage, edema and adipocytic necrosis disappeared; reconstruction of the microenvironment was suggested by the vimentin immunostaining. This is the first evidence that allogeneic MSCs infused alone can engraft in a patient with SAA. A low level of engraftment may be sufficient to induce a significant improvement of the ME.

O349

Allogeneic bone marrow transplantation using intensified conditioning regimen in multi-transfused patients with severe aplastic anemia

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Allogeneic bone marrow transplantation(BMT) is the most important therapeutic modality in severe aplastic anemia(SAA). Graft rejection as well as GVHD is the main cause of failure after BMT in patient with SAA. Graft rejection is associated with large numbers of transfusions and prolonged disease duration before transplant. We added procarbazine to the cyclophosphamide(CY) and antithymocyte globulin(ATG) conditioning regimen to overcome rejection since a synergistic immunosuppressive effect
between alkylating agents and ATG had been reported. Between January 1995 and December 2000, 113 consecutive patients received an HLA-identical sibling marrow transplant for SAA. Median age was 29 years (range, 16 to 50) and 61 (54%) were men. The median number of transfusions including RBCs compared was 80 units (range, 5 to 480). Median interval from diagnosis to transplant was 16 months (range, 1 to 216). The conditioning regimen consisted of CY 50 mg/kg/d i.v. for 4 days, ATG (1.25 mg/kg/day i.v. for 3 days, rabbit type, Pasteur Merieux, France), and procarbazine (6.25 mg/kg/day p.o. for 6 days). The GVHD prophylaxis was a combination of cyclosporin and short-term immunosuppression. The median number of more severe courses was 1.42 (0.6-11.7). 108/260. The median time to recovery of granulocytes to 0.5 10^9/L was 13 days (range, 10-49). The median time to achieve a platelet count 20 10^9/L without transfusions was 19 days (range, 10-150). The incidence of graft failure, grade II to IV acute GVHD and chronic GVHD were 14.1%, 10.5%, and 11.5%, respectively. In spite of multi-transfusions and or long disease duration, the estimated overall survival at 3 years was 88.4% with a median follow-up for surviving patients of 30 months, which does not appear to be worse than that in other series. In multivariate analysis two factors which influenced survival were graft rejection and chronic GVHD. One case of disease for a long time even though follow up for long-term sequelae is required. 

O350

Quality adjusted survival is better after BMT for patients with severe aplastic anemia compared to immunosuppression

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Bone marrow transplantation (BMT) and immunosuppression with ATG have both improved prognosis in patients with aplastic anemia. Both treatment modalities have specific advantages and drawbacks but yield similar survival rates. Additional endpoints than survival are warranted for decision analysis. In a retrospective single center study, all patients with aplastic anemia treated between 1974 and 1999 with ATG (n=155) or BMT (n=52) were compared for survival, event free survival and Quality adjusted time without symptoms and toxicity (Q-TWIST). For the Q-TWIST analysis, seven health states were chosen: Time with treatment-related toxicity (TOX), time with clonal disease (CLON), time with chronic GVHD (GVHD), time with transfusion dependency (TRANS), time with partial remission (PR), time with complete remission but still on drug medication (CR), and time without symptoms and toxicities, and free from any medication (TWIST). Probability of overall and event free survival at 15 years was similar between both treatment groups (BMT, 51% and 25%; ATG, 53% and 27%), with more early deaths in the transplant group. Important differences were found between both treatment forms in terms of mean duration in various health states. The mean time per patient spent with toxicity (0.36 years for ATG; 0.27 years for BMT), in transfusion dependency (ATG, 0.66 years; BMT, 0.1 years), in partial remission (ATG, 3.27 years; BMT, 1.42 years), and with a clonal disorder (ATG, 0.68 years; BMT, 0.04 years) was significantly longer for ATG treated patients as compared to patients treated with BMT (P = 0.001). In contrast, patients treated with BMT spent more time with extensive chronic GVHD (0.96 years for BMT versus 0 for ATG; P = 0.023), and in TWIST (BMT, 2.43 years; ATG, 1.22 years; P = 0.056). The time that patients still being on drugs spent in complete remission was not statistically different between both groups (P = 0.431). Using the predefined utility factors, the time spent in Q-TWIST was similar for the two treatment groups, i.e. 5.08 years after bone marrow transplantation, and 5.72 years after ATG treatment (P = 0.434).

In conclusion, survival, event free survival and Q-TWIST is similar after both treatment forms. Patients treated with BMT spent more time free form symptoms, while ATG treated patients had greater requirements for close medical care, transfusion support and medications.
could be successfully re-engrafted. Two patients with MDS or AML died for transplant related complications. Conclusion I: These results are rather promising especially for patients with alternative donors and advanced disease.

Autologous HSC were harvested in 15 patients for reinfusion in case of graft failure, cancer treatment or gene therapy. BM was stored in 14 patients, containing 59 x 10^8 TNC (range: 15 – 378); 21.0 x 10^6 CD34 cells (range: 1 – 62); and a viability of 72.4 % (range: 40 – 94). CB was harvested in one patient, containing 5.2 x 10^8 TNC; and 2 x 10^6 CD34 cells. Quality of residual HSC and absence of clonal aberrations are depending on the interval between diagnosis and HSC harvest. Autologous BM has been reinused in one leukemic FA-patient resulting in a partial reconstitution. Conclusion II: The preservation of autologous HSC in FA is ingenious but should be performed early after diagnosis. BM cells are favored.

**O353**

**Hemopoietic stem cell transplantation (HSCT) for paroxysmal nocturnal hemoglobinuria (PNH) and acquired severe aplastic anemia (SAA): a report from the Aplastic Anaemia Working Party of the European Blood and Marrow Transplant group (EBMT)**

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Severe aplastic anaemia (SAA) and paroxysmal nocturnal haemoglobinuria (PNH) share many clinical and possibly pathogenetic features. Allogeneic haemopoietic stem cell transplantation (HSCT) is a curative treatment for both disorders. In the present study we compare the outcome of PNH and SAA patients allografted in Europe in the past 3 decades. The patient population consisted of 2894 patients with severe aplastic anaemia (SAA) and of 113 patients with PNH, reported to the EBMT. The donor was an HLA identical sibling in 2309 SAA and 92 PNH patients respectively, an identical twin in 67 and 0, a mismatched family or matched unrelated donor in 469 and 23 patients. The median time from diagnosis to transplant was 111 days for SAA and 946 for PNH (p=0.0001) and the median age of patients 18 and 29 respectively (p=0.0001). Radiation was used in the conditioning regimen in 19% and 21% of patients (p=0.5); the source of stem cells was bone marrow in 90% and 82% respectively (p=0.02). The overall actuarial survival for HLA identical sibling HSCT 10 years after transplant is 63% in SAA vs 52% in PNH (p=0.04). Patients with PNH had more acute GVHD (p=0.04) as compared to SAA patients, also when adjusted for age and excluding radiation based conditioning. There is a strong overall effect of patient age, interval diagnosis-HSCT and year of HSCT on outcome: older age is a significant negative predictor in both SAA and PNH, whereas year of HSCT and duration of the disease appears to be significant only in SAA. The small number of PNH patients undergoing an alternative donor transplant (n=23) did relatively well with 14 patients surviving. Probability of survival in this group was not different form patients grafted from HLA-identical siblings.

Conclusion. This study confirms the feasibility of HSCT in patients with PNH. When compared to the SAA patients, there are differences in patient characteristics, disease duration and outcome of the transplant. Patients receiving a non radiation based conditioning and bone marrow as the source of stem cells, seem to be doing better.

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**Stem Cell Biology**

**O354**

**High-dose administration of allogeneic mesenchymal stem cells to immunocompetent baboons**

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Mesenchymal stem cells (MSCs) are rare cells found in bone marrow and other tissues that have the capacity to be expanded to large numbers for tissue repair. We have previously demonstrated that MSCs from humans, rats, and baboons did not elicit an alloreactive T cell response in vitro as evaluated by peripheral blood cell responses to MSCs in one-way mixed lymphocyte reactions (MLR). Furthermore, MSCs from these species were shown to suppress MLRs induced against immunogenic stimulator cells. In studies of bone repair, transplantation of relatively low numbers of allogeneic MSCs (approximately 0.5 million MSCs/kg body weight) did not induce an immune response in immunocompetent recipient baboons and the MSCs survived in recipient animals for several months. The current study was designed to evaluate the immunologic consequences of multiple administration of high numbers of allogeneic MSCs (10 million total MSCs/kg body weight) to immunocompetent recipients. MSCs from male donors were labeled with dye for tracking purposes and injected into female recipients. Immunologic analysis of recipient priming, evaluated by one-way MLR, showed that recipient peripheral blood responses to donor alloantigens did not increase after MSC administration by either the IV or IM routes. Muscle biopsies obtained 4 weeks after the second administration of DiO-labeled MSCs showed persistence of dye-labeled cells at the site of injection. PCR analysis of male Y chromosome TSPY gene in these biopsies is ongoing. These results demonstrate that high doses of allogeneic MSCs administered in multiple doses to immunocompetent recipients do not induce immune responses that result in the rejection of these cells.

**O355**

**Pediatric bone marrow mesenchymal stem cells favour the expansion of primitive over committed progenitor CD34+ cells**

A. Tocci, G. Deb, L. Luchetti, A. Donfrancesco (Rome, I)

The proliferation of repopulating human stem cells (HSC) and primitive or committed human progenitors (HPP-CFC and HPC) proceeds through the regulated amplification of a series of gradually more restricted compartments. A complete understanding of this process and the clinical application of ex vivo expansion protocols require the development of model systems that support the proliferation of HSC/HPC with no or minimal differentiation. We now report that mesenchymal stem cells (MSC) from human pediatric bone marrow (BM) support the hematopoiesis of human HPP-CFC/HPC and favour the proliferation of primitive over committed HPC. Magnetic activated cell sorting (CD34+ cells >98% phenotypically pure) were isolated from term CB and cultured on pediatric BM irradiated MSC (CD34-, CD45-, SH2+, SH3+) in serum-containing and serum-free conditions with or without supplementation of exogenous early acting cytokines (FL, TPO, SCF). Over a period of 1 week, in the presence of MSC, observed changes included (n=7): higher number of nucleated and CD34+ cells and amplification of the blast compartment with lower differentiation. Interestingly, in
comparison with MSC-free cultures, MSC sustained a modest amplification of committed HPC, coupled with a high expansion of primitive progenitors (high proliferative potential-locy colony forming cells, HPP-CFC). Interestingly, the presence of MSC allows one-half to one-third reduction of CD34+/CD38- HSC inoculation to obtain similar levels of HPC expansion. These results indicate an influence of MSC on expansion of primitive HPC and possibly repopolulating HSC and indicate the use of MSC in clinical transplantation strategies, in particular when an insufficient harvest of HPC/HSC is obtained.

O356

Multilineage differentiation of CD133+ bone marrow cells
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A subset of bone marrow and mobilized peripheral blood CD34+ cells co-express a stem cell marker, recently designated CD133. The potential of CD34+/CD133+ cells to give rise to hematopoietic clonogenic precursors has already been investigated. The aim of this study was to determine their ability in generating both endothelial (ECs) and mesenchymal cells (MCs). Bone marrow samples from five healthy donors were separated by means of immunomagnetic selection with -CD133 antibodies. FACs analysis revealed the co-expression of CD34 on 95±0.5% of the CD133+ cells and endothelial and stromal markers (VEGF receptor VEGFR-2/KDR, NGFR p75 and SH2 CD105) on 3%. The cells were then grown on fibronectin-coated flasks in M199, 10% FBS and in the presence of VEGF, bFGF and IGF-1 to promote endothelial cells differentiation: after 3-4 weeks of culture, the cells formed a monolayer with a typical EC morphology. These cells were further purified with Ulex europaeus agglutinin-1 (UEA-1)-FITC and to differentiate them into the hematopoietic and mesenchymal lineages.

O357

Long term culture of human hematopoietic stem cells - influence on homing, organ selectivity, survival and proliferation early after transplantation into NOD/SCID mice
T. Kerre, B. Vandekerckhove, F. Olffen, J. Plum (Gent, B)

Long-term culture of human hematopoietic stem cells (HSC) is clinically important for retroviral gene therapy and for expansion of umbilical cord blood (UCB) HSC to enable transplantation of older children and adults. However, reports in literature on long-term engraftment of cultured HSC in NOD/SCID mice are mostly disappointing, suggesting a decrease in HSC activity after culture. We wanted to investigate the behavior of these cells early after transplantation, and therefore introduced UCB HSC that were cultured for 2 weeks in serum-free medium supplemented with SCF, TPO and Flt-3L, into our short-term in vivo trafficking assay (T. Kerre et al, J. Immunol. 2001;167(7): 3692-8). In this model, freshly isolated anti-FITC CD34+ cells home specifically to the bone marrow and spleen, but only in the bone marrow proliferation exceeds apoptosis and cells expand, with kinetics depending on the source (UCB>mPB>BM) and the expression of CD38. In comparison, cultured CD34+ cells show an impaired homing capacity (prolonged presence in the circulation) and altered organ selectivity (homing to spleen and bone marrow, but also to liver and lung). Apoptosis on the other hand, is less pronounced. Markedly, the bone marrow homed CD34+ cells show a reduction in both the early expansion (day 3 - week1: fresh 8x vs cultured <1x) as well as the late expansion (day 3 - week4: fresh 350x vs cultured 50x). All these data show that long-term culture of HSC affects all stages in the early transplantation phase. The homing defect could be partially explained by the downregulation of CXCR4, an important homing receptor of CD34+ cells to the bone marrow. On cultured CD34+ cells, or the increased expression of other adhesion molecules on these cells, which results in homing to inappropriate sites. Studies are now ongoing to identify the phenotype of the long-term repopulating cell after culture, and to further improve homing of these cells, by investigating the influence of culture on other homing molecules in this model.

O358

Factors inherent to a human cord blood stem cell graft determine telomere dynamics after transplantation to NOD/SCID mice
H. Roelofs, W.A. Noort, A.H. Zwinderman, R. Willemze, W.E. Fibbe (Leiden, NL)

Telomere length dynamics of a cell population after stem cell transplantation is determined by multiple factors. E.g. induction of telomerase or the recruitment of immature precursors with longer telomeres might result in an increase of telomere length while the excessive expansion of stem cells following transplantation might cause telomere shortening. The net result of these factors might be dependent on intrinsic properties of the graft as well as on extrinsic factors inherent to the recipient. To differentiate between such intrinsic and extrinsic factors, we performed xenotransplantations of human progenitor cells into NOD/SCID mice. Hematopoietic progenitor cells (CD34+) were isolated from 4 umbilical cord blood samples and transplanted into cohorts of 3 – 4 recipient NOD/SCID mice. Six to eight weeks after transplantation, the mice were sacrificed and human progenitor cells (CD45+ /CD34+) as well as B cells (CD45+/CD19+) were isolated from the bone marrow. Using FlowFish analysis, we compared the telomere length of these expanded cell populations with the telomere length of the transplanted progenitor cell populations. The expanded B cell populations invariably showed longer telomerers than the transplanted progenitor cells. However, transplantation of progenitor cells from 3 out of 4 different umbilical cord blood sources resulted in shorter telomeres in all 4 recipients. Our data indicate that the change in telomere length during the expansion period after transplantation in the recipient mice depends on the specific progenitor cell source. It is unlikely that the increase in telomere length that was observed in the majority of the expanded progenitor (CD34+) and B cell populations is due to the recruitment of immature precursors (CD34+/38-) with longer telomeres since in a direct comparison between the telomere lengths of CD34+/38- and CD34+/38+ umbilical cord progenitor cells no difference was found. The differential telomere elongation/shortening cannot be explained by differences in graft size, since the grafts contained similar numbers of CD34+ cells. Our data indicate that factors that are intrinsic to the graft, e.g. the capacity of the transplanted cells to engraft and upregulate telomerase expression, influence the telomere dynamics in the recipient.
The common lymphoid progenitor protects against lethal MCMV infection in a murine model of matched unrelated hematopoietic stem cell transplantation

C. Arber, A. BitMansour, J. Higgins, E.S. Mocarski, J.A. Shizuru, J.M. Brown (Stanford, USA)

Cytomegalovirus (CMV) reactivation after allogeneic hematopoietic cell transplantation (HCT) is one of the leading causes of infectious morbidity and mortality despite available antiviral agents. Recently, a population of cells committed to lymphoid lineage development was phenotypically identified and isolated from mouse bone marrow (BM). These cells termed common lymphoid progenitors (CLP) comprise 0.02% of total BM cells and rapidly give rise to T, B and NK cells. The goal of our study was to assess the capacity of CLP to restore functional immunity when co-transplanted with purified hematopoietic stem cells (HSC) as compared to mice that were transplanted with HSC alone. CLP activity was studied in a pre-clinical murine model of CMV infection in the setting of a matched unrelated donor HCT. HSC and CLP were isolated by fluorescence activated cell sorting from C57BL/6.CD45.2 (H-2b) mice (for HSC) or C57BL/6.CD45.1 (H-2b) mice (for CLP) and transplanted into lethally irradiated BALB.B(H-2b) mice (200 rad). The recipients of either group were challenged with 5x10^6 pfu (i.p.) of murine CMV on day 14 post transplantation and examined daily for signs of CMV disease and acute graft-versus-host disease (GVHD) through day 35. Infected mice that were co-transplanted with HSC and CLP demonstrated a higher survival rate as compared to infected mice that had received HSC alone (80% vs. 27.8%, p=0.002). The severity of CMV disease reflected by mouse body weight as well as the virus load in the liver was decreased in CLP co-transplanted animals. In addition, no signs of clinical GVHD were observed in infected or uninjected mice. In conclusion, these data suggest that (1) CLP do not induce clinical GVHD and (2) co-transplantation of CLP with HSC protects against lethal CMV infection in a matched unrelated donor HCT model.

O360

Processing of preterm cord blood for transfusion of red blood cells in combination with stem and progenitor cells

J.M Van Beckhoven, P.K de Groot, A. Brand (Leiden, NL)

Objectives: Low-birth-weight preterm infants are among the most heavily transfused of patient groups during the early weeks of life. The risks associated with donor blood exposure are largely unknown. Possible immunomodulatory effects with consequences for both autologous and allogeneic HCT. We started a pilot study to investigate the possibility of collecting and processing cord blood (CB) with the aim to support post-natal transfusion requirements.

Methods: By using the collection system with a incorporated syringe of our CBBank we collected CB from preterm infants. Modification of the bag and the needles were made. CB was separated in RBC, stem cell (SC) and a plasma fraction by using a Sepax device (Biosafe). The RBC fraction was stored in SAG-M, AS3 and autologous plasma for at least 14 days and during this period concentrations of ATP, pH and MCV were measured. In addition different cell counts in the different fraction were performed. Our first aim was to evaluate the storage possibilities of RBC. Results: Mean volume of collected cord blood (excl. anticoagulation) is 28.7 ml. By using the syringe in 42% of the collections, we collected an additional extra of 5.9 ml. Sepax separation resulted in a mean recovery of RBC in the RBC fraction of 51% with a mean Hct of 0.53. The residual contamination of WBC in RBC fraction was still 29%, whereas 24% of WBC were collected in the SC fraction, which still contained a Hct of 0.22. The first storage results in SAG-M, AS3 or autologous plasma are shown in table 1. Storage results

<table>
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<tr>
<th>Day:</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
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<tr>
<td>ATP</td>
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<tr>
<td>SAG-M</td>
<td>5.22</td>
<td>4.13</td>
<td>3.34</td>
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<td>5.34</td>
<td>3.22</td>
<td>3.14</td>
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<tr>
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<td>2.67</td>
<td>3.27</td>
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<tr>
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<tr>
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</tr>
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</tr>
<tr>
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</tr>
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</tr>
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</table>

Conclusion: In all instances a RBC volume (10 ml/kg) at least sufficient for 1 transfusion can be obtained. Separation by Sepax shows promising results. Recovery of RBC and Hct can be increased by making adaptions. RBC can be stored for a period of 15-20 days in AS3. It remains to be evaluated whether expansion of committed CB cells can be further applied to support transfusion needs of the neonate.

O361

Immune reconstitution after autologous PBPC transplantation: effect of IL-15 on T-cell survival and function


Objective: To evaluate the occurrence of T-cell spontaneous apoptosis (Aspont) and its modulation in vitro by the IL-2 receptor (IL-2R) gamma-chain-signaling cytokine IL-15 in patients transplanted with autologous PBPC for hematological malignancies.

Patients and Methods: Patients were examined on days 30-60, 60-90 and 90-120 after PBPC infusion. The dissipation of mitochondrial transmembrane potential, a hallmark of T-cell apoptosis, has been detected with the fluorescent probe 3,3'-dihexyloxacarbocyanine iodide, following short-term T-cell culture in the absence or in the presence of exogenous cytokines. The expression of Bcl-2 family members has been studied by flow cytometry and reverse transcriptase polymerase chain reaction. T-cell proliferative responses to recall antigens have been estimated in autologous mixed leukocyte cultures.

Results: Aspont could be evidenced in 45±6% of CD4+ and 55±6% of CD8+ T cells cultured in the absence of cytokines; of interest, IL-15 and to a lesser extent its structural cousin IL-2 counteracted T-cell Aspont by a) inhibiting the processing of caspase-3 and b) upregulating Bcl-2 mRNA and protein levels. Cell division tracking confirmed that IL-15 did not rescue T cells from Aspont by promoting proliferation but rather it acted as a genuine survival factor. The addition of a gamma-chain-blocking antibody to cytokine-conditioned cultures abrogated both apoptosis inhibition and Bcl-2 induction by IL-15, suggesting the involvement of the IL-2R gamma-chain signal transduction pathway. Whereas cytokine-unprimed posttransplant T cells mounted inadequate responses to recall antigens, T cells conditioned with IL-15 expanded vigorously, indicating a restoration of antigen-specific proliferation.

Conclusions: T cells recovering after autologous PBPC transplantation are highly susceptible to spontaneous apoptosis in vitro; this phenomenon can be counteracted by the gamma-chain-signaling cytokine IL-15. These findings suggest that IL-15 might be a promising immunomodulating agent to improve postgrafting T-cell function.
Differ in AML or ALL patients receiving BM or PBSC from HLA.

However, acute GVHD, TRM, relapse, LFS and survival did not differ in patients receiving BM or PBSC. In multivariate analysis, PBSC vs. BM was associated with chronic GVHD in multivariate analysis included remission status at transplant, FAB groups, respectively (p=0.04).

Factors of importance were remission status, transplantation later than 1997, T-cell depletion and center effect. Acute GVHD did not differ in patients receiving BM or PBSC. However, chronic GVHD occurred in 32±2% of the AML patients receiving BM vs. 46±3% among those receiving PBSC (p=0.0001). For all patients, the corresponding days were 30 vs. 16 (p=0.00001). In multivariate analysis, PBSC was the strongest factor associated with engraftment by ANC and platelets. Other factors of importance were remission status, transplantation later than 1997, T-cell depletion and center effect. TRM, relapse, LFS and survival did not differ in AML or ALL patients receiving BM or PBSC. In multivariate analysis, PBSC vs. BM was not significant in patients receiving PBSC. Factors of importance for these outcomes included remission status at transplant, FAB M3, female donor to male recipient, center and age for AML patients and remission status, immunosuppression including methotrexate for ALL patients.

In conclusion, for patients with AML and ALL, those receiving PBSC compared to BM had a significantly faster engraftment of ANC and platelets, and an increased risk of chronic GVHD. However, acute GVHD, TRM, relapse, LFS and survival did not differ in AML or ALL patients receiving BM or PBSC. In multivariate analysis, PBSC vs. BM was not significant in patients with AML or ALL. Factors of importance for these outcomes included remission status at transplant, FAB M3, female donor to male recipient, center and age for AML patients and remission status, immunosuppression including methotrexate for ALL patients.

O363

High doses of allogeneic peripheral blood CD34+ stem cells (PBSC) correlate with better engraftment but induce a higher risk of extensive chronic GVHD: long-term results in 93 patients


The transplantation PBSC is rapidly growing in the allogeneic setting. However, the issue of the incidence of chronic GVHD (cGVHD) remains unresolved. We recently reported a higher incidence of cGVHD associated with PBSC in a comparative randomized trial. In the present report, we investigated whether a correlation exists between PBSC graft composition, especially the stem cell dose and transplant-related events such as tempo of engraftment, acute GVHD, cGVHD and survival. Thus, we undertook a retrospective analysis of clinical features and graft characteristics of 93 patients who received a PBSC graft between 1994 and 2001 and included in 2 consecutive multicentric trials. Patients and grafts characteristics are: median age 39 y. (19-52), sex (53M/40F), sex-mismatch 50 (54%), ABO compatible 65 (70%), diagnosis 32 CML (34%), 38 AML (41%), 16 ALL (17%), other 7 (8%). 77 patients (83%) were considered as standard risk, whereas 16 patients (17%) were high risk. 74 patients (80%) received Cy-TBI based preparative regimen. 75 patients (81%) received CsA and methotrexate for GVHD prophylaxis. The median doses of CD34+ and CD3+ cells were 7.9 (1.5-29.1) and 328 (104-754) x10e6/Kg. Median time for ANC and platelet to reach 0.5 and 25x10e9/L was 15 (10-32) and 14 (8-188) day. 65 patients out of 92 evaluable patients, experienced acute GVHD [17 grade I (18%), 28 grade II (30%) and 20 grade III-IV (22%)]. cGVHD occurred in 56 (15 limited and 41 extensive) out of 74 evaluable patients (7%). Increasing CD34+ cell numbers (> 7.0 x10e6/Kg) were significantly associated with accelerated ANC (P = .02) and platelet (P = .04) engraftment. The incidence of acute GVHD was not associated with CD34+ cell dose. However, high CD34+ cell doses were associated with a significantly increased risk of clinical extensive cGVHD (P = .05). In addition, among the 41 patients with extensive cGVHD, survival was significantly better in the group who received less than 7 x10e6/Kg CD34+ cells (P = .03), although DFS in the whole group was not significantly different between patients receiving < 7 x10e6/Kg vs. >7 x10e6/Kg. Collectively, these data suggest that the CD34+ cell dose affect both the engraftment kinetics and the development of cGVHD in HLA-identical sibling recipients. Thus, we conclude that efforts to further accelerate engraftment by increasing CD34+ cell numbers should be counterbalanced with the high morbidity of detrimental extensive cGVHD.

O364

Related cord blood transplant in patients with thalassemia and sickle cell disease

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Allogeneic BMT from HLA-identical family donors is an accepted treatment for both beta-thalassemia and sickle cell disease (SCD). A high percentage of patients can be cured with BMT. However, it is associated with a not negligible risk of both transplant-related mortality (TRM) and chronic GVHD (cGVHD). We analyzed 44 children given an allogeneic cord blood transplantation (CBT) for thalassemia (33 patients) or SCD (11 patients). Out of the 33 thalassemia children analyzed, 20 belonged to class I and 13 to class II of the Pesaro classification. All CB donors were HLA matched, except 3 (1 HLA-A difference). Median recipient age was 5 years (range 1-20), whereas median follow-up was 27 months (range 1-85). Twenty-six patients were transplanted after a preparative regimen including busulfan (BU) and cyclophosphamide (CY). In 17 patients, TBI was added to a preparative therapy combining either BU and CY or BU and Fludarabine (FLU). Thirty children were given Cyclosporine A (Cs-A) alone as GVHD prophylaxis, 10 received the association of Cs-A and Methotrexate (MTX, n=10), whereas the remaining 4 patients had other combinations of immunosuppressive drugs. Median number of nucleated cells infused was 4.0 x 107/kg (range 1.2-10). No patient died and 36 out of 44 children remain disease-free, with a median follow-up of 24 months (range 3-76). Only one patient with SCD did not have sustained donor engraftment as compared to 7 out of the 33 patients with thalassemia. Three of these 8 patients had sustained donor engraftment after BMT from the same donor. Four patients experienced grade II acute GVHD, only 2 out of the 36 patients at risk developing limited cGVHD. The 2-year probability of event-free survival (EFS) is 79% and 90% for patients with thalassemia and SCD, respectively. Use of MTX for GVHD prophylaxis was associated with a greater risk of treatment failure. Patients with thalassemia given the combination...
of either BU+THIO+CY or BU+THIO+FLU had a significantly higher probability of EFS in comparison to those prepared with BU+CY. Although obtained in a limited cohort, our results show that related CBT in patients with hemoglobinopathies offers a probability of remission comparable to ABMT. However, CBT was faster with PBSCT. The probability of relapse for whole group was 61% (median 2.6 y)- for marrow (n=148) 69% (median 2.3 y) vs 56% (median 3.4 y) with PBSCT (n=287); P<0.04. Median OS (P=0.9) and EFS (P=0.3) in both groups was comparable, median OS/EFS with BM 5.5/2.2 y vs 6.5/2.5 y with PBSCT respectively. Cox analysis-PBSCT (RR 0.68, P=0.005) and CR post-transplant (RR 0.70,P=0.02) were associated with lower risk of relapse; CR prior to transplant (RR 1.82,P=0.001), beta-2M 2.5 mg/L (RR 1.37,P=0.01) and PBSCT (RR 1.37,P=0.02) with superior EFS; and CR post-transplant (RR 1.85,P<0.0001) and beta2 M 2.5 mg/L (RR 1.81,P<0.0001) with superior OS. Lack of survival advantage for PBSCT was not surprising because of the availability of effective salvage therapy options. We have observed lower relapse and higher EFS with higher CD34+ cells among PBSCT recipients with myeloma (unpublished). That coupled with this study suggests-this effect is possibly mediated by rapid cellular/immune reconstitution due to higher infused cell numbers. Outcome of this single transplant group compares favorably to tandem autografts (median OS 5.7 y Blood1999:93:55-65). We conclude that sequential therapy as described results in excellent clinical outcome, and should be regarded as standard therapy of myeloma. However, an essential component of this programme is the use of PBSCT.

O365

Cord blood (CB) transplants with simultaneous infusion of highly purified haploidential CD34+ cells: different behavior of sibling and maternal CD34+ cells


Co-transplantation of more than one CB unit and of uncultured and culture-expanded fractions of a single CB unit are protocols that are being assayed seeking to reduce the period of neutropenia following a CB transplant. We have previously reported that co-transplantation of one CB unit and a limited number of highly purified haploidential peripheral blood CD34+ cells harvested from a sibling has resulted, in 3 high risks adult acute leukemia patients, in an early, efficient and transient generation of circulating granulocytes derived from the sibling donor and in an initial double (CB & haplo cells) chimerism evolving towards full CB chimerism, with no adverse effects related to the dual transplant procedure. Similar results have been obtained in two additional patients here first reported in whom the early recovery of granulocytes derived from the sibling cells seem to have been crucial to overcome serious early infectious complications. On the other hand, to four other similar high risk patients lacking a haploidential sibling donor, we have infused, together with a CB unit, similarly highly purified maternal haplo-CD34+ cells. All these 4 patients had undelayed CB engraftment, but in no one was DNA of the haplo cells observed either in BM or PB cells populations after the transplant. Data of the whole group of 9 patients are shown in the table. These different behaviors of the sibling and maternal haplo CD34+ cells in these dual transplants do not appear related to HLA-NIMA antigens. The possibility of intrauterine patient sensitization to maternal non HLA antigens could be a possibility. Further transplants using this dual model using sibling, maternal and paternal purified haplo CD34+ cells may help to confirm the apparent favorable effect of the sibling haplo-cells and to clarify the issue of intrauterine induction of sensitisation vs tolerance to maternal antigens.

O366

Reduced relapse rates in newly-treated myeloma patients by using peripheral blood stem cells over bone marrow as the source of stem cells for autologous transplantation: single-center experience of 435 patients treated with 200 mg/m2 melphalan


High-dose melphalan induces remission in large proportion of myeloma patients (McElwain&Powles.Lancet.1983:2:822-4). PBSCT alloagrafts result in lower relapse than marrow alloagrafts (Powles et al.;Lancet 2000:355:1231-7). We studied the largest single-centre series of myeloma patients treated with 200 mg/m2 melphalan(HDM200)+ 1 unpurged autograft to see if stem cell source affects outcome. 435 patients (31-75 y;median 52.7%; stage III) were treated from 1985 to 2001. 209 presented after primary therapy elsewhere and 226 were untreated. Therapy sequence was: induction therapy(VAMP/C-VAMP for untreated) till maximum response. HDM200-autograft, interferon maintenance, 25%(6%) died of treatment-related causes; all within 3 mo. 261(60%) attained CR post-transplant. Overall response rate was 92%. Progressive disease was seen in 242 patients; 164 died of disease/consequences of further therapy. 10 patients died of causes unrelated to transplant/disease progression. At last follow-up, 232 patients were alive 1 mo to 15.4 y post-transplant (median 39 mo); 154 in continuous CR/PR at 1 mo to 15.4 y(median 32 mo). Hematologic recovery was faster with PBSCT. The probability of relapse for whole group was 61% (median 2.6 y)- for marrow (n=148) 69% (median 2.3 y) vs 56% (median 3.4 y) with PBSCT (n=287);P=0.04. Median OS (P=0.9) and EFS (P=0.3) in both groups was comparable, median OS/EFS with BM 5.5/2.2 y vs 6.5/2.5 y with PBSCT respectively. Cox analysis-PBSCT (RR 0.68, P=0.005) and CR post-transplant (RR 0.70,P=0.02) were associated with lower risk of relapse; CR prior to transplant (RR 1.82,P=0.001), beta-2M 2.5 mg/L (RR 1.37,P=0.01) and PBSCT (RR 1.37,P=0.02) with superior EFS; and CR post-transplant (RR 1.85,P<0.0001) and beta2 M 2.5 mg/L (RR 1.81,P<0.0001) with superior OS. Lack of survival advantage for PBSCT was not surprising because of the availability of effective salvage therapy options. We have observed lower relapse and higher EFS with higher CD34+ cells among PBSCT recipients with myeloma (unpublished). That coupled with this study suggests-this effect is possibly mediated by rapid cellular/immune reconstitution due to higher infused cell numbers. Outcome of this single transplant group compares favorably to tandem autografts (median OS 5.7 y Blood1999:93:55-65). We conclude that sequential therapy as described results in excellent clinical outcome, and should be regarded as standard therapy of myeloma. However, an essential component of this programme is the use of PBSCT.

O367

Bone marrow or peripheral blood stem cell autotransplantation (PBSCT) for acute myeloid leukemia in first remission? A matched-pair analysis from the Royal Marsden Hospital and the European Blood and Marrow Transplant group (EBMT)


Slow hematologic recovery is hallmark of autologous bone marrow transplantation (ABMT) in AML, and is partly responsible for non-relapse mortality (NRM). This is overcome with PBSCT but there is concern over relapse rates following PBSCT in AML based on limited data. We compared outcome of ABMT from RMH (1990-2000;n=57) with PBSCT from EBMT (1996-2000;n=114). ABMT was performed after consolidation; conditioning comprised 140mg/m2 melphalan+ single-fraction TBI. Minimum total nucleated cell(TNC) dose was 2x10^8/kg. For 1 ABMT, 2 PBSCT patients were selected; 1 with myeloid karyotype, CR-transplant interval, FAB(for karyotype category D), and age; in that order. Karyotype matching was perfect, and based on categories: A [t(8;21),inv(16),t(15;17)], B [normal, other clonal], C [+8,-5q/-7q,4q11q23], and D [not done/failure]. Median CR-transplant interval was comparable (4 mo). PBSCT patients were slightly older (median 43 vs 39 y; P=0.04). Conditioning regimens were variable for PBSCT. Median TNC dose in the ABMT and PBSCT groups were 2.6 and 9x10^8/kg(P<0.0001). Hematologic recovery was significantly faster in the PBSCT group. 97% of PBSCT patients had recovered neutrophils to 0.5 x 109/L by day 50 vs 81% ABMT (P=0.0006). 79% of PBSCT had recovered platelets to 50x10^9/L by day 100 vs 44% ABMT (P<0.0001). 1 PBSCT and 7 ABMT patients died of toxicity. 11 PBSCT and 14 ABMT patients relapsed. At last follow-up, 102 PBSCT patients (89%) were alive and well at 1-70 mo vs 36 ABMT (63%) at 2-132 mo. 4-y NRM was 13% with ABMT vs 1% with PBSCT (P=0.04); 4-y relapse 31% with ABMT vs 20% with PBSCT (P=0.35); 4-y OS 63% with ABMT vs 77% with PBSCT(P=0.15). PBSCT was not associated with higher risk of relapse. Lower NRM with PBSCT is probably due to faster hematologic recovery and higher numbers of TNC infused. We have shown that NRM after ABMT in AML is higher with low TNC doses (Bone Marrow Transplant1995:16:499-506); 6/7 deaths in ABMT group occurred in the 35 patients getting <3 x 10^8 TNC/kg compared with 1 amongst those getting 3. Induction
and consolidation chemotherapy that ABMT patients received prior to transplantation as well as conditioning regimen used are at least as intense as any treatment regimens used for PBSC transplantation. The difference in the outcome of the groups is therefore probably entirely attributable to source of stem cells. We conclude that blood should replace marrow as source of autologous stem cells in patients with first remission AML.

O368

Two consecutive courses of peripheral blood progenitor cell (PBSC) mobilization induce marked telomere length shortening


Introduction. Telomeres are terminal repeated sequences of chromosomes, essential for DNA stability and integrity. During cell divisions, telomeres progressively become shorter until they reach a critical length that switches on apoptosis. As an indicator of cell aging, telomere length was evaluated in peripheral blood progenitor cells (PBPC) of patients undergoing high-dose (hd) chemotherapy with autografting. Aim of our study was to compare telomere length of PBPC harvested at different time-points of the program.

Patients and methods. Sixteen previously untreated lymphoma patients were evaluated. They all received a hd-sequential (HDS) chemotherapy regimen, including: i. 3 APO courses, as debulking; ii. sequential delivery of hd-cyclophosphamide (CY), hd-Ara-C and hd-etoposide; iii. final PBPC autografting. PBPC were collected after hd-CY and after hd-Ara-C. Telomere length was evaluated by Southern blot analysis and chemoluminescent detection on whole leukapheresis products as well as on CD34+ve cells obtained using the Miltenyi cell separation system. Results. All patients displayed high PBPC mobilization, with median peak values of circulating CD34+ve cells/ml of 238 and 175, following hd-CY and hd-Ara-C, respectively (p=0.86). Large amounts of PBPC were collected following both mobilizing courses. Despite comparable mobilization, telomere length was markedly shorter after hd-Ara-C compared to hd-CY. Mean telomere length on leukapheresis products was 8201 bp and 7003 bp, after hd-CY and hd-Ara-C, respectively (p=0.0001). Similar results were observed when telomere was evaluated on selected CD34+ve cells. Based on an age-related telomere shortening curve, an estimated 20-year loss of biological cell life was calculated in post-Ara-C compared to post-CY PBPC.

Conclusion. Two hd-chemotherapy courses at short intervals induce a marked PBPC telomere shortening. Loss of telomere is associated to increased risk of tumor transformation. Thus, this analysis should be considered whenever innovative modalities of hematopoietic cell mobilization are tested.

O369

Transplantation of G-CSF-mobilized blood stem cells from unrelated donors


A prospective trial was conducted by the U.S. National Marrow Donor Program to test the feasibility of harvesting peripheral blood stem cells (PBSC) from volunteer donors and the safety of transplanting those PBSC to patients with hematological disorders. Donors were treated daily with G-CSF 10 mg/kg, and PBSC were harvested on days 5 and 6. Cells collected on the day 5 were stored at 4C. The two-day collection was transported at 4oC and infused fresh into the recipient. This interim analysis evaluated results of 227 transplants facilitated by 55 apheresis centers and 57 transplant centers over the first year of study. PBSC were obtained in a one-day (n=47) or two-day (n=175) collection. The median blood volume processed was 12 liters per day, and 24 liters per total collection. Transplant regimens varied according to institutional protocols. The incidence of engraftment was 96%, acute graft-vs-host disease (GVHD) grades II-IV 47%, acute GVHD grades III-IV 33%, extensive chronic GVHD 36%, mortality from causes other than relapse at 100 days and 41% at one year, relapse 26%, survival 35% and disease-free survival 32% at one year. We compared outcomes of PBSC and bone marrow (BM) transplants conducted at the same institutions over the same period. Multivariate analyses were used to adjust for differences in patient age, gender, cytomegalovirus serology, performance status, diagnosis and stage, interval from diagnosis to transplantation, donor age and HLA matching, transplant center, year of transplant, conditioning and immunosuppressive regimen. PBSC were associated with a faster granulocyte (odds ratio [OR] 4.0, 95% C.I. 2.2-7.4, p=0.0001) and platelet engraftment (OR 4.5, 95% C.I. 2.7-7.7, p=0.0001), similar risk of GVHD grades II-IV (risk ratio [RR] 1.2, 95% C.I. 0.9-1.7, p=0.19), a suggestion of increased GVHD grades III-IV (RR 1.5, 95% C.I. 1.0-2.2, p=0.08), and similar rates of relapse (RR 1.0, 95% C.I. 0.7-1.5, p=0.90), survival (RR 0.8, 95% C.I. 0.6-1.1, p=0.11) and disease-free survival (RR=0.9, 95% C.I. 0.7-1.1, p=0.22). Similar findings were obtained by a matched cohort analysis. We conclude that harvest and transplantation of PBSC from volunteer donors are feasible and, within the constraints of this initial pilot study, appear as safe and effective as BM grafts.

1. Acute Leukemia

P387

Outcome of adults aged up to 60 years with high-risk de novo AML after autologous peripheral blood stem cell transplantation is comparable to that after allogeneic stem cell transplantation: results of prospective multicenter trial

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In a prospective multicenter trial a total of 305 de novo AML patients (median age 43 years) were treated according to the karyotype and response to induction course I. Patients with CBF leukemias or normal karyotype and a good response to induction I (<5% blasts in bone marrow on day 15) were considered as standard risk (SR), all others as high risk (HR) cases. Patients with a t(15;17) were excluded. Good responders to induction I (idarubicin, Ara-C, etoposide, IVA I) received IVA II starting on day 21. Patients with >5% blasts after IVA I received ID-Ara-C/m-AMSA. After early consolidation with ID-Ara-C/DRAM or m-AMSA, SR patients received HD-Ara-C/DRAM, while patients with a normal karyotype and a HLA-matched sibling were to be allotransplanted. HR patients were either auto- or allotransplanted if a HLA-matched sibling was available. PBSC were mobilized after early consolidation by filgrastim. The overall CR rate was 76% (89% in the SR vs. 60% in the HR group). In 78% of the patients who were candidates for autotransplantation a sufficient number of CD34 positive cells could be harvested with a median of 2 leukaphereses (range 1-6). This did not differ from a comparable number of SR patients who were mobilized outside the protocol. After a median follow-up of 35 months, the CCR rate at 67 months was 48% (median = 42 months) for the SR group and 29% (median = 12 months) for the HR patients. The corresponding figures for overall survival were 51% for the SR group and 26% (median = 16 months) for the HR patients. Out of 72 HR patients in CR, 45 patients (63%) were either auto- (n = 20) or allotransplanted (n = 25). Major reason for not being transplanted was relapse (16/27 patients) prior to transplantation. Eleven of the autotransplanted patients relapsed mostly within the first year after transplantation while from the allotransplanted patients 11 died during transplantation and another 3 relapsed. The probability of
Peripheral blood transplantation (PBT) after intensive chemotherapy (CT) in adult patients with primary acute myeloid leukemia (AML) according to adjusted prognostic factors: ongoing results of a prospective multicenter study


We evaluated the results of CT followed by PBT, allogeneic (allo-PBT) or autologous (auto-PBT), according to cytogenetic, number of courses to complete remission (CR) and availability of an HLA-identical sibling (HLA-id) in primary AML (M3 excluded). Between November 98 and October 2001, 192 patients (pts) were included (60 -80 years) (mean age+/−SD: 43+12 yrs; m/f: 105/87). Induction CT consisted of idarubicin, intermediate dose ara-C and VP16 (IDICE). Intensification CT included mitoxantrone and ara-C at intermediate dose. Subsequent treatment was as follows: a) pts with good prognosis (t[8;21] or inv(16)) received 1 course of high dose ara-C (HDAC) and no further therapy; b) Pts with intermediate prognosis (IP) (1 course to 4 CR and normal karyotype): auto-therapy with alpha interferon (a-IFN) combined with recombinant interleukin-2 (rIL-2) (n=9) or d-mercaptopurin (6-MP)/methotrexate (MTX) (n=1). Results: 27 patients including all 6 autografted in CR2 released between 55 and 413 (median 161) days posttransplant, another 5 died from causes other than leukemia. All patients autografted in CR 1 who received only moderate pretransplant chemotherapy and no posttransplant maintenance therapy relapsed (n=10), while 8 of 24 patients receiving either more intensive pretransplant chemotherapy or double autologous transplants and/or posttransplant maintenance therapy remain alive and well in continuing CR between 1.6 and 9 (median 4) years after transplant (p=0.02). The probabilities of leukemia-free survival for all patients, after single and after double autologous transplantation in CR1 were 22±2%, 19% ±8% and 27±13%, respectively.

Conclusions: Autologous transplantation in CR1 cures some patients and should be performed if a suitable allogeneic donor is lacking. Overall treatment intensity including pre- and posttransplant therapy appears of crucial importance.
cases by a marked and durable decrease in PLTS count lasting even 3 months.

P391
Long-term survival of patients with acute myeloid leukemia who underwent autologous bone marrow transplantation: a retrospective study in a single center
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High dose therapy (HDT) with autologous bone marrow transplantation (ABMT) is widely used in the treatment of acute myeloid leukemia (AML) as post remission therapy. In this study we report the results of HDT/ABMT in patients with AML enrolled in our center. Fiftyone patients (median age 35 years; female:24, male:27) underwent ABMT included 61% in first complete remission (CR1), 26% in second complete remission (CR2) and 13% in relapse. Preparative regimens consisted of busulfan (16mg/Kg) and cyclophosphamide (CY) (120 mg/kg) or busulfan (16 mg/Kg), CY (120 mg/Kg) and etoposide (60 mg/kg). At the bone marrow harvest all patients were in complete remission and purging were performed in 39 patients with malofasamid at a standard dose of 50 microg/ml (29) or individually adjusted (10). The median dose of mononucleated cells infused was 1.31x10^8 /Kg (range 1-8) and 3.66x10^6 /Kg for CD34+ cells. Complete remission was reached in 74% of patients and the leukemia relapse was 30%. The median time to recovery of polymorphonucleated cells to 500/mm3 and of platelets to 5000/mm3 was 22 days (range 12-73) and 39 days (range 15-232) respectively. The median overall survival (OS) was 20 months with a 5 and 10-15 years OS of 51% and 46%. The 5 and 10-15 years disease free survival (DFS) was 42% and 45% respectively. No significant difference was found in the OS and DFS between CR1 and CR2 patients. With multivariate analysis we found that age, infections have a significant impact on the outcomes of ABMT (OS, DFS and leukemia relapse) In conclusion our results suggest the long term efficacy of the HDT/ABMT in the treatment of acute myeloid leukemia.

P392
Allogeneic transplantation with melphalan-total body irradiation from HLA-identical siblings for AML in first remission: higher relapse rates with marrow-derived stem cell grafts compared to blood despite similar chronic graft-versus-host disease

59 patients over the age of 16 (17-56 years, median 36; 26F, 33M) with AML (excluding M3) in CR1 were allografted from HLA-identical siblings between 8/90 and 5/01. Source of stem cells was marrow in 40 (BMT) and G-CSF-stimulated blood (BSCT) in 19. The BMT/BSCT groups were comparable except for shorter CR-transplant intervals (median 90 vs 119 d;P=0.05) and older age (median 40 vs 34 y;P=0.09) with BSCT. Conditioning regimen comprised 110 mg/m2 melphalan on day -1 and single-fraction TBI. Uniform GVHD prophylaxis comprised cyclosporine and short course methotrexate (MTX-day 1, 3, 6, 11). MTX doses were not modified for any factor except significant renal or hepatic dysfunction. Engraftment was achieved in all evaluable patients. Actuarial probability of acute GVHD (any grade) was 82±6% for BMT and 79±9% for BSCT. 18 patients (13 BMT, 5 BSCT) experienced non-relapse mortality (NRM) at 19-268 d (median 63), 5 patients (all BMT) relapsed at 135-790 d (median 329); 4 died and 1 is alive 7 years after relapse following chemotherapy and a G-CSF-mobilized blood stem cell infusion from the donor. As of 7/01, 37 patients are alive in CR (36 in continuous CR) at 2-111 mo (median 61). Actuarial probabilities (+/-SE) of NRM, relapse, overall survival (OS) and disease-free survival (DFS) are 32+/−6%, 14+/−6%, 60+/−7%, and 58+/−7% respectively. 3-year probability of NRM, relapse, OS, DFS and chronic GVHD in BMT vs BSCT are as follows: 33+/−8% vs 28+/−11% (P=0.8); 19+/−8% vs 0%(P=0.1); 57+/−8% vs 72+/−11% (P=0.4); 54+/−8% vs 72+/−11% (P=0.4); 46+/−9% vs 48+/−15% (P=0.7) respectively. Age >35 y was found to be associated with higher NRM, and lower OS and DFS on univariate analysis. Relapse was significantly higher in the BMT group although the difference did not reach statistical significance due to small patient numbers. Chronic GVHD rates were comparable with BMT and BSCT. These key findings of this retrospective analysis of a homogeneous group of patients are in keeping with the findings of our prospective study comparing (Lancet 2000;355:1231-37). We conclude chronic GVHD rates are comparable with BMT and BSCT when the stem cell donor is an HLA-identical sibling and GVHD prophylaxis is rigorous. More powerful graft-versus-tumor effects associated with BSCT result in lower relapse rates. Blood should replace marrow as preferred source of stem cells for transplantation from HLA-identical siblings in conjunction with rigorous GVHD prophylaxis.

P393
Prospective evaluation of early allogeneic transplantation in patients with high risk acute myeloid leukemia defined by karyotype and response to first induction therapy treated in the Multicenter AML HD98A Trial
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Karyotype and response to induction therapy are the most important prognostic factors in acute myeloid leukemia (AML). In our ongoing treatment trial induction and consolidation therapy are stratified according to the karyotype and response to first induction therapy. High risk patients are defined either by karyotype (abn(3q), -5/-5q, -7/-7q, abn(12p), abn(17p), complex) or persistent AML after first induction therapy. Patients with persistent AML after ICE are assigned to second induction therapy with A-HAM (high-dose cytarabine, mitoxantrone, ATRA orally 45mg/kg/day d. 3-5 and 15mg/kg/day d. 6-28). Patients with partial (PR) or complete remission (CR) after ICE are assigned to a second cycle of ICE followed by one cycle of HAM. All high risk patients are assigned to allogeneic transplantation (Allo-Tpl) either from matched related donors (MRD), matched unrelated donors (MUD) or haploidential related donors (HRD). At the interim analysis from August 2001, 100 of 450 eligible patients were high risk patients. 30 high risk patients were defined by karyotype and 70 high risk patients by persistent leukemia after ICE. So far 55 patients received an Allo-Tpl: n=24 PBSC MRD, n=1 bone marrow MRD, n=26 PBSC MUD, n=4 PBSC HRD. The disease state before Allo-Tpl was CR, 11 PR and 16 patients with persistent leukemia. The conditioning regimens were as follows: n=23 TBI/cyclophosphamid(Cy), n=11 busulfan(Bu)/Cy (some patients with additional thiopeta or etoposide), n=13 antibody-targeted radionucleide therapy (188Re-Anti-CD66) and n=3 bisphosphonate-targeted radionucleide therapy (186Re HEDP) in combination with Bu/Cy(n=13) or TBI/Cy (n=2) or Fludarabine(Flud)/melphalan/thiopeta (n=1), n=3 Bu/Flud, n=1 Flud/Cy/idarubicin/etoposide, n=1 TBI/Flud. For rejection prophylaxis patients receiving grafts from MUD or HRD were treated with anti-thymocyte globulin. The median follow-up after Allo-Tpl is 16 months and treatment related mortality, relapse probability and disease free survival after Allo-Tpl at this time point are 27%, 45% and 28%, respectively. In univariate analysis CR before Allo-Tpl (p=0.05) and conditioning regimen with 188Re- Anti-CD66 (p=0.03) showed statistically significant better overall survival. In conclusion our approach of early Allo-Tpl in high risk AML is feasible.
P394
Results of allogeneic SCT for adult patients in the Netherlands
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Until January 1, 2001, 931 patients aged 16 years or older were given an allograft from an HLA-identical sibling for AML (N=442), ALL (N=205) or CML (N=284) in the 7 centers for allogeneic stem cell transplantation in the Netherlands. Median age of the 520 males and 411 females was 36 years (range, 16 to 61 year). In 70% of cases grafts were partially depleted of T-cells. The percentages of grafts obtained by stem cell apheresis of peripheral blood increased from 0% before 1993 to 48% from 1997 to 2001. From 1997 to 2001, the number of patients aged more than 50 years almost doubled to 46 in comparison with the period from 1993 to 1997. Recently, also nonmyeloablative transplants are performed, although the number is small (N=23). From 1993 onwards, 3-year probability of survival in patients < 40 years and transplanted for AML-CR1 or CML-CP1 (but not for ALL-CR1) significantly improved to 62% and 77%, respectively. This was caused by an impressive decrease of TRM (death in continuous complete remission) from 38% to 16% and from 49% to 21%, respectively. The results obtained with allogeneic SCT in the Netherlands are good and still improving.

P395
Treatment of leukemic relapse after allogeneic stem cell transplantation with cytoreductive chemotherapy and/or immunotherapy or second transplants

We analyzed toxicity and efficacy of chemotherapy (CT) or second stem cell transplantation (SCT) and/or immunotherapy defined as stop of immunosuppression (IS) or donor leukocyte infusion (DLI) in 51 patients relapsing with acute leukemia. Ten patients received no treatment and 14 patients were treated with CT only. In 15 patients IS was stopped and 3 of them received additional CT. Five patients received DLI after CT as consolidation and two patients as frontline therapy. Five patients received a second SCT. Median overall survival after relapse was 2 months for the untreated patients, 2 months for patients receiving CT only, 5 months in patients after cessation of IS, 16 months in DLI treated patients and 3 months in patients receiving a second SCT. Eighteen patients achieved remission after relapse, two with CT (2, 2 months), 6 with IS (3, 19, 11, 13, 35+ months), 7 with DLI (3, 8, 9, 14, 20, 36, 12+ months) and 3 with second SCT (2, 4, 6 months). Conventional CT was able to reestablish donor hematopoiesis and patients achieving remission showed a significant better survival than patients with refractory disease. Patients who were brought into remission by (DLI) or cessation of IS had a significant better survival than patients who achieved remission with CT alone or a second SCT. We conclude that a selected group of patients achieving remission with regeneration of donor hematopoiesis following CT might benefit from immunotherapy as consolidation.

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Outcome of autotransplantation in 118 adult acute myeloid leukaemia patients in first complete remission: impact of consolidation chemotherapy (CC)

Though biological variables like karyotype affect outcome of AML patients profoundly, it is important to identify variables which can be modified in clinical practice. 118 adult patients (16-62 y median 37M:44 F) with AML in CR1 were autografted between 11/85 and 1/01 using unpurged marrow (n=108;BM) or PBSCs (n=10) after 140 mg/m2 melphalan (110 mg/m2 in 18) and 1050 cGy single- fraction TBI(950 cGy-S). The CR-transplant interval was 5-246 d (median 118) was known in 81 patients: 38 favorable karyotype and 43 other (including normal). The total nucleated cell number (TNC) infused was 1.12-22.66 x 10^8/kg (median 2.45). Actuarial prob of recovering platelets at 1 y was 55 %. Neutrophil recovery was significantly faster with PBSC and with >=2x10^8TNC/kg, but not platelet recovery. 19 patients experienced non-relapse mortality (NRM) at 15-1603 d (median 124). 42 patients relapsed at 50-1306 d (median 229); 41 died and 1 is alive in CR2 13 y after relapse. The 5-y prob of relapse is 44%. 58 patients are alive (median survival 154+ months) post-transplant. The 5-y prob of OS and DFS are 40% and 42%. The following factors were analyzed in univariate fashion for their effects on outcome, and if significant (P<=.05) were added to Cox model: gender, age, FAB , karyotype, type induction therapy, courses of induction therapy to achieve CR, courses of CC prior to collecting autologous cells, exposure to high-dose ara-C, CR-auto interval (<120>/>120 d), TNC dose, source of cells (BM/PBSC), cryopreserved/fresh cells and melphalan dose. Patients who received >=2 CC had a significantly lower relapse rate (RR-2.34;P=0.02), OS (RR-1.88;P=0.03), DFS (RR-2.28; P=0.02) in the Cox analysis. The other variables that impacted favorably for a longer DFS were one induction course to attain CR (RR-2.01;P=0.02) and TNC dose of >=2x10^8/kg (RR-2.35;P=0.03). No factor affected NRM independently. The lack of impact of karyotype on outcome may have been due to missing karyotype results in 1/3rd of the patients. Outcome of the BM and PBSC groups was comparable. The TNC dose was comparable for patients who had received 0/1 vs >= 2 cycles of consolidation. We conclude that adequate consolidation therapy (in vivo purging) is important prior to the collection of cells and autotransplanting in AML and it is important to infuse an adequate quantity of nucleated cells. PBSC may be better source of cells quantitatively but longer-term data are needed on relapse rates and DFS.

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Risk factors for chronic GvHD after allogeneic peripheral blood stem cell transplants (allo-PBSCT) in patients with acute leukemia (AL)
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Although the reports of the comparisons between BM and PB as a source of HSC have not been conclusive, one concern of using allo-PBSCT is the probable high incidence of chronic GvHD (cGvHD). In order to study risk factors for cGvHD, we analyzed 371 adult recipients of HLA identical non T depleted PBSCT for AL (surviving more than 100 days and with sustained engraftment). Transplants were performed from 01/94 to 2001 and reported to EBMT registry. Median follow-up time was 20 months (3.3-72) and median age 35 years (4). Two hundred sixty-six (72%) patients had AML and 105 (28%) ALL: 231(62%) were transplanted in first CR, 58 (16%) in CR2 and 82 (22%) in more advanced phase of the disease. Median age of the donor was 36 years. There were 99 (27%) female donor to male recipients. CMV serology prior to PBSCT was positive (CMV+) in 51 (14%) donors.
and recipients, and negative in both in 108 (29%). Conditioning regimen varied according disease status and centers protocols, however irradiation based regimen was used in 163 (44%) patients and busulfan containing regimen in 125 (34%). GvHD prophylaxis was the most frequently used in CSA associated to MTX. Median number of nucleated cells infused was 9.8 x10^8/kg. Acute GvHD (I-II) was observed in 116 patients (31%). Estimate probability of cGvHD at one year was 51 ± 3%. Among patient, disease-, donor and transplant-related factors analyzed, the following variables statistically influenced the occurrence of cGvHD: i) female donor (60% versus male donor 43%, p<0.0002); ii) female recipient to male recipient (45% versus 46%, p<0.0001) and iii) the combination CMV+ donor to a CMV- recipient (67% versus 52%).

Previous presence of acute GvHD, as a time dependent covariate, also influenced the occurrence of cGvHD (RR:1.91, p<0.0001). In a multivariate analysis the most important factors were presence of acute GvHD (grade >II) (RR:1.83, p=0.001), CR1 status at transplantation (RR:0.63, p=0.016), female donor to male recipient (RR:2.43, p<0.0001) and CMV+ donor to a CMV- recipient (RR:1.45, p=0.043). In conclusion, chronic GvHD after allo-PBSCT is influenced by already known factors. We can speculate that the increased incidence of chronic GvHD after PBSC compared to BMT is probably related to specific biological characteristics of PBSC.

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**Unrelated hematopoietic stem cell transplantation for acute myeloid leukemia - A single center experience**

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Transplantation of hematopoietic stem cells (HSCT) from unrelated donor is increasingly used for the treatment of acute myeloid leukemia (AML). AML patients (pts) lacking a suitable sibling donor have a probability of approx. 80% to find an HLA-compatible unrelated donor. Between 1994 and 2000, 68 pts with primary AML received an unrelated HSCT in our center. We analysed the outcome, i.e. overall and disease-free survival in this group of patients. In addition, the outcome of this group of pts was compared to the results of 65 patients with AML treated with HSCT from related donors. Both groups were matched with regard to age, FAB classification, stage of the disease and HLA-compatibility of the donor. Median age of pts was 36 (range 16 to 55; unrelated) and 40 (range 22 to 61; related) years, respectively. The male to female ratio was 33/32 and 33/35, respectively. Conditioning for HSCT consisted either of a busulfan-based regimen in 65 pts (30 related, 35 unrelated) or total body irradiation in 68 pts (35 related, 33 unrelated). Graft-versus-host disease prophylaxis consisted of cyclosporine and prednisolone without (related transplants, 72%) or with methotrexate (unrelated transplants, 84%). Seventy nine % of pts with unrelated HSCT and 77% of pts with related HSCT have received an HLA-A, -B, and -DRB1 loci matched transplants. Median and average post-transplant follow-up was 8 and 18 months, respectively, with the longest overall survival being 6 years. Disease free survival (DFS) in the unrelated group of pts at 5 years was 54% for transplants transplanted in first complete remission (CR1) (n=16), 47% in second CR (CR2) (n=16) and 13% for advanced stages of the disease (>CR2) (n=36). Overall survival at 5 years was 47% (CR1), 36% (CR2) and 9% (>CR2). The cumulative incidences for relapse in the unrelated group were 13%, 19% and 44% for the three groups, respectively. DFS in pts treated with related transplants at 5 years was 42% (CR1) (n=25), 31% (CR2) (n=9) and 25% (CR2), (n=31). The cumulative incidences for relapse in related group were 20%, 22% and 48% for the three groups, respectively. In conclusion, the results of this comparative study confirm that unrelated HSCT is an important treatment option for patients with standard and high-risk AML, if sibling donors are not available.

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**Comparison of intensive chemotherapy (CHT), autologous (ALLO) or autologous (AUTO) stem cell transplantation (SCT) in children with very high-risk acute lymphoblastic leukemia (ALL). Results of prospective randomized PETHEMA-ALL93 trial**


Objective: to analyze the preliminary results of protocol PETHEMA ALL93 for children with very high-risk ALL (any of the following: age<1yr., WBC>200x10^9/L, (I<22 or 11q23 rearrangements, or slow response to induction therapy).

Design: Five-dose induction (CPV+PDN+DNR+ASP+CPM) followed by three consolidation cycles (including high-dose MTX, HD ARA-C and HD ASP). Patients with a HLA-identical sibling were assigned to ALLO-SCT and the remaining were randomized to AUTO-SCT or intensification CHT (the same three consolidation cycles) plus standard maintenance therapy for 2 yr.

Characteristics of patients: 57 evaluable patients, 9 hospitals, 39 males, mean age 6(SD4)yr., pro-B ALL 10, common+pre-B 21, T 25. Cytogenetics: t(9;22) 4, 11q23 8, other rearrangements 3, hypodiploidy 4, normal karyotype 19.

Response to therapy: CR 54(97%). 5-yr.DFS probability 53%(95%) 39-67). By intent-to-treat analysis, no differences in DFS were found for ALLO-SCT (n=16, DFS 60%,40-80), AUTO-SCT (n=18, DFS 58%,40-76) or intensification + maintenance CHT (n=19, DFS 41% (29-54).TRM was 18% for ALLO-SCT and 0% for AUTO-SCT.

Conclusion: the preliminary results of the PETHEMA ALL93 protocol for children with very high-risk ALL are promising. No differences in outcome have been observed according to the three options of post-remission therapy (ALLO-SCT, AUTO-SCT or intensification + maintenance CHT)

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**P400**

**Early data using fludarabine and IV busulfan ± ATG as myeloablative, yet reduced toxicity conditioning regimen for allogeneic transplantation in AML and MDS**

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We combined IV Busulfan (Bu) with Fludarabine (Flu) as conditioning therapy for allogeneic transplantation in AML and MDS because of a perceived potentiation by Flu of both the immunosuppressive and DNA damage repair inhibiting activities of Bu. Fludarabine, 40 mg/m2 IV was given daily for 4 days, each dose immediately followed by Busulfan, 130 mg/m2 IV over 3 hr (days −6 till −3). Ten patients with an unrelated donor received equine ATG on days −3 to −1. Stem cells/marrow were given on day 0. Results: We treated 20 AML and 3 MDS patients, 8 were refractory to chemotherapy, 3 in untreated relapse, and 9 in CR. There were 12 males and 11 females with a median age of 43 years (21-57). Clinically all patients engrafted (ANC 500) at a median time of 13 days (10-22), the time to 20,000 platelets/µL was 13 days (9-50), 2 individuals never had <20,000 platelets/µL. Assessed by PCR-based microsatellite DNA polymorphisms 17 patients were complete chimeras by day 30 while 2 had 97 and 98% chimerism, in 4 patients results are pending. There was no serious CNS- or lung toxicity and no VOD (Jones’ criteria). Transient hyperbilirubinemia was recorded in 2 patients unrelated to GVHD. Mucositis grade 2 was seen in 9 and grade 3 in 2 patients. One patient developed grade 3 hemorrhia, and one had transient pneumonitis. There was no treatment-related mortality up to day 100, but 3 patients died of recurrent AML before day 100. Pharmacokinetic analyses yielded mean busulfan plasma

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clearance of 2.4 ml/min/kg (1.3-3.9), average daily AUC of 5.516 µMol-min (3.990-8.271) without accumulation from day 1 and on, and a T½ of 3.1 hours (1.6-6.1), all of which are similar to the parameters obtained with IV Bu at 0.8 mg/kg/dose every 6 hours for 4 days. We conclude that IV Busulfan-Fludarabine is a well-tolerated, low-risk conditioning regimen for AML/MDS patients. Longer follow-up is needed to properly estimate disease-free survival in patients treated in CR vs. those having active disease.

P401
High-dose idarubicin and busulphan as conditioning regimen to autologous stem cell transplantation in acute myeloid leukemia
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Autologous stem cell transplantation (ASCT) is increasingly used for the treatment of acute myeloid leukemia (AML) in first complete remission (CR); however, following ASCT 30-50% of patients still relapse. We previously investigated the feasibility of a new conditioning regimen, called IBu, consisting of the combination of high dose idarubicin, given at 20mg/sqm as 3 days continuous infusion from day -3 to -1 and busulphan (4mg/kg from day -5 to -2). Here we report our updated experience on a series of 21 AML patients autografted in first CR and conditioned with IBu regimen. Patients with t(8;21) and inv(16) as well as those with M3 acute leukemia were excluded from the study. Twenty-one patients with a median age of 50 years (16-71) were treated. Fifteen had normal karyotype, 6 showed unfavorable karyotype. All transplants were performed using peripheral blood stem cells (PBSC) collected after consolidation treatment plus G-CSF. The median interval between diagnosis and ASCT was 4 months (3-6). The median number of CD34+ve cells infused was 6.2 x 10^6/kg (9.4 x 10^6/kg in all patients) ventricular ejection fraction (LVEF) was evaluated before and after ASCT. The median number of days with granulocytes <500/cmm and of platelets <20000/cmm was 11 (7-21) and 12 (9-95), respectively. The median number of platelet and blood units transfused was 3 (1-6) and 3 (0-12), respectively. Extra-hematological toxicity consisted of grade WHO III/IV stomatitis (86%), while 1 patient had grade III hepatic toxicity. Furthermore, 20 patients had F/UO, while one experienced fungal infection. No ASCT related death occurred; LVEF examination post-ASCT did not reveal cardiac toxicity in any patient. After a median follow up of 12 months (1-29), 16 patients are in continuous CR1, while 5 have relapsed at median time from ASCT of 8 months (3-8). Among relapsing patients, two had secondary AML with complex karyotype and one was aged 71; 3 patients died from progressive disease, while 2 achieved CR2 by salvage treatment. Median overall and disease free survival have not yet been reached after a median follow up of 12 months from transplantation. In conclusion, our data confirm the low toxicity of the IBu regimen in a series of AML patients with a median age of 50 years and suggest a possible reduction of relapse rate. Of note, patients with favorable cytogenetics were excluded from the study. These very encouraging results need to be confirmed in a larger series with longer follow-up.

P402
Busulfan, Vp-16, cytarabine, and G-CSf as conditioning regimen for patients with acute myeloid leukemia undergoing autologous stem cell transplantation. Preliminary results of Spanish PETHEMA study

Leukemic relapse is still the most frequent cause of treatment failure in patients with acute myeloid leukemia (AML) undergoing autologous stem cell transplantation (ASCT). The administration of new conditioning regimens is an alternative strategy to improve current clinical results. Between August/99 and October/01, 35 patients (15M/20F, median age, 45 years) with de novo AML were included in the Spanish PETHEMA LMA99 multicenter study. All the patients underwent ASCT in first complete remission. Conditioning regimen consisted of busulfan (1mg/kg/6h, p.o., days -8 to -5), VP-16 (200mg, i.v., days -4 and -3), Ara-C (3g/m2/12h, i.v. days -3 and -2), and G-CSF (10 mcg/kg, days -9 to -2). The median (range) number of days to neutrophil and platelet recovery was 11 (5-90) and 17 (6-112), respectively. The toxicity most frequently observed was gastrointestinal (24 cases) and cutaneous (9 cases). Two patients developed renal toxicity (1 grade 4) and two presented cardiac toxicity. With acute relapses developed fever (9 microbiologically documented, 9 clinically documented, and 8 fever of unknown origin). Median duration of hospitalization was 28 days (20-90). There were seven posttransplant deaths, four due to relapse, two treatment-related deaths (cerebral hemorrhage and multorgan failure), and one patient died 172 days after ASCT while in CR due to a respiratory infection. Eight patients relapsed between 1 and 15 months after transplant. Actuarial probability of disease-free survival and overall survival at 2 years is 62% and 75%, respectively. These preliminary results confirm a similar toxicity profile of this regimen as compared with the standard BUCY combination. More patients and a longer follow-up are required to know the anti-leukemic efficacy of this scheme.

P403
Impact of cytogenetics on the outcome of autotransplantation for acute myeloid leukemia in first remission: Is the benefit of intensive pre-transplant therapy limited to patients with good karyotypes?

81 patients (16-62 y, median 36) with AML and a known karyotype undergoing unpurged autografts in CR1 after melphalan-TBI were studied to explore the impact of cytogenetics (CG) on outcome. The CG groups were Favorable [t(8;21), inv(16), t(15;17); n=38] and Other (all the rest including normal; n=43). "Intermediate" and "adverse" karyotypes (Grimaldi et al. Blood 1998;92:2322-33) were combined because there were only 2 patients with adverse CG. 0 (n=7), 1 (n=19), 2 (n=51) or 3 (n=4) cycles of consolidation chemotherapy were administered prior to harvest and transplant. 12 patients experienced non-relapse mortality (NRM) at 15-1603 d (median 202). The 3-y actuarial probabilities of NRM, relapse, EFS and OS were 19, 36, 54, and 53% respectively. 26 patients relapsed at 60-1306 d (median 242) and died. 43 patients were alive and well at the last follow-up at 55-4681 d (median 1341). Infusion of 2.3 x 108 total nucleated cells/kg resulted in higher NRM (3-y probability 34 vs 5% for >2.3; P=0.02). In Cox analysis, favorable CG and lower-intensity consolidation (0 vs 1 cycles, 0-1 vs 2 cycles, or 0 vs 1 vs 2 cycles) were independently associated with lower relapse rates, and higher EFS and OS. The 3-y (0) relapse/EFS(OS in patients with favorable CG and 2 cycles of consolidation therapy were 9/83/87 compared to 71/17/25 in those with favorable CG and 0-1 cycles (P=0.0001<0.0001<0.0001). With the limitation of small patient numbers, it appears there is added benefit for patients with favorable CG with every additional cycle of consolidation therapy; thus, patients receiving 1 cycle of consolidation fare better than those receiving none, and those receiving 2 fare better than those receiving 1. However, in patients with other CG, while there is a clear benefit from 1 cycle of consolidation compared with none, the impact of further consolidation therapy is unclear because the outcome of patients receiving 1 cycle of consolidation is similar to those receiving 2 cycles. We conclude that administration of consolidation chemotherapy prior to harvest (in vivo purging) is essential to the success of autotransplantation in AML. While it is possible to enhance the benefit of consolidation in patients with favorable CG by delivering 2 cycles, the usefulness of this intensification in patients with other CG may be limited. Perhaps a completely

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novel approach is needed for the second consolidation cycle in these patients.

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Potential for therapy with leukemic dendritic cells derived from patients with acute myeloid leukemia

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Dendritic cells (DC) can facilitate immune responses that might help in the induction of effective antitumor T cell responses. We reported previously that leukemic blasts from selected patients with acute myeloid leukemia (AML) were able to differentiate in vitro into cells with mature DC features. However, despite the use of a wide variety of cytokine combinations, leukemic DC could not be obtained from all AML patients. In this study, we investigated in a wide range of AML patients (n=30), the nature and functional characteristics of the blast compartment that can be induced to acquire DC features in vitro.

Our results demonstrate that leukemic DC generated in the presence of GM-CSF, IL-4 and CD40L, are composed of two major subsets: leukemic DC derived from CD14+ blasts and leukemic DC derived from in vivo expanded circulating blood myeloid DC (MDC). Leukemic DC of both subsets exhibited DC morphology, had a phenotype of mature DC, and could induce a potent primary T cell response of naive CD4+ T cells. Moreover, both subsets produced large amounts of IL-12.

These results can be considered as a prerequisite before the design of vaccine immunotherapy protocols for the adjuvant treatment of AML patients.

P405

Salvage therapy with STI571 (glivec) prior to allocineous stem cell transplantation (Allo Sct) in relapsed or refractory Philadelphia-chromosome positive acute lymphoblastic leukemia (Ph+All)


Objectives: In Ph+ ALL failing chemotherapy, the ABL-tyrosine kinase inhibitor STI571 induces high initial remission rates, although responses are usually short. As allo SCT presently is the only curative treatment option for Ph+ALL, we investigated whether salvage therapy with STI571 may facilitate allo SCT in advanced Ph+ALL.

Patients: Of 67 consecutive Ph+ALL patients whom we enrolled in phase II studies of STI571, 31 were considered eligible for allo SCT based on performance status and age. Of these, 18 pts. underwent allo SCT, in 4 pts. allo SCT is scheduled within the next month. Reasons for not performing a transplant in 9/31 pts. were primary resistance to STI (n=5), relapse before SCT (n=2), no suitable donor (n=1) and patient refusal (n=1). Disease status at start of STI571 in n=18 transplanted pts was: refractory (n=11) or relapsed (n=7) (1st relapse n=5, 2nd relapse n=2) Ph+ALL.

Results: Median duration of STI571 was 48 (range 22-96) days. Allo SCT was performed a median of 66 (range 34-152) days after initiation of STI571. STI571 induced a complete cytologic response (CCR) in 12 (67 %) and a partial remission in 3 (17 %) of the 18 pts. subsequently transplanted. These responses were not sustained; however; at the time of allo SCT, 7 pts. were in CCR, 1 pt. in PR, and 10 pts. had relapsed or were transplanted with refractory disease. STI was discontinued 2 days prior to conditioning therapy. All pts. achieved engraftment with ANC >0.5/µl after a median of 14.5 (range 8-23) days and unsupported platelet counts > 50 x 10^9/l after a median of 18 (range 14-34) days. Two pts. undergoing a 2-antigen mismatch transplant experienced secondary graft failure due to grade IV GVHD and due to toxic reasons. Six transplant-related deaths occurred due to respiratory failure (n=2, d +32 and +72), secondary graft failure (n=2, d +52 and +60), multi-organ failure (n=1, d +5) and disseminated aspergillosis (n=1, d +103); transplant-related mortality correlated with the number of prior chemotherapies. 6/18 pts. developed grade III-IV acute GVHD. 6/18 pts. remain in CCR with a median follow-up of 202 (range 13-574) days. Probability of DFS is 54.6 ±16 % at 19 months post allo SCT.

Conclusion: STI571 is a promising salvage therapy for pts. with refractory or relapsed Ph+ALL scheduled for allo SCT, although the time interval available between start of STI571 and allo SCT is short, due to developing resistance.

P406

Imatinib (STI571) in preparation for allogeneic transplantation and donor lymphocyte infusions (DLI) in patients with Philadelphia positive acute lymphoblastic leukemia

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Imatinib (STI571) is effective in the treatment of Philadelphia positive acute lymphoblastic leukemia. However the remissions achieved are of only short duration. Allogeneic transplantation is a potentially curative treatment for these patients but the prognosis is significantly worse if performed with active disease rather than in remission. We have investigated the use of Imatinib to induce or maintain remission prior to allogeneic transplantation. Seven patients were included in the study. 4 male, 3 female, median age 29.5, range 16-43. 6 pts. had Ph positive ALL and two had CML in lymphoid blast crisis. Four patients had allogeneic transplantation from HLA-matched siblings and three from matched-unrelated donors. Six were given Imatinib prior to transplantation and one prior to DLI. Imatinib was administered to induce remission after failure of prior chemotherapy (n=5), and to maintain remission during a search for an unrelated donor (n=1) or while being treated for fungal pneumonia (n=1). Imatinib was given at 600 mg/day and was well tolerated. Response was achieved in all patients within a median of two weeks. Six patients maintained complete hematological and cytogenetical remission for a median of 4 weeks (range, 2-16) prior to transplantation or DLI. One has relapsed after two months of treatment while waiting for unrelated donor transplantation but was successfully re-induced with further chemotherapy prior to transplantation. With a median follow-up of 6 months (range, 4-10) 5 of 6 patients treated prior to transplantation are in continuous CR and 1 is too early to evaluate. The patient treated prior to DLI relapsed after 4 months. He was induced into remission again with Imatinib and awaits further therapy. In conclusion Imatinib (STI571) is effective in achieving remissions prior to allogeneic transplantation or DLI. It can also be used to maintain remission while searching for a unrelated donor or while treating a complication, considered a temporary contraindication to transplantation. Although Imatinib can not be considered curative treatment in these settings it opens a window of opportunities for transplantation in a more favorable status.

P407

Autologous transplantation of not cryopreserved bone marrow - A good option for high-risk adult ALL patients

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Between 1991 and 2001 autologous bone marrow transplantation (ABMT) was carried out in 114 high risk ALL patients (CR1 – 89 pts, CR2-2 – 25 pts; median age 23, range 15-53). Conditioning regimen (CAV) consisted of cyclophosphamide 60 mg/kg on days –3, –2, etoposide 800 mg/m2 d. –3, –2 and cytarabine 1000 mg/m2 d. –3, –2, -1. Bone marrow was not cryopreserved but stored in temporary contraindication to transplantation. Although Imitinib (STI571) is effective in achieving remissions prior to allogeneic transplantation or DLI. It can also be used to maintain remission while searching for an unrelated donor or while treating a complication, considered a temporary contraindication to transplantation. Although Imitinib can not be considered curative treatment in these settings it opens a window of opportunities for transplantation in a more favorable status.
20-72) or 19 (14-58) days when calculated since the date of ABMT. The probability of overall survival at 9 years was 61% for patients in CR1 and 28% for patients in CR2; the probability of relapse equalled 46% and 79%, respectively. None of the analyzed variables (age, sex, WBC at diagnosis, and phenotype, time to achieve CR1) was found to influence the outcome, except of the stage at transplantation (CR1 vs. CR2, p=0.02).

Conclusions: ABMT without cryopreservation, using CAV regimen seems to be a good option for patients with high risk ALL in 1st CR who don’t have sibling donor. Fast recovery and low transplant related mortality makes it an alternative to PBSCT.

**P408**

Radioimmunotherapy to intensify conditioning prior to stem cell transplantation in pediatric high-risk leukemias


Radioimmunotherapy with radiolabeled monoclonal antibodies (MABs) represents an attractive concept to intensify the preparative conditioning prior to haematopoietic stem cell transplantation (SCT). We used Rhenium-188 labelled CD66 (NCA-95) antibodies in 9 pediatric patients aged between 1 and 16 years. All presented with high risk leukemias: refractory AML (n=2), early relapsed AML in CR2 (n=1) or PR2 (n=2), relapsed AML after stem cell transplantation (n=1), ALL in PR2 (n=1) or PR3 (n=1), biphrenotypic leukemia in CR1 (n=1). Radiation dose to bone marrow was >12 Gy (12.5 to 34.5 Gy) in 4 of 9 patients, with acceptable doses to liver (1.8 to 10 Gy), kidney (4.05 to 11 Gy) and lung (0.29 to 1.4 Gy). Following radioimmunotherapy, patients received standard conditioning, followed by allogeneic stem cell transplantation from HLA-identical related donors (n=4) or matched unrelated donors (n=5). Eight patients achieved stable haematopoietic engraftment. Death was due to treatment related mortality in 5 patients (PCP-pneumonia n=1, toxicity n=1, aGVHD n=2, haemolytic uremic syndrome n=1) and due to relapse in only one patient. Three patients are alive 1, 2, and 6 months after transplantation. One relapsed with ALL on day +137.

Our experience demonstrates the feasibility of this approach which may enhance the antileukemic effect of conditioning in patients at high risk for relapse following stem cell transplantation.

**P409**

Unpurged autologous peripheral blood stem cell transplantation for acute myeloid leukemia - Influence of the dose of collected nucleated cells on post transplant outcome

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This study evaluated the impact of the dose of nucleated cells collected from peripheral blood for autografting, on leukemia free survival (LFS), relapse incidence (RI) and transplant related mortality (TRM), in adult patients (>16 years old) with acute myeloid leukemia (AML) in first complete remission (CR1) receiving high dose therapy and unpurged autologous peripheral blood stem cell transplantation (PBST).

A total of 372 patients reported to the EBMT and transplanted from January 1988 to December 2000 (median: 1996) were analysed. The median age was 46 years old, sex ratio (M/F) 1, and median leucocyte count at diagnosis 14x10^9/l, median time from diagnosis to CR1: 36 days, median time from CR1 to PBST: 110 days. 78% of patients received one induction course of chemotherapy to reach CR1. 59% of patients received 0 or 1 consolidation course of chemotherapy before collection and 29% two courses. 44% of patients did not received additional consolidation course of chemotherapy from collection to PBST and 38% one to two courses. Total body irradiation was part of the conditioning regimen for 85% of patients. The dose of peripheral blood nucleated cells collected for transplantation ranged from 0.06 to 313x10^8/ kg (median value: 8.41x10^8/ kg).

Univariate analysis showed that the dose of collected nucleated cells from peripheral blood was a prognostic factor on outcome: patients with a dose below the median value (8.41x10^8/ kg) showed a LFS of 42±5% and over the median value 52±4% (p=0.016). For RI results were 53±4% and 45±4% (0.048) respectively. No difference was observed for TRM: 9±2% and 6±2% (p=0.12) respectively. The number of consolidation course of chemotherapy before and after collection did not influenced outcome. By multivariate analysis collected nucleated cell dose remained a prognostic factor on LFS: p=0.006, RR:0.83 (0.52-0.96).

Concluding, the dose of peripheral blood nucleated cells collected for autografting in adult patients with AML in CR1, is a prognostic factor on post transplant outcome by univariate and multivariate analysis: higher doses of nucleated cells are related to a higher LFS and a lower RI.

**P410**

Does donor-recipient ABO-incompatibility protect against relapse after allogeneic bone marrow transplantation in first remission acute myeloid leukemia?

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We have previously reported lower relapse rates with donor-recipient ABO-incompatibility in a group of 85 adults and children with AML in CR1 (Bone Marrow Transplant 1996; 18:741-6). We have now studied this in a total of 119 AML patients (15 y) allografted in CR1 from HLA-identical siblings after Cy-TBI(n=72) or Mel-TBI(n=47). GVHD prophylaxis in the Cy-TBI group comprised cyclosporine alone, and in the Mel-TBI group, cyclosporine-methotrexate. All patients received non-T-cell depleted marrow which was depleted of red cells and/or plasma if there was donor-recipient ABO mismatch. PBSC recipients were excluded because of lower risk of relapse (Lancet 2000; 355:1231-37). 15 Mel-TBI and 41 Cy-TBI patients received 950 cGy TBI, 32Mel-TBI and 25 Cy-TBI patients 1050 cGy TBI, and 6Cy-TBI patients 1150 cGy TBI. 18 relapses were seen amongst 76 patients with ABO-matched donors, and 4 relapses were seen amongst the 43 patients with ABO-mismatched donors. Incidence of acute and chronic GVHD, non-relapse mortality was not significantly different between ABO-match and mismatch groups. The 3-y probability (%) of relapse/OS/EFS in patients with ABO match (n=76) was 30/48/46 compared to 9/70/67 for those with ABO mismatch (n=43), P=0.03/0.06/0.04 respectively. In Cox analysis, donor-recipient ABO mismatch was the only factor found to be independently associated with a reduced risk of relapse (RR=0.27; 95% CI, 0.07-0.89; P=0.04). Donor-recipient ABO mismatch was also associated with superior OS and DFS in Cox analysis in addition to age<35 and Mel-TBI conditioning. It is possible that minor (O/B/AB, A/BB) and bidirectional (AB, BA) ABO incompatibility, where the donor possessed hemagglutinins in addition to age<=35 and Mel-TBI conditioning, it would be interesting to see if donor-recipient ABO mismatch contributes any further to the enhanced anti-tumor effects already seen with allogeneic PBSC.
Early recovery of total white cell (WC) count is an independent prognostic predictor for outcome and length of CR in newly-diagnosed patients with AML treated with intensive induction chemotherapy - A single center study of 103 patients

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Assessment of WC count recovery after the first course of intensive induction chemotherapy in de novo patients with AML could be a surrogate marker of outcome. 103 patients over the age of 15 (15-62 y, median 37-40F, 63M) with AML received induction with BF12 (Mehta et al; Seminars in Hematol 1996;33:18-23; Cytarabine 2g/m² i.v. bd, d 1-5; Etoposide 100 mg/m² i.v. od d1-5; Idarubicin 5 mg/m² i.v. d 1-3). The WC response to induction was evaluated in those patients who attained CR and died (sepsis, 3 bleeding). 86/103 received 1-3 (median 2) courses of consolidation chemotherapy and 11 more entered CR. 80 (78%) patients (73 in CR1, 7 refractory disease) had a stem cell transplant (1 syngeneic, 19 allogeneic, 60 autologous) at 35-692 d (median 325). The median overall survival (OS) is 58 mo: the 4-y probability being 50% (95% CI, 49-72%); median has not been reached yet; 4-y event free survival (EFS) 43% (95% CI, 47-57%). Fifty-two patients are alive at a median follow-up of 2 y (2 d – 11.3 y). Fifty-two patients are alive at a median follow-up of 2 y (2 d – 11.3 y). The following factors were analysed in univariate and multivariate fashion for their effects on OS, EFS, RFS and duration of CR: gender, age, FAB, WC, platelet count, WC and platelet on d 16 and 23 post BF12 (corresponding to d 21 and 28 from start of BF12) and karyotype. The median WC on day 16 post BF12 was 0.9 x 109/L (range, 0.1-24.1). In the Cox analysis, patients with favorable cytogenetics (RR-4.3; P=0.02) and recovery of WC to >0.9 x 109/L on d 16 (RR-2; P=0.03) had longer OS; favorable cytogenetics (RR-5.1; P=0.005) and recovery of WC to >0.9 x 109/L on d 16 (RR-0.3; P=0.01) had longer EFS; recovery of WC to >0.9 x 109/L on d 16 predicted for a longer relapse-free survival (RR-0.3; P=0.01) and longer duration of CR (RR-0.4; P=0.03). Similar results were obtained with WC on day 23 post-BF12. In conclusion, our data suggest that poor WC recovery post-BF12 is associated with an increased risk of relapse and could be the basis for early planning of alternative treatment.

Quantitative evaluation of the MDR1, BCRP, WT1 gene expression in acute leukemia

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The Multidrug Resistance (MDR) phenotype is frequently found in patients with myeloid acute leukemia (AML). Results from qualitative molecular assays are often contradictory; so, we tested the expression of MDR1 and BCRP, already described as genes involved in the Multidrug Resistance phenomenon, by a quantitative technique (Genescan PCR). The WT1 expression was also tested, according to the clinical prognostic role recently shown for this gene by several authors. Thirty-five AML patients (15 male, 20 female, median age 58 years) were evaluated in this retrospective study. FAB subtypes resulted: 2 M0, 5 M1, 13 M2, 7 M3, 2 M4, 3 M5, 1M7. With a median follow up of 68 months (range 7-131), 23% of patients are still alive. Thirty-one patients were evaluable for response to induction therapy. CR, achieved in 48%, did not correlate with age, sex and FAB subtype. MDR1 gene was expressed in 60% and both BCRP and WT1 in 66%.
HL is defined as leukocyte count of >=100x10^9/L. Diminished deformability, altered size and surface adhesion molecule expression makes blasts "sticky" and this with HL results in leukostasis which can cause catastrophic vascular events. We leukaemise AML patients with HL at presentation. A retrospective analysis was done to assess the impact of this practice. Of 518 previously untreated AML patients, 72 (14%) had HL (101-513 x 10^9/L; median 174). 50% of the patients with HL had FAB M4/M5 subtypes compared with 45% of the remainder (P=0.02). 15% of the patients with HL (11/72) died within 14 d compared with 5% of the remainder (24 of 446; P=0.0002). Amongst 72 patients with HL, the 11 patients dying early had higher leukocyte counts (124-513 x 10^9/L; median 257) than remainder (101-429 x 10^9/L; median 168; P=0.0004). There was a significant correlation between increasing leukostosis and the 14-day death rate: 100-199 (7%), 200-299 (17%), 300-399 (40%), 400-499 (100%). HL in leukapheresis patients was associated with poor outcome. The leukocyte count in these patients was reduced to 49-326 x 10^9/L (median 95) post-apheresis. Only 1/35 patients with successful reduction in the count died early compared with 9/26 with persistent HL (P=0.001). The most recent 110/518 patients treated on uniform induction regimen comprising idarubicin, high-dose ara-C and VP-16 were studied to determine effect of HL on remission. 11/110 patients had HL. 2 of 11 died early within 2 weeks (P=0.03). CR rates with 1 cycle of induction chemotherapy (7/11 vs 70/99; P=0.5) and overall CR rates (8/11 vs 85/99; P=0.3) were not affected adversely by HL. No correlation was seen between cytogenetics and HL. Despite intensive consolidation therapy comprising alogeneic or autologous transplantation, 4/7 HL patients relapsed within 6 mo of attaining CR (2-y probability of relapse 67%). For the 70 patients without HL, the 2-y prob of relapse lower at 24% (P=0.0004) and the total number of relapses was 15 as of 7/01. We conclude that HL is a critical risk factor for early mortality in patients with AML and should be considered as oncologic emergency. Lowering the leukocyte count to rapidly by leukaapheresis is vital for reducing early mortality. Biologic immunophenotyping of HL in terms of high relapse rates is unaltered by leukaapheresis, and need to be studied further in context of karyotype abnormalities.

Relapse after HSCT in childhood acute leukemias is related to higher in vitro drug resistance to most drugs except for etoposide, treosulfan and thiopeta


Current possibilities to potentiate antileukemic effect of HSCT include: protection of GvHD, introduction of adoptive immunotherapy, use of more intensive or tailored preparative regimen (prep-reg) before HSCT or modulation of drug resistance of residual leukaemic cells against agents used in prep-reg. The aim of this study was the analysis of in vitro drug resistance profile in aspect of leukemia relapse after HSCT. A total number of 24 children with acute leukemias (15 ALL, 9 AML), aged 1.9-17 years, who underwent HSCT, were included into the study. Drug resistance profile was done by the MTT assay in reference laboratory for all children. Leukemic cells of each child were tested for cytotoxicity of up to 26 drugs. In 15 children HSCT was performed from HLA identical sibling, in 2 from MUD, in 1 from MMRD and 6 children had autotransplantation. Children with ALL were prepared to HSCT with: FTBI/VP/CY (6 patients), BU/VP/CY (9 patients) or BU/CY/MEL (1 child). ATG was also given before MUD-HSCT. AML children were conditioned with one of the following regimens: BU/VP/CY, BU/CY/MEL, BU/VP/CY, TREO/VP/CY, TREO/FLU/ATG, TRE/FLU/MEL. 8/24 children relapsed after HSCT. Children who relapsed after HSCT showed higher in vitro resistance of leukemic blasts to most of tested drugs including cyclophosphamide (3.3-fold), fludarabine (2.3-fold), cytarabine (2.4-fold), mafosfamide (2.3-fold) and doxorubicin (6.5-fold). Chemosensitivity of relapsed patients was better only for 4/26 tested drugs: treosulfan (1.7-fold), thiopeta (4.6-fold) and mercaptopurine (1.7-fold). Relapsed patients more often than others received etoposide in prep-reg (75% vs 62.5%). Only 1/6 children with ALL prepared with FTBI relapsed after HSCT. The results of this analysis might suggest that relative sensitivity of leukemic blasts to treosulfan and etoposide is not sufficient to prevent patient from relapse after HSCT. It is possible that in vitro drug resistance profile might give a suggestion for choosing prep-reg before HSCT or DLI in children with acute leukemias.

Internal tandem duplication and D385 mutation analysis of Flt3 gene in AML patients

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The Flt3 gene encodes a tyrosine kinase receptor that regulates proliferation and differentiation of hematopoietic stem cells. A somatic internal tandem duplication of the Flt3 gene in the juxtamembrane domain-coding sequence (Flt3/ITD) has been reported in acute myeloid leukemia (AML) and seems to be more frequent in patients with poor prognosis compared with the general AML population 

Internal tandem duplication and D385 mutation analysis of Flt3 gene in AML patients from 1995 to date, for the presence of the Flt3/ITD. Exons 14 and 15 (and not 11 and 12, as previously named) of the Flt3 gene were amplified by genomic polymerase chain reaction (PCR). Three samples (25%) were positive for the Flt3/ITD and the location and the size of the duplication varied from sample to sample. Nucleotide sequencing of the abnormal PCR products is in progress. One patient had a single aberrant PCR product indicative of the Flt3/ITD and at the moment we are investigating the loss of heterozygosity of chromosome 13 containing the Flt3 gene. None but one of the Flt3/ITD positive patients had unfavorable cytogenetic marker and there was not predominance of a particular FAB class. The remission induction rate (CR) was 66% in the Flt3/ITD positive patients compared with 73% in the Flt3/ITD negative ones (P=0.3). Overall survival for patients with the Flt3/ITD was 33% compared with 77% for patients without the Flt3/ITD (P=0.028). The event-free survival at 1 year for patients with and without Flt3/ITD were 33% and 55% respectively (P=0.04). In conclusion, Flt3/ITD was not associated with the overall CR rate and patients with a Flt3/ITD tended to have a lower overall survival and disease-free survival, although these differences were not so highly significant, since the sample number was not enough to make statistical difference and the median follow-up period of our patient population is 8.5 months, which limits the predictive value of our data. Moreover, we confirmed that the presence of a Flt3/ITD is strongly associated with high absolute leukocyte count, a very poor prognostic factor in AML, since 2/3 patients with the Flt3/ITD had hyperleukocytosis compared with 4/9 patients without the Flt3/ITD. At the moment, we are carrying out the analysis of the novel mutation of the key regulator residue D385 of Flt3 gene in the same cohort of AML patients, although it is still not demonstrated that D385 mutation significantly affect any clinical variable or prognosis.
A, B, and C are potent mitogens for endothelial cells expressing regulatory molecules. Vascular endothelial growth factors (VEGF) endothelium. These cell lineages could also have common transplantation, relapsed a few months after autologous BMT. undetectable (2 patients) or very low (1 patient) before and after remission with a follow up ranging between 10 and 14 months. In their receptors (VEGFRs). Hypoxic tumor cells secrete the molecules in human acute leukemia and CML. PolyA RNA was extracted from the BM or PB mononuclear cells of consecutive hematopoietic colony stimulating factors in human vascular VEGF has also been reported to stimulate expression of increased angiogenesis has been reported in many tumor types angiogenesis through endothelial cell migration and proliferation. VEGFs, which stimulate the receptors resulting in tumor hypoxia. Direct effects in normal hematopoiesis. The results show that VEGF-A, B and C are widely expressed in human leukemia. However, their functional role in this setting remains unclear. In the few cases where the corresponding receptors were detected, an autocrine loop could exist. However, aberrant expression of VEGF without a functional role in leukemogenesis cannot be ruled out. On the other hand, no specific effects on cellular kinetics could be detected when VEGF-A or C was applied to human LT-BM cultures, suggesting that these growth factors do not have major direct effects in normal hematopoiesis.

Immune CD34+CD19- cells in TEL/AML1-positive childhood ALL are normal - implications for stem cell purging and immunotherapy

Background: Childhood ALL is assumed to originate in a lymphoid progenitor cell. However, recent molecular analyses in high risk patients provided evidence for the involvement of a more primitive leukemic process. A or C was applied to human LT-BM cultures, suggesting that donor allograft. A longer follow up and enrolment of a larger number of patients will be necessary to establish the role of autologous antileukemic activity in the control of minimal residual disease in ALL patients as well.

Expression of vascular endothelial growth factors and receptors in acute leukemia

R. Alitalo, S. Mustjoki (Helsinki, FIN) Hematopoietic and endothelial cells have been shown to have a common precursor, the hemangioblast. This cell has not been well characterized, but incidental data from transplant settings have provided evidence that PB or BM derived cells can generate hematopoiesis. These cell lineages could also have common regulatory molecules. Vascular endothelial growth factors (VEGF) A, B and C are potent mitogens for endothelial cells expressing their receptors (VEGFRs). Hypoxic tumor cells secrete the VEGFs, which stimulate the receptors resulting in tumor angiogenesis through endothelial cell migration and proliferation. Increased angiogenesis has been reported in many tumor types including hematopoietic malignancies, e.g. multiple myeloma. VEGF has also been reported to stimulate expression of hematopoietic colony stimulating factors in human vascular endothelial cells.

We have studied the mRNA expression of several angiogenic molecules in human acute leukemia and CML. PolyA RNA was extracted from the BM or PB mononuclear cells of consecutive patients with sufficient material in the diagnostic sample. The diagnosis and type of acute leukemia were based on morphology and immunophenotype. Northern blotting and hybridization with radioactive probes were used for mRNA detection. 26/26 cases of AML were positive for VEGF-A and C, 3/28 for VEGF-C. Sporadic cases were positive for VEGF-receptors 2 or 3 (1/27 and 4/25). Also ALL blasts in all cases expressed VEGF-A (3/3) and -B (5/5); VEGFR-3 RNA was found in 3/5 whereas VEGF-C or receptor-2 were not detected (0/5). In CML mRNA for VEGF-A was found in 4/4, -B in 6/6 and -C in 1/4 cases; all were negative for VEGF-2 and -3.

The results show that VEGF-A, B and C are widely expressed in human leukemia. However, their functional role in this setting remains unclear. In the few cases where the corresponding receptors were detected, an autocrine loop could exist. However, aberrant expression of VEGFs without a functional role in leukemogenesis cannot be ruled out. On the other hand, no specific effects on cellular kinetics could be detected when VEGF-A or -C was applied to human LT-BM cultures, suggesting that these growth factors do not have major direct effects in normal hematopoiesis.
Additional topics to this topic

Allogeneic hemopoietic stem cell transplantation for acute myeloid leukemia in first complete remission - Low transplant mortality despite increasing age

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Transplant mortality (TRM) is the limiting factor when discussing treatment strategy in patients with AML in first complete remission. We have analyzed 174 patients salvaged from an HLA identical sibling in our Unit between 1980 and 2001. Most patients (n=162) were conditioned with cyclophosphamide 60 mg/kgx2 and total body irradiation (3.3 Gyx3), whereas GvHD prophylaxis consisted of methotrexate (MTX) alone (n=2), cyclosporin (CyA) alone (n=81) or CyA+MTX (n=74) . 7 patients received in vivo T cell depletion. The source of stem cells was bone marrow (n=167) or peripheral blood (n=7). Patients were divided in 3 groups according to the year of transplant : (a) <=1988 (n=67), (b) 1989-1993 (n=55) and (c) 1994-2001 (n=52). The median age (range) in the 3 transplant eras was respectively 24 years (1-46), 29 years (8-46) and 38 (17-59) (p=0.00001 between <=1988 and >1993). The median dose of cells infused was <dose TRM was 28%, 16% and 4% (p=0.002), survival 43%, 71%, 81% (p=0.0001). Relapse was unchanged 30%, 18%, 21% (p=0.02). Acute graft versus host disease grade III-IV developed in 13%, 5%, 2% of the patients (p=0.04) and extensive chronic GvHD in 18%, 34%, 38% (p=0.06).

The only significant difference in the 3 groups was median bone marrow cell dose: 2.4x10^8/kg, 4.5x10^8/kg and 4.6x10^8/kg. We confirm significant reduction of TRM despite increasing patient age. The current actuarial 3 year TRM of 4% makes allogeneic stem cell transplant an attractive therapeutic option for patients in first complete remission.

Allogeneic stem cell transplantation (allo-SCT) in patients with advanced-phase acute leukemia

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Allo-SCT remains the most potent therapy for acute leukemia offering the possibility of cure even to patients with refractory or relapsed disease. In order to determine which particular subgroup of these patients may benefit from the procedure, we retrospectively studied the outcome of allo-SCT in 41 patients, aged 12-57 years (median, 37.5) with acute leukemia (AML in 28, ALL in 8 and other type in 5 patients), whose disease was in an advanced phase at the time of transplant (primary refractory: 13, untreated relapse: 17, resistant relapse: 11 patients). The conditioning regimen was combination chemotherapy in all cases. The patients received non-T-cell-depleted grafts from HLA-identical siblings (n=28), other family members (n=9) or matched unrelated donors (n=4). Of 39 evaluable patients, 37 achieved a complete remission after allo-SCT. Eleven continue to be alive and disease-free for 24-108 months. There were 11 deaths due to transplant-related complications, and 19 patients relapsed. The projected 3-year disease-free survival (DFS) for the whole group of patients is 27% (SE, 7%). In univariate analysis, transplantation from an HLAmatched sibling donor, diagnosis of de novo AML, percentage of blasts in bone marrow less than 20%, and absence of blasts in peripheral blood at transplant correlated favorably with outcome. Consequently, for the 21 patients with de novo AML who received a transplant from an HLA-identical sibling donor, 3-year DFS was 42% (SE, 11%). In conclusion, our study confirms that certain patients with advanced-phase acute leukemia can be cured by allo-SCT. The outcome of the procedure is superior in patients with AML possibly due to a more potent graft-versus-leukemia effect. Allo-SCT is mandatory in patients with advanced-phase leukemia although in patients with refractory ALL other experimental treatments can be explored.

Factors influencing hematopoietic recovery after autologous peripheral blood stem cell transplantation (PBSCT).


We analysed factors influencing hematopoietic recovery in patients treated with high dose chemotherapy (HDC) followed by PBSCT. We retrospectively evaluated 410 transplants performed in our Center from February 1993 to October 2001. Mean age of patients was 45.2 +/- 12.3 and 63.6% of patients were female. Fifty-three percent were affected by hematological malignancies (34 acute leukemias, 72 lymphomas, 103 multiple myelomas, 2 chronic myeloid leukemias, 6 other malignancies) and 47% by solid tumors (mainly breast cancer). High dose chemotherapy consisted of Melphalan (~69.7%), Thiotepa (~15.4%) and Busulfan-containing regimens (~8.3%). We infused a mean dose of 6.6 x 10E6/Kg + 3.7 CD34+ cells. G-CSF (5mcg/Kg/d) was administered in 363 cases after PBSCT at day +3. Mean time to ANC > 0.1 and 0.5 x 10E9/L was 9.4 and 10.3 days, respectively. Mean time to platelets > 30 and 50 x 10E9/L was 13.2 and 15.8 days, respectively. Mean requirement of RBC and platelet transfusion was 1.9 and 4.7 units, while the mean duration of febrile episodes was 2.4 days.

In hematological diseases, factors influencing engraftment of platelets >30 x 10E9/L and ANC > 0.1X10E9/L in multivariate analysis were the type of malignancy, with a poorer prognostic impact for acute leukemia. In solid tumors, the only predictor of platelet recovery >30 x 10E9/L was the number of CD34+ cells infused, while no factor, amongst those analysed, independently influenced neutrophil recovery, possibly due to the high number of CD34+ infused and G-CSF post-transplant in this group of patients. In conclusion, the infusion of a larger number of CD34+ cells accelerates engraftment after PBSCT. Slower recovery in acute leukemia patients may be related to functional stem cell defects.

Extramedullary relapse of acute myeloid leukemia after the second allogeneic stem cell transplantation, first manifested as an intussusception of the intestine

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Intussusception is a rare complication of acute leukemia, occurring mainly in children with acute lymphoblastic leukemia, occurring during or after chemotherapy. The complication is extremely rare in adult patients with acute myeloid leukemia (AML). We present the case of 38 year old AML patient with relapse after the 2nd allogeneic stem cell transplantation (alloSCT), first manifested as an intussusception of an intestine. To our knowledge this is the first case of this kind of relapse in an AML patient after allogeneic stem cell transplantation.

The patient developed abdominal cramps, diarrhoea, nausea and vomiting 6 months after the 2nd alloSCT. His peripheral blood and bone marrow examinations at this time were normal. The ultrasonography revealed the presence of pathologial mass (129x58x58 mm) at the beginning of the transverse colon and the liver bend of the colon, with a feature of target sign and sandwich sign which were suspected to have been caused by an intussusception of the colon. The explorative laparotomy had shown massive infiltration of the colon and the root of mesentery with segmental occlusion of the colon due to invagination. Histopathological examination of the biopsy revealed the leukemic infiltration (CD45+, CD34+, myeloperoxidase-positive). The patient died 3 weeks after the operation as a result of infectious complications and the progression of leukemia. Awareness of the possibility of this kind of complication may alert the physician to perform an ultrasound examination at an earlier time, and to possibly avoid the need for late surgery. An operation performed earlier may, in some cases, be helpful to precisely diagnose an isolated relapse. Timely diagnosis and early re-induction treatment
would probably result in prolonging the life of some patients in these rare cases of AML relapse limited to bowel.

**Fludarabine and cytosine arabinoside as salvage therapy in patients with relapsed acute and chronic leukemia after stem cell transplantation**

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Background: Chemotherapy with Fludarabine and Cytosine Arabinoside is an effective treatment option for relapsed ALL. The aim of this study was to evaluate its use in relapsed patients with acute and chronic leukemia after allogeneic stem cell transplantation.

Patients and methods: 14 patients (10 male, 4 female) with relapsed AML (n=5), ALL (n=4) and CML (n=5) after BMT (n=3) or PBSCT (n=11) were studied. The median age of patients was 36 years (range 19-57 years). Patients with CML were transplanted in 1st CP (n=2), acceleration (n=1) or blast crises (n=4) and relapsed in blast crises (n=4) or stable chronic phase (n=1). Patients with acute leukemia underwent transplantation in 1st CR (n=1), 2nd CR (n=1), 2nd partial remission (n=1) 1st relapse (n=1) or 2nd relapse (n=5). Relapse after transplant occurred at an average time of 386 days (range 59-1773 days). Chemotherapy consisted of Fludarabine 30mg/m2 and Ara-C 100mg/m2 for 5 days. Post chemotherapy 10 of 14 patients received also unfractioned DLI.

Results: Thirteen (93%) patients achieved complete remission. But 9 of 13 patients (69%) relapsed again. Duration of complete remission ranged from 80 to 781 days with a median of 314 days. Up to date 7 of 14 patients are alive. The median survival was at 362 days. Four of the 7 patients who are alive are still in complete remission, the median survival in this group is at 473 days. The main side effect was hematotoxicity and worsening of GVHD of skin and liver.

Conclusions: Chemotherapy with Fludarabine and Cytosine Arabinoside is an effective antileukemic regimen in patients with advanced relapse stages of leukemia after allogeneic stem cell transplantation, which can induce long lasting complete remissions.

**Stem cell transplantation for acute myeloid leukemia**


Annual stem cell transplantation (SCTx) number is increasing despite special circumstances dominated by limited economic resources in health care system in Slovakia. Acute myeloid leukemia is the second most frequent indication for alloTx and the first for autoTx in University Hospital Bratislava. Results of first 50 patients are presented.

Allo SCTx: total 32 HLA-identical sibling SCTx in 29 patients for acute myeloid leukemia since 1990: 16 in CR1; 13 in >CR1. 2nd SCTx with peripheral stem cells from original donor was performed to 3 pts due to leukemia relaps after 1st SCTx. Busulfan based conditioning (BuCy2/E-BuCy) and standard GVHD (MTX+CsA) prophylaxis were used. At the time of analysis (October 31, 2001) 16 patients are alive (11/16 in CR1 and 5/13 with >CR1) and 13 patients died (5/16 in CR1 and 8/13 with >CR1), median time of observation 12 months. The estimated probability of disease free survival in patients with >CR1 AML is 36% only, but 65% in the group transplanted in CR1.

For those patients lacking an HLA-identical sibling autologous HSC transplantation (ASCT) is one of the treatment possibilities. Results of 21 patients with AML are presented. There were 10 males and 11 females in the age of 20-61 (median 38) years. 9 were transplanted in CR1 and 12 in more advanced stages (>CR1). Bone marrow (BM) was used in 5 cases, peripheral blood stem cells (PBSC) in 11 and both BM+PBSC were used in 5 cases. For conditioning busulphan (Bu) based regimens were used (Bu alone or in combination with cyclophosphamide, melphalan or etoposide). Two patients died 15 and 9 days after ASCT. From the remaining 19 patients 9 relapsed and 7 died. 12 patients are alive in complete remission 10 – 70 months after ASCT (7/9 in CR1 and 5/12 in >CR1). The probability of 1 year survival is equal with the 5 year survival and is 63% in the CR1 group and 31% in the > CR1 group.

**Conclusion.** Results in this small group confirm that sibling SCTx in early stage acute myeloid leukemia is connected with better non-relapse survival. ASCT in AML is a feasible treatment option with an acceptable risk and results are significantly better in comparison with the control group of non-transplanted patients.

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**2. Myelodysplastic Syndrome**

P420

**The successful treatment of the idiopathic hypereosinophilic syndrome with nonmyeloablative allogeneic peripheral progenitor cell transplantation**

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We report two patients (pt) with progressive course of idiopathic hypereosinophilic syndrome who was treated with nonmyeloablative allogeneic peripheral progenitor cell transplantation (NST). Patient #1 (age 43) had hypereosinophilia resulted in treatment-refractory reactive airway disease and splenomegaly. Patient #2 (age 18) had the clinical involvement of the heart (thrombotic mass in left and right ventricles), kidney, lungs, liver, spleen, skin and central nervous system in different times during a year of sustained eosinophilia before NST. Both bone marrow were cyogenetically normal. Patient #1 received glucocorticoid and 2-CdA and pt #2 received glucocorticoid before NST with partial clinical response but eosinophilia persisted with symptoms. The NST was performed from HLA-matched, unrelated donor for pt #1 and related donor for pt #2. The conditioning regimen included fludarabine 30 mg/m2 daily (-6, -5, -4, -3, -2); melphalan 70 mg/m2 IV daily (-3, -2). GVHD prophylaxis consisted of tacrolimus for pt #1and cyclosporine A for pt# 2. Engraftment with neutrophils > 500/mm3, platelets > 20,000/mm3 was achieved on day+20 (pt#1), +26(pt#2) and day+27(pt#1), +10(pt#2), respectively. Complete chimerism was confirmed on day +30 for pt #1 and +22 for pt #2. During the post-transplant period, pt #1 developed acute skin GVHD (Grade II) and pt #2 developed acute GVHD (Grade II) with skin and bowel involvement that was both successfully treated with steroid, etc. Patient #1 is currently does not have any GVHD with PS of 0. Patient #2 developed chronic GVHD with liver and skin damage occurred that is being treated with Daclizumab (Zenapax, Roche), low doses of prednisolone and cyclosporin (because of nephropathy). The normal amount of eosinophils on bone marrow biopsy, peripheral blood and complete donor himerism were revealed 12 months with pt #1and 8 months with pt #2 after NST. This is the first report of successful treatment of HES with NST.

P421

**Single center Experience with BMT in patients with myelodysplasia**


Bone marrow transplantation for myelodysplastic syndromes has developed some capability of cure, even if results in term of overall survival, EFS and mortality are still unsatisfactory. We report single centre results of unmodified marrow transplants performed in a cohort of 23 patients affected with MDS in various FAB subtypes.
17 of 23 received a transplant from HLA identical donor (identical sibling n=14; id. MUD n=3) and 6 patients from 2-3 Ag mismatched mother.

Age ranged between 1 and 57, median 3.5 (1-9) in 6 HLA haploidentical and 39 (4-57) in 17 HLA identical recipients. Pre transplant FAB morphology was: RA in 5, RAEB in 8, RAEBT in 9 patients (with leukemic transformation and pre transplant chemotherapy in 6, chronic MDS-like in 3 pts), and CMML in 1. 6 pts had hypoplastic, and 17 had hyperplastic marrow, 7 of whom in blast transformation. Percentages of pre-transplant blasts in 1 were <=5% in 8 pts, 10 to 20% in 8 pts, 25-30% in 7 pts. Cytogenetic abnormalities were detected in 3, others had normal karyotype or not valuable. IPSS score was low in 2, Int-1 in 7, Int-2 in 5, high in 9 patients. Conditioning regimens included standard BUCY (BU=14-16 mg/kg+CY120-200 mg/kg) used in 14 patients, intensified regimens (IR) in 5 pts (either increased BU, or added TT or AraC), RIR (reduced intensity regimens) given to 4 pts (TT+CY or low BUCY). Mostly IR was given to young pts (1-30) and RIR to older pts (49-57). GVHD prophylaxis: CSA+Pred in 4 and sMTX+CSA ± Pred in 19 patients. AGVHD II-IV developed in 10 pts, and was severe in 7 pts.

Overall 8 of 23 are alive (34.8%), 2 relapsed and 12 died of TRM, mostly for infections and toxicity, 4 died of GVHD. Exceedingly high mortality was observed independently from IPSS, HLA compatibility, % of pre-transplant blasts, FAB subtype, IPSS, dx-GVMT interval or conditioning regimen used. Overall, occurrence of acute GvHD and cell dose below 3x10^8/Kg appear to increase TRM and decrease EFS, even if significance was not reached. Only 2 pts over 50 y.o given RI regimen relapsed. Engraftment problems were frequent and related to toxicity or infections. In conclusion, BMT can cure MDS, but in older patients less toxic conditioning regimens need to be investigated.

**P422**

**Long-term results of allogeneic stem cell transplantation (SCT) in patients with myelodysplastic syndrome (MDS) and secondary acute myeloid leukemia (sAML) at a university hospital**


Between 1987 and 2001 thirty-seven patients, twenty-eight with primary MDS/sAML (RA=2, RAEB=6, RAEBT=6, CMML=4, sAML=10) and 9 with therapy-related MDS/sAML (RAEBT=2, sAML=7) with a median age of 40 (range, 18-70) years underwent allogeneic transplantation at the University Hospital of Vienna. Eleven patients received peripheral blood stem cell and twenty-six marrow grafts. Donors were siblings (n=28) and unrelated persons (n=9), in 6 cases an HLA-mismatch was detected. At the time of transplantation, there were 15 patients in complete remission and 22 with active disease ( refractory: n=8, untreated: n=14). For conditioning the majority of patients received total body irradiation (TBI) with cyclophosphamide (n=32). Four patients, undergoing dose-reduced allogeneic SCT, received Fludarabine and TBI of 2 Gy. In the majority graft-versus-host-disease prophylaxis consisted of cyclosporine with methotrexate.

All patients had rapid and sustained hematologic reconstitution. Treatment-related mortality (TRM) was high in both primary (36%) and therapy-related MDS/sAML (56%). Probability of relapse was especially high in patients with sAML (71%). Substantial overall survival (43%) was attained in primary MDS patients. At a median follow-up of 5 (range, 1-13) years, 13 patients (35%), twelve with primary and only one with therapy-related disease, are alive, twelve of them in continuous complete remission.

Although relapse-rate and TRM are high, especially primary MDS patients attain considerable overall survival. Additional therapeutic strategies (e.g. immunotherapy) are necessary to decrease relapse rates. Allogeneic SCT with dose-reduced conditioning is under clinical investigation, hopefully decreasing TRM and thus, improving overall outcome in the future.

**P423**

**Conventional myeloablative and nonmyeloablative blood stem cell transplantation in patients with poor risk MDS - the Duesseldorf experience**

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**Background:** High-dose chemotherapy and allogeneic blood stem cell transplantation can cure patients with poor risk myelodysplastic syndrome (MDS). Recently new non-myeloablative conditioning regimens have been developed.

**Methods:** Between 10/89 and 11/01 28 patients with poor risk MDS were treated with an allogeneic blood stem cell transplantation at our centre. Of the 22 patients (initial diagnoses RA/RARS 4, RAEB/RAEB-T 17, CMML 1) who were prepared with a conventional myeloablative conditioning regimen (MAC, 14 TBI based, 8 chemotherapy based) 14 received bone marrow (BM) and 8 received mobilized peripheral blood stem cells (PBSC) from HLA identical sibling (14) or matched unrelated donors (MUD, 8). At the time of transplantation 11 patients were in 1st or 2nd CR and 6 were in PR, progression or relapse after conventional chemotherapy. Five were untreated. Of the six patients who were prepared with a non-myeloablative conditioning regimen N-MAC, all fludarabine 3x 30mg/m² and 26Gy TBI. 5 patients received PBSC from HLA-identical sibling donors and 1 received BM (MUD). Two patients were in 1st CR (both RAEB-T) 1 was in 1st relapse (RA) and 3 were untreated (1 RARS, 1 RA, 1 MDS with fibrosis).

**Results:** Following MAC 9 patients (41%) died of treatment related causes and 2 (9%) died of recurrent disease. Both patients who died of relapse were not in CR at the time of transplantation. Karyotype could not be identified as a risk factor for relapse. After a median follow-up of 2364 days (range 3-3844) 11 patients (50%) are alive and disease free. Following N-MAC no patient died of treatment related causes. Two patients died of progressive disease (1 RARS, day +383, 1 MDS with fibrosis, day +65). The remaining 4 patients are alive (3 in remission) after a median follow up of 413 days (range 18-794). Both patients with RAEB-T, who received N-MAC in first remission are long term survivors (day +794, day +732).

**Conclusion:** Allogeneic blood stem cell transplantation using MAC or N-MAC can induce long term remission in patients with high-risk MDS. Patients who achieve CR with conventional chemotherapy have the greatest chance to become long term survivors. However, N-MAC carries a significantly lower risk of TRM and should be considered for older patients or patients with comorbid conditions.

**P424**

**Unrelated donor transplant in MDS: analysis of 59 cases from GITMO**


From January 1990 to December 1999, 59 patients affected with MDS underwent transplant from a HLA identical unrelated donor in Italy. Data were reported by 14 GITMO Centers .The median age of patients (pts) was 16 years (range 1-53), 29 were males and 30 females. They were affected with RA (7 pts), RAEB (16 pts), RAEBT (18 pts), CMML (13 pts), 5 were with unclassified MDS. The median time elapsed from diagnosis to transplantation was 350 days (range: 30-4471). At the time of transplantation 11 patients were in 1st or 2nd CR and 6 were in PR, progression or relapse after conventional chemotherapy. Five were untreated. Of the six patients who were prepared with a non-myeloablative conditioning regimen N-MAC, all fludarabine 3x 30mg/m² and 26Gy TBI. 5 patients received PBSC from HLA-identical sibling donors and 1 received BM (MUD). Two patients were in 1st CR (both RAEB-T) 1 was in 1st relapse (RA) and 3 were untreated (1 RARS, 1 RA, 1 MDS with fibrosis).

**Results:** Following MAC 9 patients (41%) died of treatment related causes and 2 (9%) died of recurrent disease. Both patients who died of relapse were not in CR at the time of transplantation. Karyotype could not be identified as a risk factor for relapse. After a median follow-up of 2364 days (range 3-3844) 11 patients (50%) are alive and disease free. Following N-MAC no patient died of treatment related causes. Two patients died of progressive disease (1 RARS, day +383, 1 MDS with fibrosis, day +65). The remaining 4 patients are alive (3 in remission) after a median follow up of 413 days (range 18-794). Both patients with RAEB-T, who received N-MAC in first remission are long term survivors (day +794, day +732).

**Conclusion:** Allogeneic blood stem cell transplantation using MAC or N-MAC can induce long term remission in patients with high-risk MDS. Patients who achieve CR with conventional chemotherapy have the greatest chance to become long term survivors. However, N-MAC carries a significantly lower risk of TRM and should be considered for older patients or patients with comorbid conditions.
3. Lymphoma

Out of 59 pts 19 are alive, relapse occurred in 15 pts (28.8%), 18 pts (30.5%) died of transplantation. The actuarial probability of survival was 30% at ten years, the actuarial probability of relapse and TRM were 45% and 35% respectively. In univariate analysis DFS and relapse rate were influenced by FAB classification and status of disease at transplant; there was a trend for a lower TRM (<20 years 30%, >21 years 49%) and a better DFS (<20 years 30%, >21 years 19%) in younger patients.

Additional abstracts to this topic

Prognostic factors in myelodysplastic syndrome

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Myelodysplastic syndrome (MDS) is heterogeneous acquired clonal hematopoietic stem cell disorders, characterized by great prognostic variability. International prognostic scoring system (IPSS) could predict survival and AML evolution and determine the treatment program. The aim of the study was to reveal the significance of IPSS in survival and therapy choice in MDS patients, treated in St.-Petersburg Pavlov State Medical University during last ten years.

Materials and methods: 61 patient with MDS were included in the study. The median age of patient was 62 (3 -83) years. Male/female ratio: 28/33. The cohort was divided according WHO classification as following: RA-12 patients, RAS - 2 patients, RCMD - 19, RAEB - 18, 5q-syndrome - 10. IPSS score was calculated for each patient. Non-transplant group of patients received palliative or low- or intermediate intensity chemotherapy. 4 patient underwent hematopoietic stem cell transplantation (3-related, 2 with myeloablative and 1 with non-myeloablative conditioning regimen, 1-unrelated). Kaplan-Meier plots were used for survival analysis.

Results: The 5-year survival in non-transplanted group was following according to MDS subtypes: RA-45%, RCMD-45%, 5q-syndrome -24%, RAEB -0 (p=0.002); according to IPSS: low-risk group-55%, intermediate-risk I-32%, intermediate-risk II-15%, high-risk-10% (p=0.002). In RA group 86.6% patients had low-risk score and 13.3% - intermediate I, in RCMD 9.5% patients belonged to low-risk group, 76.2% - to intermediate I, 14.2% - to intermediate II, in 5q-syndrome group 12.5% of patients had low-risk, 50%- intermediate I, 12.5%- intermediate II, and 25%-high-risk score, in RAEB group 1% of patients had intermediate-risk I, 22% revealed intermediate-risk II and high-risk group consisted of 72%. In transplanted group 3 patients had RAEB (2-high risk, 1-intermediate I) in one patient, 17 years old, had RCMD with intermediate risk I. Two patient are alive in complete remission 25 months and 10 years.

Conclusions: IPSS is correlated with MDS subtypes according WHO classification in outcome predicting. IPSS could be useful in selecting patients with MDS to more intensive treatment, including hematopoietic stem cell transplantation.

3. Lymphoma

P425

Autologous transplantation of non-cryopreserved hematopoietic stem cells with short (1 day) regime of chemotherapeutic conditioning: 3.5 years experience

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It's known, that HSCs could survive viability without cryopreservation during 72 h in autologous plasma under 4 C. There are some HDCT-regimes which could be given during not more than 24 h without increasing extrahematologic toxicity. In this case, DMSO-toxicity could be omitted and the general cost of the procedure could be markedly reduced.

From June,98 to September,01 totally 28 auto-transplantation procedures with non-cryopreserved HSCs after 1-day HDCT were made in 27 pts (1 pt with double procedures) in BMT-unit of Department. 3 of 28 were BMT, 2-BMT/PBSC and 23 - PBSC. Among 27 pts 7 - ALL (2 - CR1; 4 - CR2; 1 - CR3), 6 - HD (not CR1), 5 - AML (2 - CR1, 3 - CR2), 5 - solid tumors (2 - ES, 2 - RMS, 1 - BC), 3 - NHL (1 - CR1, 1 - CR2, 1 - PR3), 1 pt - URA. PBSC-mobilisation were made by combination of CT+G(M)-CSF, BM-collections were made according to known methods. PBSC-collections were made with "Haemonetics MC53" or " Fresenius AS104" by LV-apheresis during 1-2 procedures. BM and PBSC-concentrates were stored in autoplasma without processing in thermostat for not more than 72 h without agitation.

Just after HSCs-collection pts had HDCT based on 3 agents in myeloablative doses: BCNU, Melph., VP-16. In pt with URA 2-days HDCT was used: BCNU 300 mg/m² + CTX 60 mg/kg + VP-16 1000 mg/m² + ATG 15 mg/kg. HSCs were rein infused not early than 24 h after finishing HDCT but no more than 72 h of storage.

Control of collection quality uncluded calculations of NCs, MNCs and CD34+. CD34-caluation was made with flow cytometer. Median number of rein infused NCs - 5 x 10/8/kg, MNCs - 3,5 x 10/8/kg and CD34+ - 3,4 x 10/6/kg.

Neutrophil engraft was achieved in 26 from 28 procedures (median time - D+14); platelets engraft (PLT >=20000/mkl) - in 25 from 26 patients (median time - D+23). TRM till D+100 - 3/27 pts (all cases because of ARDS). NRM after D+100 - 1 pt (not-VOID heparr insufficiency). 11 pts relapsed, all after D+100. 2 pts had resistant to HDCT disease (RMS and ES). 2 pts were LFU before D+100.

Conclusion: autotransplantation of non-cryopreserved HSCs after short HDCT could be considered as choice for pts with lymphoproliferative disorders with bad prognosis after non-myeloablative CT, first of all - NHL and HD.

P426

Relapsed low grade non-Hodgkin's lymphoma (NHL) treated with 2- CDA and high dose chemotherapy (HDC) with autologous peripheral blood stem cells (PBSC)


Dissemintated or relapsed low-grade NHL remains an incurable disease. Therefore, new strategies have been developed including HDC with PBSC rescue. Because purine analog as 2-CDA have demonstrated activity in pts with relapsed or resistant low-grade NHL we used this drug in a sequential treatment including 2 cycles of CHOP-like regimen followed by high-dose CTX to collect PBSC. After leukapheresis pts received 3 cycles of 2-CDA (0.14mg/kg s.c. for 5 days every 28 days) and then HDC (mitoxantrone 60mg/sqm day -5 and melphalan 180mg/sqm day -6) with PBSC support and G-CSF 5mg/kg from +5 to ANC>1000/mmc. Between January 1996 and October 2001, we enrolled 24 pts (22 follicular NHL, 2 LLC) with a median age of 50y (range 31-52) relapsed after a median number of 2 regimens of chemotherapy. At diagnosis 8 pts were stage 2, 5 stage 3 and 11 stage 4. All pts were evaluated for bcl-2/IgH translocation in bone marrow biopsy (11 pts were positive) and during the treatment. 22 pts completed the study (2 pts fail to collect CD34). Two pts received only 2 cycles of 2-CDA: 1 patient for severe neutropenia and thrombocitopenia and the other one for urticaria due to 2-CDA administration. Before PBSC transplant 5 pts were in CR, 14 in PR, and 3 in SD. Engraftment were 11 days (6-19) and 15 days (10-80) for ANC>500/mmc and platelets >30000/mmc respectively. 36% (8/22) of pts experienced a grade 3 mucositis and 41% (9/22) a microbiological or/and clinically documented infection. One patient died for legionella pneumonia after transplant. With a median follow-up of 1.5 (0.4-5.3) years, 47%
(10/21) of pts are in CR, 14% (3/21) in PR and 33% (7/21) in PD. Two pts died for PD and 1 patient, still in CR, died at +78 for cerebral toxoplasmosis complication. All except one relapsed patients were bcl-2 negative at follow-up. Moreover we observed 7/21 (33%) of late pneumonia. Alligation at HDCT, 39% with transplant related complications, suggest a careful monitoring of these pts.

P427
Autografting in aggressive non-Hodgkin’s lymphoma - A 15 year experience
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We report retrospective outcome data for all autograft procedures performed for aggressive NHL between 1985 and 2000 in 13 centres. 63 patients underwent a total of 64 transplants (one patient had 2 procedures). Median age at transplant was 47 years (range 19-68). 36 patients were male, 27 female. Histology was: diffuse large-B-cell (n=35), anaplastic (n=10), T cell rich B cell (n=5), follicle centre cell grade III (n=3), lymphoblastic (n=3), others (n=8). Status at transplant: CR1 (n=19), CR2 or greater (n=14), PR1 (n=14), PR2 (n=8), progressive disease (n=4), Complete response elsewhere: BEAM (n=5), BCNU-Etoposide Phosphate-Melphalan (n=1), Etoposide/TBI (n=1), LACE (n=1). Stem cell source: PBSC (n=50), BM (n=14). TRM was 6.3% for all patients - 2 patients died of infection, one of pneumonitis following TBI and one of myocardial infarction. Of the 4 patients who died at transplant 3 received bone marrow (TRM=21.4%) and one received PBSC (TRM=2%). Median follow up was 17 months (range 0 to 143 months). 12 year overall survival post autograft was 43.6% with relapse free survival of 42.4%. Patients in CR at transplant had 12 year overall survival of 64% with relapse free survival of 65.9%. Patients in PR at transplant had 12 year overall survival of 31.8% with relapse free survival of 27.2%. Patients with diffuse large B-cell lymphoma had 12 year overall survival of 61.9% with relapse free survival of 58.0%. All patients with chemotherapy resistant disease died within one month of the procedure. In conclusion autografting is safe, effective therapy in patients with aggressive NHL particularly those with diffuse large B-cell histology, responsive disease and who receive peripheral blood as the stem cell source.

P428
High-dose chemotherapy and ABMT in Hodgkin’s disease in an oncology institute: the Lisbon experience
High dose chemotherapy (HDCT) with autologous haematopoietic stem cell transplantation (AHSCT) has been used in refractory or recurrent Hodgkin disease. We performed a retrospective analysis of 78 patients admitted for HDCT and AHSCT in our BMT Unit between August/92 and October/01 (40 females, 38 males). The median age at diagnosis was 25 y.o (4-49) and at transplant date was 28 (7-51). At diagnosis the Ann Arbor stage was I in 2 pts, II in 32, III in 12 and IV in 32 (40 pts with B symptoms). At the start of HDCT, 19 pts were in CR, 25 in PR and 15 with PD. The HDCT regimens used were BEAM in 55 pts, Busulfan-Melphalan in 1, Etoposide-Melphalan in 15, BCNU-Etoposide Phosphate-Melphalan in 2, ICE in 1, Melphalan in 1, BCNU-Etoposide-Melphalan in 6 and TBI-Melphalan in 3. The haematopoietic support was PBPC in 41 pts, PBPC + BM in 11 and BM in 26. Seventy-seven patients were evaluable (1 death whilst on HDCT) with a median follow-up of 3 years (max 7.8 years). The median of hospital stay was 30 d (12-178). There were 4 toxic deaths. After AHSCT, 5 pts were irradiated. At the median follow-up the OS was 84.5% and EFS was 60.1% (medians not reached). The patients were divided in 2 groups, according to staging at the diagnosis date; localized disease (IA and B, IIA and B and IIA), and extensive disease (IIB, IVA and B), respectively. At the median follow-up Os for localized and extensive disease was 86% and 88% respectively and EFS was 64% and 62%, respectively. They were also put in 2 groups according to disease status at transplant: group I (first response), group II (second or posterior response). At 3 years the OS and EFS for group I was 95% and 73% respectively, and Group II OS and EFS was 89% and 65% respectively. OS and EFS for primary refractory patients, at 3 years post AHSCT, was 68% and 42% at 5 years. At the median follow-up the OS for chemotherapy sensitive and refractory disease was 91% and 69%, and the EFS was 68% and 40%. In our series, stage at diagnosis or disease status at transplant did not play a significant role in prognosis. We can conclude that in our series a substantial number of Hodgkin’s disease patients refractory to first line or salvage conventional treatment could be saved with HDCT, with more than 1/3 of them still in remission.

P429
Outcome of high-dose chemotherapy followed by autologous bone marrow (BMT) or peripheral blood stem cells transplantation (PBSCT) in poor-prognosis Hodgkin’s disease (HD) patients. A single center experience
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We compared in retrospective studies the outcome of 78 patients (pts) with HD who underwent high-dose chemotherapy (HCT) and BMT or PBSCT. The selection of the pts for PBSCT was bone marrow involvement at the time of diagnosis or ineffective marrow recovery. All pts received a modified Dexamethasone (168 mg/m2, BCNU 300 mg/m2, Etoposide 800 mg/m2, Cytarabine 6000 mg/m2, Melphalan 140 mg/m2). In the BMT-group, which comprised 44 pts, 23 had refractory disease, 12 sensitive relapse and 9 high-risk at the time of diagnosis. The PBSCT-group consisted of 34 pts. Among them 11 were with resistant disease 14 with relapse and 9 had high-risk disease. At the time of grafting, in the BMT-group 18 pts were in complete remission and 26 had partial response. In the PBSCT-group complete remission had 18 pts and partial response 16 pts. The transplanted material in the BMT-group contained 4.5 (2-6.7)x10^6/kg and in the PBSCT-group 5.1 (2-12.5)x10^6/kg of CD34 (+) cells. The granulocyte and platelet recovery was significantly faster in the PBSCT-group than in the BMT-group; with granulocytes >0.5G/1 in a median of 11 (7-19) days and 20 (11-36) days, respectively; and platelets >20G/1 at a median of 17 (7-25) days and 25 (13-46) days respectively. With a median follow-up of 68 months (1-92) months the overall survival and progression-free survival in the BM-group were 81% and 67%, respectively. In contrast, in the PBSCT-group the overall survival and progression-free survival were 91% and 85%, respectively. For the particular prognostic groups of pts in the BMT-group and PBSCT-group the overall survival in pts with refractory disease is 60% and 81%, with relapse 92% and 92%, and with high-risk disease 90% and 100%, respectively. The progression-free survival in the BMT-group and PBSCT-group in respect to the pts with resistant disease is 42% and 70%, with relapse 69% and 86% and in high-risk pts 90% and 100%, respectively. We can conclude that HCT and PBSCT are giving promising results in poor-prognosis pts with HD. However, for final conclusion the multicentre prospective and randomised studies are needed.
High-dose chemotherapy and hematopoietic stem cell transplantation (ASCT) in poor-prognosis non-Hodgkin's lymphoma


Since 1989, ninety-nine patients (pts) with poor prognosis non-Hodgkin's lymphomas received autologous (94) or allogeneic (5) stem cell transplantation, source of stem cells were the following: autologous PBSC 87 pts, autologous BM 6 pts, both 1 pt; allogeneic stem cells were: PBSC in three cases, BM in two. The median age was 44 years (range 15-67) and M/F = 65/34. Thirty-two had diffuse large B-cell lymphoma, twenty-seven follicular lymphoma, fifteen mantle-cell lymphoma, five mature B-cell neoplasms, marginal zone B-cell lymphoma and Burkitt's lymphomas, ten mature T-cell neoplasms, two linnphoblastic lymphoma. Median time from diagnosis to transplant was 10 months (3-90). Significant factors predicting poor prognosis were: advanced stage (III-IV, 81 pts), B symptoms (30 pts), bulky disease (30 pts), extranodal disease (58 pts), elevated LDH levels (17 pts); >1 CR (8 pts), PR (38 pts), relapse (5 pts), resistant disease (7 pts). High dose therapy consisted of BEAM (n=46), CBV (n=16), TEAM (n=13), melphalan (n=6), mitox + melphalan (n=11), Bu-Cy (n=6). The overall survival was 87% and DFS 78% at median follow-up of 49 months (7-157). After transplant 80% (n=80) of the pts was in CR. Among these 21% (n=17) relapsed at median of 12 months (2-104) from transplantation. Thirteen (13%) died at median of seven months (1-37). Causes of death were: disease progression (85%) and TRM (15%). Our results show that High-Dose therapy followed by stem cell rescue seems to ensure a chance of cure in High-risk NHL.

Autologous stem cell transplantation for 149 aggressive non-Hodgkin's lymphoma patients

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Background and Objectives: To retrospectively analyse the outcome of 149 aggressive non-Hodgkin's lymphoma patients submitted to high-dose chemotherapy and autologous stem cell transplantation after conventional chemotherapy. Design and Methods: Patients were considered as having an high risk disease if they present at least one of these: stage III-IV; B symptoms; elevated LDH; slow response to first line treatment (third generation regimen). Median age at diagnosis was 39 (15-60) years; male/female ratio – 1.6. They were affected by diffuse large B cell lymphoma – 60 (40.0%); anaplastic large cell lymphoma T or null cell type – 22 (14.5%); T precursor lymphoma – 9 (6.0%); follicular lymphoma – 28 (18.5%); peripheral T cell lymphoma unspecified – 10 (7.0%); marginal zone lymphoma – 10 (7.0%); mantle cell lymphoma – 10 (7.0%). Seventy-eight out of 149 (52.5%) patients were in stage III-IV, 90 (60.5%) had a bulky disease and 76 (51.5%) had B symptoms. Distribution according to the I.P.I. was: low (L+LI) – 94 (63.0%) patients; high (HI + H) – 55 (37.0%) patients. Status at transplant was: complete remission – 46 (31.5%); patients; sensitive relapse – 16 (10.5%); resistant – 16 (10.5%). ASCT was performed using BAVC as conditioning regimen. Results: As by November 2001, with a median follow-up from transplant of 48 months (1 – 132), 76.0% (113/149) of the patients were in CR, 13.5% (20/149) are alive with disease and 10.5% (16/149) died (2 for transplant related complications). After ASCT, 35 PR and 10 sensitive relapse patients entered a CR with a conversion of the response of about 57.5%. OS and PFS of the whole population are respectively 82.5% and 64.0% with a median follow-up from transplant of 48 months (1 – 132) and 42 months (1 – 132).

Autologous hematopoietic stem cell transplantation in Hodgkin's disease patients - Long-term outcome and prognostic factors in a single center experience

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Between 1992 and 2000 one hundred patients with high risk Hodgkin's disease (HD), aged 27,5 (16-50) years, were treated with autologous hematopoietic stem cell transplantation (AH SCT). Forty-five patients were partially refractory (45%) or refractory (15%) to initial chemotherapy, 55 patients were in relapse. Most of the patients were heavily pretreated with the median cycles of pre-transplant chemotherapy – 12 (4-38) and concomitant radiotherapy in 72% of cases. The source of stem cells used for transplantation was peripheral blood (n=71), bone marrow stored for 3 days in 4degC without cryopreservation (n=27) or both peripheral blood and bone marrow (n=2). Conditioning regimen was: BEAM +/- procarbazine – (n=40), other modalities (n=5). Transplant related mortality was 1/100. Five years probability of overall survival and progression-free survival equaled 71% and 63% for resistant/sensitive relapse patients – 55.0% (p<.000001). PFS according to status at transplant: CR – 92.5%; PR – 70%; resistant/sensitive relapse patients – 29.5% (p value = <.000001).

Conclusions: If we considered patients transplanted in response after chemotherapy (either complete or partial), our results seems to be in excess of what would be expected in a group of patients with the same characteristics treated with standard chemotherapy only. Moreover, to be at least in partial response at transplant seems to be enough for predicting a favourable outcome.
primary refractory patients, 63% and 41% for relapsed patients. Factors found to be associated with higher probability of overall survival in Cox model were: no hepatomegaly at diagnosis (p=0.003), subtype other than NS (p=0.006), CR or PR vs. NR at HDT (p<0.002), bone marrow vs. peripheral blood as a source of stem cells (p=0.02).

We conclude that AHSCST is safe and efficient treatment option for HD patients who cannot be cured with conventional-dose therapy. Further efforts should be focused on optimization of the timing and details of the procedure.

P434

Cox multivariate analysis identifies age, CD34+ cells and time to WBC recovery as independent predictors of survival post autologous stem cell transplantation (ASCT) in 102 patients with relapsing or resistant malignant lymphomas (ML)


Objectives: To evaluate ASCT in ML patients (PTS) by assessing its: 1) toxicity in terms of transplant related mortality (TRM), hematopoietic recovery. 2) efficacy in terms of CR, PR an NR achieved, and long-term efficacy expressed as both disease free (DFS) and overall survival (OS).

Patients and Methods: 102 pts with ML including 62 non-Hodgkin lymphoma, and 40 Hodgkin lymphoma pts, (55 male, 47 female; age 17-67, median 34) received BEAM myeloablative treatment followed by ASCT. Four pts were transplanted in CR, all others had active disease at time of transplantation. All pts received heavy prior treatment with a median of 2 lines of chemotherapy (range 1-6), median of 8 chemotherapeutic cycles (range 2-31), and average of 10.6(range 0.8-59.2) SD 11.5)x10 6/kg CD34+ cells was reinfused and G-CSF (5 microgr/kg) was administered during leukopenic period to all but one patient (median and mode of 9 days).

Results: median time to WBC recovery (> 1x10 E9/L) was 10 days (range 8-20), while platelets recovered ((>20x10 9/L) in median of 8 days (range 0-20). A median of 3 febrile days (range 0-20) was experienced neutropenic fever and blood cultures were positive in 48.5% (range 0-85). A median of 8 (range 4-14) blood cultures were positive in 24.5% (range 0-81). A median of 2 (range 1-4) CR+PR=98%. Projected DFS of 57.3% at 8 years in this group of pts is highly satisfactory. Independent predictors of survival were: age, number of infused CD34+ cells reinfused and time to WBC recovery.

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High-dose therapy supported by autologous peripheral blood stem cell transplantation (PBSC) in patients with HIV-associated lymphoma (HIV-Ly) refractory to or relapsed after intensive first-line combination chemotherapy (CT)


The recent introduction of highly active antiretroviral therapy (HAART) has allowed the evaluation of aggressive therapeutic approaches also in HIV-positive patients (pts). We started a program of PBSC transplantation after HDT in pts with HIV-Ly refractory or relapsed after intensive CT. Eligibility included sensitivity to 1/more courses of second-line standard-dose CT, available in 19 pts with active disease at a time of transplantation ASCT toxicity can be minimized, HDT may not be optimal approach in patients with EATL. We conclude that AHSCT is safe and efficient treatment option for limited, HDT may not be optimal approach in patients with EATL.

In addition, BEAM may not be the conditioning of choice in these patients.
Autologous stem cell transplant (ASCT) in HIV-1+ high risk lymphoma patients. HIV infection does not preclude ASCT for lymphoma
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AIDS has become a chronic disease after high activity antiretroviral therapy (HAART) with prolonged survival rate. Furthermore, this treatment has allowed for an increase in the intensity of treatment used in AIDs-associated lymphoma, that is becoming similar to the treatment of patients with lymphoma-HIV negative.

We present the results of Hematopoietic Stem Cells (HSC) collected from mobilized peripheral blood progenitor cells in 6 male patients (pts) with AIDS (stage C3) and high-risk Lymphoma (5 NHL, pts 2-6, 1HD, pt 1), and the outcome after ASCT performed on 5 of them. One patient died before ASCT (pt 6).

HAART was maintained during mobilization and ASCT, except during the conditioning in patient 1, which was necessary to resume due to an increase in HIV load. The pt 5 was not strict with HAART therapy. All pts were candidates for ASCT; due to the presence of their lymphoma disease (pt 1 HD & pt 6 NHL-B), or high risk histology at diagnosis (Burkitt-Lymphoma, pts 3 & 5) or not achieving a complete remission (CR) after the 1st line of treatment (pts 2 & 4 NHL-B). All pts were in CR before mobilization and ASCT. We used high doses of G-CSF (20 mcg/kg/d) in the protocols of mobilization in six pts, plus CTX 1.5 gr/m2 x 1 day in three pts. HSC were programmed cryopreserved and stored in an isolated chamber at –80ºC. The conditioning regimen was BEAM. We used G-CSF in all pts post-ASCT.

Conclusions: The collection of HSC obtained from our patients shows an adequate number of CD34+ cells after the use of high doses of G-CSF. The mobilization and conditioning programmes do not increase the viral load, as long as HAART is maintained. Engraftment is slower than in HIV-negative patients, supporting the use of G-CSF in immediate post-transplant period. The ASCT is not associated with increased opportunistic infections, ASCT may be applied with guarantees on patients diagnosed with lymphoma and AIDS, in a similar manner to the HIV-negative setting.

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Autologous stem cell transplantation in patients with peripheral T-cell lymphomas
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Objectives: Peripheral T-cell lymphomas (PTCL) are rare in Western countries where they constitute about 10-15% of all lymphomas. Although PTCL are a heterogeneous group there are in general clinically aggressive with a poor prognosis after conventional therapy. Due to the low incidence of PTCL the role of autologous stem cell transplantation (ASCT) in these patients is still not clear.

Methods: From April 1997 until October 2001 12 patients, 8 with PTCL unspecified, 4 with anaplastic large cell lymphoma (ALCL), median age 40 years (range 31-59) underwent ASCT. At that time 8 patients were in CR, 2 in PR and 2 showed progressive disease. Conditioning regimens were BEAM in 5, TBI/Cy in 4, CBV in one and augmented CBV in 2 patients. Two patients received radiation therapy after ASCT.

Results: The first restaging showed CR in 10 of 12 patients and PR in 2 patients. At a median follow-up of 12 months (range 1-56) 10 patients are alive. The disease-free survival is 65%. Six of 8 patients transplanted in CR have a continuous CR. One patient transplanted specified PTCL at time of diagnosis relapsed as AILD 14 months after ASCT with very slow progression and the other one with ALCL relapsed 4 months later and died because of progression. Two patients with PR achieved CR. The 2 patients with PD at the time of ASCT achieved CR but one of them died 4 months after ASCT with acute leukemia.

Conclusion: Although a larger number of patients and longer follow-up are warranted these are promising results in patients with PTCL and more intensive treatment seems to be useful.

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High-dose therapy supported by autologous stem cell transplantation (ASCT) in patients with peripheral T-cell lymphoma (PTCL): A nation-wide survey from Finland
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Objectives: Peripheral T-cell lymphomas (PTCL) have poorer prognosis than in most types of B-cell lymphomas. High-dose therapy (HDT) supported by ASCT has been suggested as a treatment option in patients with PTCL, but only limited experience is available on the feasibility and efficacy of this approach.

Patients and Methods: Forty-two adult patients with PTCL received HDT supported by ASCT in six Finnish centers in 1990-2001. There were 24 men and 18 women with a median age of 42 yrs (16-68).

Histology: anaplastic large cell lymphoma (T/null) (ALCL) 16 pts, unspecified PTCL 14 pts, enteropathy-associated T-cell lymphoma 5 pts, others 7 pts. At diagnosis 57 % of the patients had stage III-IV disease, 40% B-symptoms and 19% bone marrow involvement. Transplantation was done as first-line treatment in 22 pts (52%) and later in 20 pts. The conditioning was BEAC (N=24) or BEAM (N=18) and was supported with blood stem cells (N=38), bone marrow (N=2) or both (N=2).

Results: Four patients (10%) died early (< 100 d) from transplant-related reasons. With a median follow-up of 19 months 15 patients (36%) have relapsed or progressed. The median time from ASCT to relapse/progression was 4 months. Estimated 4-year overall survival (OS) for all patients was 57%. OS at 4 years was 70% in patients who received ASCT in first CR/PR vs. 44% in patients who received ASCT later (NS). Patients with ALCL had superior OS when compared to other subtypes (85% vs 47%, p=0.01).

Conclusions: ASCT is feasible in patients with PTCL although transplant-related mortality seems relatively high. Patients with ALCL had better prognosis than other subtypes. Patients transplanted after relapse had a relatively good outcome. Prospective studies are necessary in order to better define the role of ASCT in patients with PTCL.

P440

In vivo purging and autografting of CD 34+ cells in B - cell non-Hodgkin's lymphoma
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Objectives: Elimination of tumor cells from hematopoietic stem cell products is a major goal of bone marrow - supported high dose cancer chemotherapy. In patients(pts)with low - grade lymphoma,
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Uptake of stem cell transplantation with the rituximab/TBI/CY
dose regimen is an effective treatment for mantle cell
lymphoma

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The purpose of this study was to investigate if incorporation of 2
375/m² doses of the CD20 antibody rituximab into a myeloablative
regimen consisting of total body irradiation and high-dose
cyclophosphamide (R-TBI/CY) might improve the results of
autologous stem cell transplantation (SCT) in patients with
 disseminated mantle cell lymphoma (MCL). 35 patients (median
age 56 (38-67) with stage III/IV MCL were treated with a
sequential dose-escalating therapy comprising 2-6 cycles of
CHOP for remission induction, intensive Dexamethasone
chemotherapy for mobilization of peripheral blood stem cells, and
high-dose therapy with SCT. Whereas 20 patients received
TBI/CY alone, 15 patients were irradiated with the myeloablative regimen in
the last 15 patients (R-TBI/CY). Results: In all 15 R-TBI/CY
patients a complete or partial remission was achieved after
CHOP/Dexamethasone. Stem cell collection was successful in all
cases, yielding 10,8x10⁶/kg (4.1-34.5) CD34⁺ cells. However,
flow cytometry revealed MCL contamination of the collection
products in 11/15 patients. R-TBI/CY with SCT was not associated
with unexpected toxicity and resulted in prompt engraftment
(median days to neutrophil recovery >0.5x10⁹/µl: 10 (8-10);
median days to platelet recovery >2x10⁹/µl: 10 (6-13)).
Hematopoietic reconstitution was not significantly different from
the TBI/CY only group. With a follow-up of 12 (3-25) months post
transplant all patients treated with R-TBI/CY are alive with no
clinical, immunological (13/13 tested by flow cytometry) and
molecular (7/7 tested by CDR3 PCR) evidence of disease.
At present, however, it is unclear if this might translate into an
improvement of event-free and overall survival over SCT
with standard TBI/CY, which was 52% and 95% at 3 years
(median follow-up 38 (24-65) months). Conclusions: R-TBI/CY is
a safe and effective high-dose regimen for upfront SCT in patients
with advanced MCL. Longer follow-up is needed to prove that
the addition of rituximab to TBI/CY can improve the outcome of SCT
by eliminating residual MCL cells surviving conventional myeloablative therapy.

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Efficiency of in vivo purging with rituximab followed by high-
dose therapy (HDT) with autologous peripheral blood stem
cell transplantation (PBSCT) in B-cell lymphomas. A single
center study

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HDT with PBSCT is a treatment option for patients (pts) with
advanced follicular, mantle and mantle cell lymphoma. In this
setting, frequent contamination of PBSCT harvests by tumor cells
may contribute to relapse. Anti-CD20 monoclonal antibody
(Rituximab) induces clinical response in such lymphomas
(McLaughlin JC 18:28225, 1998; Foran JCO 18:317, 2000) and
is efficient in removing circulating B-cell from the peripheral blood
(PB). We therefore hypothesized that Rituximab may be a useful
in vivo purging agent prior to transplant therapy. From May 98 to
March 01, 14 pts with relapsed follicular lymphoma (n = 11),
marginal zone (n = 2) and mantle cell (n = 1) lymphomas with a
PCR-detectable molecular marker were treated with Rituximab,
375mg/m² once weekly, for a total of 4 infusions. All patients
except one had stage IV disease. At the time of treatment, pts with
MCL and follicular lymphoma had received no prior chemotherapies
or radiotherapy. For the 3 other pts. Rituximab was administered after a
1st line anthracyclin-containing regimen. With a median delay of 2
months after the 1st infusion of Rituximab, a mobilization regimen
was delivered, consisting in Cyclophosphamide (Cy) 4.5g/m² and
VP16 450 mg/m² at d1 with G-CSF (5mg/kg) from d5 until the end of
leukaphereses. In all pts a median number of 6 106 CD34/kg
(3-21) was harvested with a median of one leukapheresis (1-3).
All pts completed HDT with Cy (50mg/kg d-6 and d-5), VP16
(300mg/m² d-6 to d-4) and a single dose total body irradiation
(TBI) at d-1 (8Gy). In all the pts, PCR analysis was performed in the
leukapheresis product with bcl2-JH (n = 9), Bcl1-JH (n = 1) and
CIR DII/III-specific primers (n = 4). Harvests were free of PCR-
detectable molecular marker in 11 out of 14 pts (79%).
The median time to neutrophils over 0.5 10⁹/L was 14 days (12-17),
and to platelets over 50 10⁹/L 12 days (9-18), similar to historical
data for NHL not receiving pre-transplant Rituximab. With a
median follow-up of 2 years, the 14 transplanted pts are alive, 12
in clinical complete remission (PCR leukapheresis product/PCR
follow-up: -/-, +/+ 2, +/- 2, +/+ 1) and 2 with progressive disease
(-/- and +/+). We conclude that combination of Rituximab and HDT
is effective in attaining complete clinical (86%) and molecular
(71%) response. A longer follow-up will determine the outcome of
these patients.

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Frequency and clinical characteristics of non-Hodgkin's
lymphoma (NHL) and Hodgkin's disease (HD) patients
relapsing before day-100 after high-dose therapy with
autologous stem cell transplantation (ASCT)

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ASCT is widely used for lymphoid malignancies as consolidation in
chemosensitive patients. Relapse before day-100 post-transplant is rare, we aimed to assess its frequency and patients'
characteristics. Among the 609 patients who underwent ASCT in
our department from Apr 1985 to Jan 2000, 250 pts (41%) relapsed, in 55 pts (9%) relapse occurred before day-100. At
diagnosis, 41 NHL pts (5 PTCL, 18 B-DLCL, 4 Burkitt-L, 2
Lymphoblastic-L, 6 FL, 1 SLL, 1 MALT, 4 others), and 14 HD pts;
median age 39 yrs (20 -64), M/F 30/15. 33 pts (60%) had extra-
gonal sites with 15 (27%) having more than 2 organs involved.
10 pts had marrow involvement. 40 pts (72%) had 2 lines or more
of chemotherapy, 26 (47%) 3 lines or more, in addition 19 (34 %)
were irradiated. CR had been reached at least once in 35 pts
(64%). Median number of progressions was 2 (1-6). 4 pts were
grafted in first CR. Stem-cells source was PBSC for 45 pts (82%).
6 pts had immunological purging. At time of harvest only 13 pts were in CR (24%), 4 had marrow involvement. Chemotherapy conditioning regimens were associated with TBI in 10 pts (18%). Status before graft was: CR 22 pts (40%), PR 6 (11%), no response 27 (49%), 54 pts (96%) engrafted successfully. After graft, 36 pts (65%) relapsed in previous involved sites. 48 pts (87%) had multiorgan relapse, 22 with marrow involvement. The majority received mono-chemotherapy or supportive care. Only 5 CR and 2 PR were achieved. Median overall survival was 6 months, with a 2-year-OS at 10% (+/- 8%). There was no survival difference between remission or non responding patients. In conclusion, ASCT patients relapsing before day100 are unlikely to be easily isolated. As the relapse presented with multiorgan involvement even if CR has been achieved, it suggests stem-cell contamination and haematogenous dissemination or failure of the immune system.

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Treatment for primary refractory Hodgkin’s disease; a comparison of high-dose chemotherapy followed by ASCT with conventional therapy


The choice of optimum therapy for the treatment of primary refractory HD is unclear. The use of ASCT as a treatment modality has proven clinical effectiveness, but few comparative studies with conventional treatments have been carried out. We decided therefore to conduct a retrospective analysis of all patients treated in 16 hospitals throughout Poland between June 1991 and June 2000 either with an ASCT or with conventional chemotherapy (CHEMO). 64 patients with primary refractory HD were treated with high dose chemotheraphy and ASCT. These patients were matched to a cohort of 64 conventionally treated patients, selected from 82 on the basis of previously published prognostic factors (IPFI, LDH, disease stage at diagnosis, symptoms). Conventionally treated patients were included only if they survived the minimum time to treatment with ASCT from diagnosis (200 days). Comparison of ASCT and CHEMO groups showed no statistical differences with regards to patient gender, disease stage, IPFI, LDH, disease symptoms. The ASCT group was however younger than the CHEMO group (26.5y vs 33.5y, p=0.001). Kaplan Meier analysis of survival showed a statistically significant advantage for patients treated with ASCT when compared with CHEO (p=0.008). 5yr values 54.8% (95%CI 41-68) and 27.0% (95%CI 16-42). Univariate analyses of all potential prognostic factors revealed statistical significance only for disease symptoms at diagnosis. In multivariate analysis, the relative risk of death following treatment with CHEMO was 1.92 (95%CI 1.2-3.2) times higher than that for ASCT when adjusted for disease symptoms at diagnosis. High dose chemotherapy with haematopoietic cell transplantation offers improved long term survival when compared with normal chemotherapy for patients with primary refractory HD.

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The patients who achieved PCR negativity after high-dose therapy with autologous stem cell transplantation for B-cell lymphoproliferative disorder (B-LPD) seem to have a better prognosis


Objectives: The high-dose therapy (HDT) followed by autologous stem-cell transplantation (ASCT) represent an effective treatment modality for patients (pts) with B-LPD. In our study we compared the outcome of pts with B-LPD according to their PCR status after transplant.

Patients and Methods: We performed clinical and molecular follow up of pts treated with ASCT in samples of bone marrow, peripheral blood and available peripheral blood stem cell (PBSC) harvests. Touchdown PCR was used for t(14;18) and t(11;14) and one step PCR for CDRIII detection. Out of 64 pts tested by PCR before ASCT 35 were PCR informative and 25 were eligible for further monitoring. Median age of pts was 54 years (29-63), 14 were females (56%). 9 pts had follicular cell lymphoma (FL), 2 diffuse large cell lymphoma (DLCL), 4 mantle cell lymphoma (MCL), 7 chronic lymphocytic leukemia or CLL type of NHL (CLL), 3 others.

Disease status at the transplant was evaluated as follows: 2 pts were in 1st CR, 7 were in 1st partial remission (PR) and 16 in chemosensitive relapse. The median number of previous therapy lines was 2 (1-5). 10 pts received rituximab (R) as an in vivo purging, in 7 cases the PBSC were purged ex vivo. The median follow up of living pts was 24 months (1-82).

Results: Out of 25 pts monitored by PCR 8 pts (32%) were positive for t(14;18), 1 patient (4%) for t(11;14) and 16 pts (64%) for CDRIII. 18 (64%) out of previous positive pts reached the PCR negativity. Out of 22 PBSC harvests tested 4 were PCR negative. Neither ex vivo or in vivo purging nor the PBSC contamination had any significant influence on the disease progression. In the PCR negative group 2 (11%) pts relapsed and we observed 4 (57%) relapses and 2 (29%) progressions after ASCT in the PCR positive group within the follow-up period. The estimated progression free survival (PFS) of patients who reached PCR negativity was significantly better than in the PCR positive patients both after ASCT (89% vs 33% at 2 y) (p<0.001). There was one death among the PCR positive pts.

Conclusion: We conclude that ASCT leads to PCR negativity in a considerably high number of patients with B-LPD and this appears to improve their prognosis. Data from this our small trial will require confirmation in larger trial and further follow-up.
Allogeneic and autologous stem cell transplantation for patients with relapsed or primary refractory lymphoma. A single center experience

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The majority of patients with malignant lymphomas are not cured with conventional chemotherapy. Ongoing studies are actually evaluating the role of high-dose chemotherapy as first or second line treatment, as well as the graft-versus-lymphome effect after allogeneic transplantation. We retrospectively analysed nifty-five lymphoma patients receiving either autologous (n = 81) or allogeneic (n = 14) stem cell grafts at our institution between 1991 to 2001.

Patients: Fourteen patients with Non-Hodgkin-Lymphoma (intermediate grade NHL n = 2; high-grade NHL (n = 10) and low grade NHL n=2) were treated by allo-PBSCT since October 1991. The median age was 34.4 years. All patients were either primary refractory to first line chemotherapy or in first or subsequent relapse. In order to achieve a remission patients received chemotherapy according to the B-ALL-protocol (GM-ALL Trial), one patient with CLL was treated with CVP and another patient with mantle cell lymphoma received regimens consisting in TBI and cytoxan (n=4), I-CVB (idarubicine, BCNU, cytoxan and etoposide) (n=3), CVB (n=4), BEAM (n=1). Two patients received non-myeloablative conditioning with fludarabine and TBI. The median follow-up is 43 months. Twelve patients are alive and in complete remission. One patient relapsed and died from progressive disease after 3 months.

Eighty-one patients were autografted (intermediate grade NHL n= 29; high-grade NHL, n = 52). The median age was 50.2 years. Autologous peripheral blood stem-cells were collected subsequent to B-ALL or HAM protocol and G-CSF. High-dose chemotherapy consisted of I-CVB, CVB or BEAM. The median follow up after autologous stem cell transplantation is now 17 months. 45 are still alive, 44 patients are still in CR, one patient achieved a PR. 33 patients relapsed after 2 to 17 months after autologous PBSCT, 32 of them died subsequently.

Toxicity: Three patients died of toxicity (sepsis n=1, Cardiac toxicity n=1, toxic colitis n=1). All patients developed mucositis (WHO grade IV).

Conclusions: Our data confirm the effectiveness of high-dose chemotherapy, but many of them still die from recurrent disease. Although the number of patients is small, the outcome after autologous grafting is promising. These data show that transplantation of hematopoietic stem cells (HSC) can represent a curative treatment for lymphomas, possibly due to the immunological effect of the donor cells, the graft versus lymphoma effect.

High-dose chemotherapy without stem cell support followed by an allogeneic low-intensity-transplant in refractory lymphoid neoplasia

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Background: Treatment of refractory lymphoma is not satisfactory. Allogeneic Mini-stem cell transplant (mSCT) has been shown to induce a possible graft-vs-lymphoma effect but this lacks the potential benefit of high dose chemotherapy (HDC). BEAM with autologous SCT for tumor debulking followed by allogeneic mSCT is a concept currently tested in clinical trials. We report on 2 cases undergoing BEAM without stem cell support followed an allogeneic mSCT to treat refractory lymphoma.

Patients: Patient 1, F, 51y of age was diagnosed 4 years ago with low grade Non Hodgkin Lymphoma stage IVA. After initial response she relapsed several times, and was treated with 7 different types of chemotherapy including CHOP and Rituximab. She progressed rapidly while undergoing salvage chemotherapy with DHAP. Patient 2, M, 25 y of age was diagnosed 2 years ago with nodular sclerosing Hodgkin's disease stage IIA with mediastinal bulk. He obtained CR on LOPP/ABV and irradiation but relapsed 6 months later. He was then treated with further chemotherapy and an autologous SCT, but relapsed 3 months later with rapidly progressing mediastinal bulk.

Both patients were treated with BEAM chemotherapy using the same dosage as for autologous SCT but without SC-support, followed by an mSCT from an HLA-identical sibling 28 days later using Fludarabine and 2Gy of TBI for conditioning and Cyclosporine and Mycophenolate for GvHD prophylaxis.

Results: Both patients had bone marrow aplasia lasting from the start of the BEAM chemotherapy until day +15 and day +10 after mSCT. Patient 1 developed grade II skin GvHD responsive to steroids. At 12 months after mSCT he is a complete chimera, without evidence of disease progression with a regressing CT-residual mass by PET.

Conclusions: Sequential administration of HDC without stem cell support followed by an allogeneic mSCT is feasible. When an allogeneic mSCT is planned, autologous stem cell support may not be necessary after HDC. Patients with refractory lymphoma may benefit of the bulk reducing effect of HDC separated in time from the graft-vs-lymphoma effect of the mSCT. This approach warrants further study.

Allogeneic transplant for Hodgkin's and non-Hodgkin's lymphomas: a report from the GITMO (Gruppo Italiano Trapianto Midollo Osseo) Allogeneic Marrow Transplant Registry

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Patients with Hodgkin's and non-Hodgkin's lymphomas have high chances of disease control with conventional or high-dose chemotherapy. Nevertheless, autologous bone marrow transplant may provide an additional treatment opportunity, suitable for patients with refractory or progressive disease. To evaluate the role of allogeneic marrow transplant as salvage therapy in relapsed/refractory lymphoma, we reviewed data from 373 patients reported to the GITMO (Gruppo Italiano Trapianto Midollo Osseo) allogeneic marrow transplant registry from 1988 and 1999. The median year of transplant was 1998, indicating that over 150 patients were allografted in the last 3 years (53 in 1998, 75 in 199, and 98 in 2000). Overall, detailed records on 305 patients with non-Hodgkin's lymphoma (NHL) and 68 with Hodgkin's lymphoma (HL) receiving an allogeneic marrow transplant for relapsed or refractory disease were reviewed. Median age was 34 (12-74) and 28 (14-51) years, in NHL and HL respectively. Most patients (73%) were grafted with cells from HLA-identical siblings while few patients (9%) underwent a transfant from an unrelated donor. The autologous bone marrow in 188 patients (50%) and marrow in the other 50%. Transplant related mortality (TRM) was overall 40% for NHL, (38% vs 42% (1998, p=0.1) and 40% for Hodgkin disease (42% vs 38% (1998, p=0.7). With a median follow up of 446 days (13-5470) for surviving patients 141 (46%) NHL and 35 (51%) HL patients are alive. The actuarial risk of relapse at 2 years for NHL was 36% and 74% for patients with Hodgkin. For NHL the relapse was 26%, 23%, 6% for patients with absence limited extensive chronic GvHD (p=0.05), and for HD it was 42%, 54%, 60% (p=0.7). No significant differences were observed among follicular, mantle cell and high-grade NHL subtypes in terms of overall and disease-free survival. In conclusion, the data indicate (i) a growing interest for allogeneic transplants in lymphoma; (ii) the persisting high TRM and (iii) a GVH-mediated antilymphoma effect in NHL, but not in HD patients. Taken together, these observations support ongoing studies with reduced intensity preparative regimen in patients with non Hodgkin lymphoma.
Salvage therapy with rituximab plus GM-CSF and CHOP is effective in lymphoma patients relapsed after auto PBSC transplant. Interim analysis from open prospective study in 25 patients


Relapse post-PBPCT in NHL patients is characterized by poor prognosis and in this setting of patients both poor PS and poor hematological tolerance to chemotherapy often make troublesome any salvage options. We evaluated the response and tolerability of RTX associated with GM-CSF ± CHOP in 25 patients affected by CD20+ NHL, relapsed after PBPCT.

Twenty-five patients, 13 with HG-NHL, 3 mantle cell lymphoma and 9 with LG-NHL, relapsed after PBPCT (one after allogeneic PBPC). received RTX alone (9) or RTX + CHOP (16); for a median of 4 administrations (range: 1-14). The GM-CSF administration has been started 4 days before the second RTX administration and immediately after the CHOP administration, in order both to upregulate the CD20 expression, and to increase the phagocytic activity of CD16+ cells and finally to reduce the leukopenia. Twenty-two patients are now evaluable for response; the ORR was 72.7% (respectively 71.4% and 75%, in aggressive and indolent lymphoma) with 7 CR (31.8%) and 9 PR (40.9%); 4 patients (18.1%) died for disease progression while 2 patients (9.1%) died after the first RTX infusion and are not evaluable for response. The clinical outcome of 21 patients evaluable, with a median follow-up of 6 months (range: 1-31), shows that 19 patients are alive, 9 in CCR (46.2%) and 3 with stable disease; 7 are relapsed. The toxicity of this treatment was acceptable but not negligible, especially in patients receiving the association RTX+CHOP: we observed grade III-IV neutropenia and thrombocytopenia respectively in 33% and 35% of cases, compared with 8% and 15% of patients receiving RTX alone; finally grade III-IV extrahematological toxicity was quite low in the 2 groups (11.3% vs 15.3%) as the incidence of acute grade III-IV events (11% vs 5%).

In conclusion the association RTX+CHOP+GM-CSF seems feasible also after PBPC and highly active in this subset of poor prognosis patients.

Erythropoietin is effective in patients with relapsed lymphoma treated with the Cologne high-dose sequential chemotherapy regimen


Introduction: Patients treated with aggressive chemotherapy and stem cell support usually develop severe anemia requiring multiple red blood cell (RBC) transfusions. Erythropoietin (EPO) has been shown to reduce the number of transfusions in chemotherapy-induced anemia. This study evaluated the effects of EPO on RBC transfusions, hemoglobin (Hb) levels and quality of life in patients with relapsed lymphoma treated with an aggressive high-dose sequential chemotherapy (HDSTC) regimen.

Patients and methods: Sixty patients with early or late relapsed Hodgkin's lymphoma (HD) or first relapse of aggressive Non-Hodgkin's lymphoma (NHL) were treated in a randomized multicenter study involving 23 centers. Patients were stratified according to gender, age (<40 vs. >40 years [y]) and Hb level before therapy (<10 vs. >10 g/dL). Pts in both study arms received two cycles DHAP; patients with PR or CR then received cyclophosphamide 4g/m², followed by PBSC harvest; methotrexate 8g/m² plus vincristine 1.4mg/m² and etoposide 2g/m². The final myeloblative course was BEAM followed by autologous stem cell support. Pts in the experimental arm additionally received 10,000 IE EPO (Neorecombin® 3x/week) from the start of therapy until the end of VP-16. Primary end-point of the study was number of RBC units needed during HDSTC. Hb levels and quality of life were measured.

Results: The median age of the 29 male and 31 female patients was 37y (19-60y). Thirty-nine patients (65%) had HD and 21 patients (35%) NHL. Eighteen patients had stage I/II disease and 42 patients had stage III/IV at relapse. There was no difference in terms of age, gender and Hb level between the two groups. The mean number of RBC units given in the EPO arm was 4.5 compared to 8.9 in the control arm (p=0.0494). Five patients (25%) in the EPO arm and 1 patient (5%) in the control arm did not require transfusions. Twelve patients (55%) in the control arm received >= 8 RBC units compared to 4 patients (20%) in the EPO arm. The mean Hb levels during therapy were 10.4 g/dL in the EPO arm and 9.7 g/dL in the control (p=0.022). Quality of life (QOLQ30) and fatigue (MFI) assessment, however, showed no significant difference.

Conclusion: This prospectively randomized study shows that EPO is effective in decreasing RBC transfusion requirements in patients with relapsed lymphoma undergoing aggressive chemotherapy and stem cell support. Quality of life and fatigue was not different in both treatment arms.

First line treatment in patients with mantle cell lymphoma - A three-phase therapy with ASCT

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Mantle cell lymphoma, with median of OS about 36 months, belongs among unfavourable high-risk malignancies. In an attempt to improve its bad prognosis, new treatment regimes are being presented. Recently, interesting results have been published within the "VAD+Chlorambucil" induction regimen (Gressin, 1997, 2000) and also sensitivity of MCL to anti-CD20 immunotherapy has been proved (Foran, 2000). ASCT done in 1st remission seems to prolong its duration (Blay, 1997). Assuming that complex first-line treatment could lead to a better treatment results and concurrently to prolonged EFS and OS, we have defined a three-phase-treatment strategy taken in the above-mentioned regimes (1/ VAD+Chlorambucil, 2/ Anti-CD20, 3/ ASCT). In 2000-01, 16 MCL patients (9 newly diagnosed, 7 in 1st relapse, age median 62y (42-74), st. IV Ann Arbor 13(81%), BM involvement 13(81%), PB involvement 7(43%), IPI 3 and more - 6(40%), bulky over 10 cm - 5(31%)) were indicated for "VAD+Chlorambucil" chemotherapy with post-treatment response rate as follows: 2x CR (12%), 9x PR (58%), 4x SD (24%), 1x progress (6%). No better response was observed in newly diagnosed pts compared to those pretreated. In the second phase of the complex treatment, 11 pts (2x CR, 7x PR, 2x SD) were indicated for anti-CD20 therapy (Mabthera 375 mg/m² in 1st, 8th,15th,22nd day) with response rate as follows: 4x CR, 3x CRu, 3x PR, 1x SD. In the third phase of the complex treatment, 5 pts (3x CR, 2x PR) were indicated for ASCT (BEAM) with response rate as follows: 4x CR, 1x CRu. Median FU of the whole group of 16 pts is 12 m (6-22) in 9/2001, and PFS, EFS, OS cannot be evaluated properly yet. To evaluate the VAD+Leukeran effectiveness we compared our data with those of group of MCL patients (n=18) treated with "CHOP" regimen in 1996-99. Both groups were comparable in their quality.

Conclusions: For the present, when comparing historical data we cannot prove VAD-Leukeran induction regimen to be more effective than that of CHOP. Our preliminary data show that our complex first line treatment with anti-CD20 therapy and ASCT (BEAM) leads to further treatment response improvement. However, to get definite answers to our original assumptions it is necessary to carry out more observations.
A single center experience of autologous stem cell transplant for follicular lymphoma


The role of autografting in follicular lymphoma remains uncertain. We report a single centre experience of 40 stem cell transplants between 1993 and the present date. The median age at transplant was 46 (range 26-66), 23 patients were male, 17 female. 5 cases had transformed at the time of transplant. 11 patients were stage I or II at diagnosis, 21 were stage III or IV and 8 were unknown. The median time from diagnosis to transplant was 36 months (range 9-211). 18 patients were in complete remission at the time of transplant (range CR1 to CR4), 20 were in partial remission and 1 patient had progressive disease. All patients received autologous peripheral blood stem cells. 10 patients underwent CD34+ selection. The median CD34+ dose for unselected stem cells was 3.37 x 10^6/kg (range 0.8-15.8), for selected stem cells was 1.4 x 10^6/kg (range 0.4-7.6). Conditioning for the autologous transplants was with BEAM (BCNU, Etoposide, Ara C and Melphalan) in 37 patients and Cyclophosphamide with total body irradiation in 1 patient. The median time to neutrophil engraftment (neut+>0.5 x 10^9/l) was 16 days (range 10-20) and to platelet engraftment (platelets >20 x 10^12/l) was 16 days (range 8-43). There was no transplant related mortality. Post transplant 28 patients achieved a CR, 5 patients were in partial response and 7 patients had progressive disease, 6 patients are too early to assess. 6 patients have died, 5 of progressive disease (median time to death 19 months (range 4 to 49)) and 1 of suicide at 6 months post transplant. 34 patients remain alive, with an overall survival of 83% at 36 months median follow up and disease free survival of 60%. 10 patients have relapsed from CR at a median of 7.5 months (range 0-44), 18 continue in CR at a median of 32 months (range 4 - 92). We found no significant difference in overall survival or progression free survival for CD34+ selection, stage at diagnosis, stage at transplantation or with number of previous treatment modalities. Our findings are in line with other published series for follicular lymphoma. Although autologous stem cell transplants appear to be safe, we have found no benefit from CD34+ selection or transplantation early in the course of the disease. Further investigation is required into methods of purging and the timing of autologous stem cell transplantation in follicular lymphoma.

Autologous stem cell transplantation in the treatment of lymphoma

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Introduction: Autologous stem cell transplantation (auto SCT) improves survival in patients (pts) with previously untreated multiple myeloma (MM) and relapsed, chemotherapy-sensitive, aggressive non-Hodgkin lymphoma (NHL) and Hodgkin disease (HD).

Methods: Between February '97 and August '01, 34 pts received auto-SCT for lymphomas: 17 NHL (aggressive histology 82.3%; IPI-score 3/4 78.5%); 13 HD and 4 pts with MM. Median age was 46 (range 26 - 66), 23 patients were male, 17 female. 5 cases had transformed at the time of transplant. 11 patients were stage I or II at diagnosis, 21 were stage III or IV and 8 were unknown. The median time from diagnosis to transplant was 36 months (range 9-211). 18 patients were in complete remission at the time of transplant (range CR1 to CR4), 20 were in partial remission and 1 patient had progressive disease. Twenty one pts (61.8%) remain alive with a median follow-up of 15 (range, 2-52) months and in CR are still 16 pts. The 2.5-years probability of survival for all pts was 46 (95% CI, 38-56%). The probability of progression-free survival at 2.5 years was 44.5 (95% CI, 38-53%).

Conclusion: High-dose chemotherapy with auto SCT has shown efficacy as consolidation therapy in high-risk patients and in chemosensitive relapse. Adequate approach for resistant disease needs further investigation.

Pre-transplant therapeutic activity and mobilizing potential of a vinorelbine (VNR), ifosfamide (IFX) and cytarabine (ARA-C) regimen (VIHA) as consolidation or salvage therapy in poor prognosis non Hodgkin’s lymphoma (NHL)

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Background:The identification of active regimens sharing clinical and mobilizing activity is warranted in NHL pts at high risk at onset of relapse, as induction therapy before transplantation (PBSCT), VNR, IFX, and ARA-C are of proved efficacy in this setting.

Methods: 37 pts (median age 47, high grade 34, low-grade 3) were given the VIHA regimen: VNR 25 mg/m² day 3, IFX 2500 mg/m² days 1-3, and ARA-C 2 gm/m² bid days 1-2. Pts older than 60 years were given the same regimen at 75% of doses. Cycles were repeated every 21 days with GCSF support from day 7 to day 20. 17 pts were mobilized with the third one, provided at least PR was achieved after cycle 2. All cases with at least PR after VIHA and with a CD34+ cell yield of > 2 x 10^6/Kg were then candidated to PBSCT. Results: At accrual, 3 pts were in 1st CR, 14 in 1st PR, 4 had primary refractory disease or resistant relapse, and 16 were relapsed after a median of 12 months. VIHA converted PR in CR in 1pt with 1st PR, and improved the quality of PR in 4 of 3 pts in 1st CR, 2 remained in CR and 1 progressed. Of 4 pts with resistant disease, 1 showed an objective response. Ten out of 16 cases with relapsed disease (62%) responded (4 CR and 6 PR). 13 pts did not undergo leukapheresis due to early disease progression (2) or to previous PBSC collection with other agents. Median number of CD34+ cells collected was 7.4x10^6/Kg (range 0.5-45) after a median of 2 (1-3) apheresic procedures. Two pts could not mobilize an adequate amount of CD34+ cells. Up to 11/01, 22 pts have received PBSCT and at the end of the treatment program 10 were in CR and 4 in PR.

Toxicity: G IV neutropenia and thrombocytopenia occurred in 100% of cases, with all pts but 1 requiring PLT transfusions. RBC transfusions were not required in 12 cases. G II-III fever complicated 60% of cycles and 9 pts had documented infection with 1 CMV pneumonia. Overall, 24 pts were hospitalized for management of toxic effect.

CONCLUSIONS: this regimen shows clinical and mobilizing activity. However, it should be reserved to highly selected cases with particularly poor characteristics at relapse, in order to justify its toxicity profile. Reduced dose of ARA-C could allow a better management of the regimen, especially concerning thrombocytopenia.

Immunol-chemotherapy with rituximab, high-dose therapy and autotransplant of in vivo purged stem cells as treatment of advanced follicular and mantle cell lymphoma


We combined chemotherapy, immuno-chemotherapy, mobilization of in vivo purged stem cells, high-dose therapy and autotransplant.
for the treatment of 14 patients with advanced, refractory or relapsed, follicular (n=10) or mantle cell (n=4) lymphoma (median age 51, range 42-59; 13 stage III-IV and 1 stage II bulky; 8 with marrow involvement; 6 bcl-2+ and 4 bcl-1+ in blood and marrow). This program was organized in a sequence of 4 phases, each designed to play a specific role in tumor eradication: Phase 1 was a debulking phase consisting of 6 weeks of VACOP-B; Phase 2, immuno-chemotherapy, consisted of two six-day courses with Rituximab on day 1, vincristine on day 2, and cyclophosphamide from day 2 to day 6. Phase 3, in vivo purging and stem cells mobilization, coupled Rituximab on days 1 and 9 with high-dose cyclophosphamide and melphalan (140 mg/m2) and rituximab. Phase 4 consisted of high-dose therapy with BECOP followed by rescue with in vivo purged autologous progenitor cells and two doses of Rituximab on day 14 and day 21 after transplant. After phase 1, 4 patients reached a CR, 9 a PR, and 1 less than PR. No patient PCR-positive for bcl-2 or bcl-1 rearrangement in blood and marrow achieved a molecular response. After phase 2, 8 patients obtained a CR, and 6 a good PR. Of 10 patients PCR-positive for bcl-2 or bcl-1, 8 showed disappearance of the signal from blood and marrow. Phase 3 was effective in mobilizing an adequate number of progenitor cells (median number of CD34+ cells harvested: 15.7±10(6)/kg), that were PCR-negative in all informative cases. Autograft was performed in 13 patients; 1 patient with mantle cell lymphoma progressed before transplant. The median time to neutrophils over 500 was 12 days, and to platelets over 20,000 was 28 days. Two Rituximab infusions post transplant had no side effects and did not affect hemopoietic recovery. After a median follow-up of 12 months (2-18) after transplant, all 13 patients are in CR. Of 9 patients bcl-2 or bcl-1 positive, 8 remained PCR negative and one became bcl-1 positive 5 months after transplant. This sequence of chemotherapy, immuno-chemotherapy, stem cell mobilization and in vivo purging and autografting is simple to administer and devoid of toxic effects. It permits rapid attainment of clinical and molecular response and enables the harvest of lymphoma-free peripheral blood progenitor cells even in heavily pretreated patients.

BECP (BiCNU, etoposide, chlorambucil, prednisolone) salvage treatment for Hodgkin’s disease (HD) post autologous hematopoietic cell transplantation (AHCT)

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Relapse remains the major obstacle to a successful outcome of AHCT in HD. The management of this resistant entity requires combined chemo ± radiotherapy. We retrospectively analyzed the outcome of 7 patients with a median age of 34 years (15-42) who had undergone AHCT for primary refractory or relapsed HD. Histological subtype was nodular sclerosis in 6 and lymphocytic predominance in one patient. Five out of 7 patients were refractory to the intermediate intensity mobilization regimen administered pre-AHCT. The BECOP (BiCNU: 100 mg/m2 day 1; Etoposide: 100 mg/m2 days 1-5; Chlorambucil: 18 mg/m2, days 1-5; Prednisolone: 100 mg/m2 days 1-5) regimen was used as salvage treatment for patients with relapse (3/7; 1 early, 2 late) and resistant disease post AHCT (4/7 patients); a median of 6 (2-8) cycles were administered. BECOP was initiated at a median of 22 (7-54) months post AHCT; disease stage at the onset of BECOP treatment was I/II (1/7), IVA (5/7), IVB (1/7), with bulky disease in two patients. Three out of seven patients had already received other chemoradiotherapy regimens post AHCT. The BECOP regimen proved feasible at the outpatient clinic and was well tolerated by the patients. One heavily pretreated patient developed grade 3 (WHO) hematologic toxicity, while another, with a history of congenital thrombophilia, had an episode of deep venous thrombosis successfully managed with anticoagulants. Two patients had complete remission lasting, respectively, 6 and 50+ months, 4 attained partial remission (PR) and there was one treatment failure. Five patients (71%) achieved major response and are alive a median of 25 (17-112+) months post AHCT. Two patients (one PR case plus the failed case) died of disease progression, at 4 and 24 months post chemotherapy. In conclusion, the BECOP regimen seems to be effective in the management of HD relapse post AHCT, for which treatment options are generally unsuccessful. The immediate toxicity of the regimen was acceptable and no major late effects or secondary myelosuppression were observed at the longest follow up.

Allogeneic stem cell transplantation with reduced intensity conditioning for poor prognosis lymphoma (a single center experience)

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Graft versus lymphoma effect is a principal anti-tumor mechanism of allogeneic stem cell transplantation (SCT). Allogeneic SCT using traditional conditioning regimens (BEAM, BUCY2) is associated with unacceptable transplant related toxicity and mortality, but reduced intensity conditioning with allogeneic SCT is well tolerated in elderly and high-risk patients (pts).

Patients and methods: We report six pts with NHL lymphoma (2 follicular, 2 diffuse large B-cell, 1 peripheral T and 1 T lymphoblastic). All pts had either relapse (3 pts) or refractory disease (3 pts). Tumor mass was reduced by salvage chemotherapy regimen (3 pts-ESAP) or high dose chemotherapy regimen (5 pts-ALP). All allogeneic SCTs received stem cells from HLA identical sibling, after preparation with Flu-Cy regimen (cyclophosphamide 300mg/m2 on days 4-3,2 and fludarabine 30mg/m2 on days 4-3,2). Cyclosporine A and methotrexate were used for GVHD prophylaxis. Results: One pt died during SCT of NHL progression. The treatment was well tolerated and toxicity grade 3 or 4 was not observed. Mixed chimerism was documented in 5 pts on day +30, complete chimerism was documented in all pts three months after SCT. Acute GVHD grade 3 or 4 occurred in 1 pt, extensive chronic GVHD occured in 1 pt. Pts with extensive chGVHD died (in CR) of GVHD complication. CR is documented in 2 pts and stable disease in 2 pts. Median follow-up was 14 months (7 – 21 months) in 4 surviving pts.

Conclusion: These early data suggest that reduced intensity conditioning followed by allografting is a safe and feasible procedure in pts with poor-prognosis NHL and has a high proportion of complete or partial remission.

Predictive factors for stem cell mobilization in lymphomas

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High-dose therapy supported by autologous stem cell transplantation is a widely used treatment modality for lymphomas. Blood is the most popular source of autologous stem cells. A prerequisite for stem cell transplantation is a sufficient mobilisation of stem cells so that adequate yields can be harvested. The blood CD34+ cell concentration predicts accurately the CD34+ cell content in the leukapheresis product and can be used as a measure of the mobilisation success. To characterise those factors that predict a mobilisation failure in lymphomas we analysed our 26 consecutive patients with failed mobilisation of CD34+ cells (definition of failure: maximal blood CD34+ cell count <20 x 10^6/L), and compared them with 71 patients who had successful mobilisation. CD34+ cell counting was performed with flow cytometry, and the data were evaluated according to standard statistical methods (SPSS software). The patient characteristics for the groups with failed and successful mobilisation were (median values): age 53.5 and 48 (P=0.02), time from diagnosis to mobilisation 14.5 and 8 months, number of previous chemotherapy cycles 11 and 8 (P=0.04), and number of previous chemotherapy regimens 2 and 2 respectively. In the failure group more patients had diffuse large-cell NHL (46 vs 31 %) and follicular NHL (39 vs 30 %), whereas all patients with Hodgkin’s disease were in the group of successful mobilisation (18% of patients). The disease status between the respective patient groups did not differ: 96 and 91 % of patients were in CR or PR. In addition to older age and higher number of previous
chemotherapy cycles, other significant pre-mobilisation factors predicting mobilisation failure were higher serum LDH (P=0.0008), and higher International Prognostic Index (P=0.001). The mobilisation-related factors predicting mobilisation failure were lower platelet nadir (P=0.0003) and longer duration of platelet count <20x10^9/L (P=0.002) after mobilisation therapy. The predictive factors may be useful in identifying those patients who need more potent mobilisation agents and those with sufficient harvests for stem cell manipulations.

**Rituximab in CD20+ lymphoproliferative disorders as in vivo purging in the context of PBSC transplantation**

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Patients with aggressive NHL showing progression on the 1st and 2nd line chemotherapy have extremely poor prognosis, also the patients with indolent lymphomas who relapsed after the 4th course of treatment. Monoclonal antibodies to CD 20 (Rituximab) may diminish the risk of relapse after HDC with AutoSCT in pts. with B-cell lymphoma due to possibility in vivo stem cell purging and adjuvant immunotherapy of minimal residual disease.

Since April 2000 to October 2001 8 pts. (6 m. and 2 f., median age 36 (21-60), with poor prognosis B-cell NHL (DLCL with progression on the 1st and subsequently the 2nd line chemotherapy - 6 pts., follicular lymphoma with the 4th early relapses - 2 pts.) were included in HDC + Rituximab (Rit) protocol. Pts. were treated with 1-2 Dexto-Beam chemotherapy courses followed by G-CSF and Rit. 375 mg/m2 one day before PBPC collection or BM withdrawal in heavily pretreated pts. After the 4 collection patients were treated with Rit. 375 mg/m2 followed one day later BEAM HDC. After the neutrophil recovery (2-3 weeks post SCT) 2 additional Rit. infusions were performed in next two weeks. In case of platelet recovery 3rd post HDC infusion performed with DHAP chemotherapy and 4th Rit. infusion, performed after the recovery, followed by radiotherapy on residual mass or bulky disease regions.

To date 7 pts finished the protocol and SC collection were performed in 1 pt. CR was reached in 3 (43%) and PR in 4 (57%). Two pts with DLCL and PR progressed and dyed 2 and 3 month after the cessation of treatment. 4 pts showed no signs of progression or myelodysplasia 3-14 month after the treatment. One pt. relapsed in 12 m. and was successfully treated with CHOP + Rit chemotherapy (CR after the first course).

We conclude that Rituximab after the HDC is well tolerated and may have additional role in the treatment of patients with resistant lymphoma.

**Rituximab in CD20+ lymphoproliferative disorders as in vivo purging in the context of PBSC transplantation**

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Bone marrow involvement in low grade lymphoproliferative disorders is a common finding which is rarely eliminated by standard treatments. The introduction of Rituximab as specific immunotherapy of CD-20+ lymphomas has provided the opportunity to eliminate the disease from the BM and several studies are in progress. This report is based on Rituximab as in vivo purging therapy in low grade lymphoproliferative disorders with BM involvement. The therapy consisted of CHOP 4 courses, Cytoxan 7gr/sqm, Rituximab at 375mg/sqm single dose as purging in vivo, first collection of CD34+ cells, AraCytin 8gr/sqm and Rituximab as second course of purging in vivo, second collection of CD34+ cells, PBSC transplantation following BEAM therapy by using the best purified collection of CD34+ cells. Up to October 2001 15 pts entered the evaluation; 5 pts had stage B (3pts) and C (2pts) Chronic Lymphoid Leukemia (CLL), 2 Follicular Center Lymphoma (FCCL), 3 Lymphoplasmacytic Lymphoma (LP), 1 Centroblastic Lymphoma (CB), 1 Mantle Cell Lymphoma (MCL), 1 Splenic Marginal Cell Lymphoma (SMCL) and 1 Prolymphocytic Leukemia (PL) and 12 pts are now valuable because either completed or stopped the program and 4 pts are under therapy. Data are presented on the 12 valuable pts: mean age 54 years (range 37 – 62 ), 8 pts male and 4 female. Two pts stopped the program because a re-activation of HBV infection following Rituximab with acute hepatitis in one pt. All pts entered clinical, pathological and immunologic remission after the purging and mobilization therapy, the only valuable pt with FCCL and blc-2 molecular positivity resulted molecularly negative after the second purging approach. Except for the two pts who stopped the procedure and had immunologic relapse at 12 and 18 months, respectively, but still clinical CR, all pts did PBSC transplantation and remained in clinical CR 3-24 months. 9 out 10 pts in immunological CR; the only patient who had an immunophenotypic relapse was a CLL stage C previously treated. The entire procedure was uneventful and well tolerated. We conclude that a sequential therapy including Rituximab as in vivo purging procedure in low grade lymphoproliferative disorders warrants durable CR in most of patients and exerts a safe profile.

**Autologous stem cell transplantation (ASCT) in lymphomas:**

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A retrospective analysis was performed on 153 patients (pt) with NHL or HD consecutively treated with the BEAM regimen and ASCT at our centre. The aim of the study was to investigate the impact of previous chemotherapy, CD34+ cell dose, and several variables at diagnosis and at transplant, on engraftment, transfusion requirements, days of hospitalization, and incidence of infections. Using multivariate analysis, the predominante prognostic factors negatively affecting hematological recovery were: bone marrow as source of stem cells on neutrophil recovery to >0.5 x 10^9/L (p < .0001), and >100 x 10^9/L (p < .0001), RBC transfusions (p = .019), platelet transfusions (p < .0001), and days of hospitalization (p < .0001); dose of VP-16 received before transplant (850 vs. >850 mg/m2) on neutrophil recovery to >0.5 x 10^9/L (p = .042), platelet recovery to >20 x 10^9/L (p = .0002), and >50 x 10^9/L (p = .0003), RBC transfusions (p = .005), and platelet transfusions (p = .005); and dose of doxorubicin (300 mg/m2 vs. >300 mg/m2) on platelet recovery to >20 x 10^9/L (p = .002), and >50 x 10^9/L (p = .02), and RBC transfusions (p = .006). However, age at transplantation (<40 vs. >40 years) was the single factor that significantly increased the incidence of grade 3-4 infections (1.5% vs. 11.5%, p = .019). When peripheral blood (PB) stem cell transplants were separately analyzed, the predominante factors negatively affecting neutrophil and platelet engraftment were: CD34+ cell dose infused (<1.6 vs. >1.6 x10^6/Kg), VP-16 dose (850 vs. >850 mg/m2), and extranodal involvement at diagnosis. Factors affecting transfusion requirements in PB transplants were: CD34+ cell dose infused (<1.2 vs. >1.2 x10^6/Kg), VP-16 dose (850 vs. >850 mg/m2), and doxorubicin dose (300 mg/m2 vs. >300 mg/m2); while patients with HD, and patients >40 years, had a higher incidence of severe infections. The principal factors adversely affecting CD34+ cell collection (<1.2 x10^6/Kg) were VP-16 dose previously received (850 vs. >850 mg/m2), and extranodal involvement at diagnosis. We conclude that exposure to stem-cell toxic drugs, specially etoposide, is a critical factor adversely affecting CD34+ cell collection and hematological recovery after transplantation. However, the predominante factors negatively affecting engraftment did not increase the incidence of severe infections in our series.
4. Multiple Myeloma

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Use of infusional chemotherapy (C-VAMP) for patients who relapse after original C-VAMP and high-dose therapy (HDT)

When patients with myeloma relapse, it is unclear whether they should receive the same infusional chemotherapy (IC) they had at diagnosis or something different. i.e. has drug resistance occurred? We studied 82 patients who were originally treated with C-VAMP (cyclophosphamide, vincristine, doxorubicin, and methylprednisolone; 16 with added verapamil), followed by a single autograft after 200 mg/m2 melphalan (HDM200), and then received C-VAMP again at relapse. Initial CR rate following C-VAMP was 9/62 (15%) with an overall response rate of 57/62 (92%). Post HDM200, total CR rate was 56/62 (78%). Time to relapse after the transplant was 3.83 mo (median 29), and the interval between the initial and salvage C-VAMP 9.121 mo (median 35). 57/62 patients (92%) had responded to 1-8 (median 5) cycles of initial C-VAMP, whereas 31/62 patients (50%) responded to 1-7 (median 4) cycles of salvage C-VAMP (P<0.0001). 3 (5%) patients died of toxicity after salvage C-VAMP. There was no obvious correlation between response to initial and salvage C-VAMP. Of 31 responders to salvage C-VAMP, 24 were previously in CR compared with 23 of 31 non-responders (NR). Being in first CR hence made no difference to predicting response to salvage C-VAMP at relapse. No other factor predicted for resistance to salvage C-VAMP. Patients not responding to C-VAMP received alternative therapy including a salvage transplant using previously cryopreserved cells (n=16) or allograft (n=2). 23/31 patients responding to salvage C-VAMP received a second transplant as consolidation. Of the 31 responders, 3 were already in CR after salvage C-VAMP and an additional 8/23 (35%) attained CR after second HDT. Of the 31 NR to salvage C-VAMP, 6(19%) attained CR after salvage HDM. The final CR rate to HDT was therefore, 11/23 (48%) in the responders to salvage C-VAMP and 6/18 (33%) in the NR (P=0.3). Total CR rate after second HDT was 17/41(41%). We conclude that there is no evidence that resistance develops to IC when used again in a salvage setting. Practically speaking, its not possible to predict which patients will respond to salvage C-VAMP, so all patients should be candidates to receive it at relapse. Undoubtedly, an additional benefit to salvage C-VAMP is that it acts as platform for subsequent salvage chemotherapy, so all patients should be candidates to receive it at relapse. No other factor predicted for response to salvage C-VAMP. Patients not responding to C-VAMP received alternative therapy including a salvage transplant using previously cryopreserved cells (n=16) or allograft (n=2). 23/31 patients responding to salvage C-VAMP received a second transplant as consolidation. Of the 31 responders, 3 were already in CR after salvage C-VAMP and an additional 8/23 (35%) attained CR after second HDT. Of the 31 NR to salvage C-VAMP, 6(19%) attained CR after salvage HDM. The final CR rate to HDT was therefore, 11/23 (48%) in the responders to salvage C-VAMP and 6/18 (33%) in the NR (P=0.3). Total CR rate after second HDT was 17/41(41%). We conclude that there is no evidence that resistance develops to IC when used again in a salvage setting. Practically speaking, its not possible to predict which patients will respond to salvage C-VAMP, so all patients should be candidates to receive it at relapse. Undoubtedly, an additional benefit to salvage C-VAMP is that it acts as platform for subsequent salvage chemotherapy, so all patients should be candidates to receive it at relapse.

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Administration of weekly cyclophosphamide as part of infusional induction chemotherapy (C-VAMP) does not affect subsequent peripheral blood stem cell collection in patients with myeloma

We have shown that 500 mg cyclophosphamide (absolute dose) given on days 1, 8 and 15 of a 21-d cycle of infusional chemotherapy with vincristine, doxorubicin, and methylprednisolone (VAMP) results in significantly higher CR rates in patients with myeloma (Raje et al. Br J Haematol 1997;97:153-160) and improves EFS in patients with light chain disease (Sirohi et al. Bone Marrow Transplant 2001;28:29-37). C-VAMP is administered until the achievement of a plateau. As chronic administration of low-dose cyclophosphamide administered to newly-diagnosed myeloma patients during C-VAMP chemotherapy affected the number of CD34+ cells harvested after mobilisation with G-CSF, 80 previously-untreated patients presenting between 11/94 and 12/00 received 2-8 (median 5) cycles of C-VAMP chemotherapy prior to leukapheresis. G-CSF was used as single agent to mobilise stem cells and collections were performed on 2 consecutive days on Cobe Spectra cell separators. Since the leukocyte count had to be >2 x 109/L and the platelet count >100 x 109/L for Cy administration during C-VAMP, most patients did not receive all scheduled doses of Cy (median number of doses administered: 75%; range, 8-100%). Number of Cy doses administered ranged from 1 (500 mg)-19 (5.5g) with median of 10 (5 g). The number of CD34+ cells collected was 0.26-11.64 x 106/kg (median 2.56). There was no correlation between the number of CD34+ cells collected and the cumulative dose of Cy administered (r2=0.004;P=0.6), the number of cycles of C-VAMP administered (r2=-.001;P=0.8), or the proportion of the scheduled doses of Cy administered (r2=0.003;P=0.6). The total amount of Cy, the number of C-VAMP cycles, and the proportion of the scheduled doses of Cy received were comparable for patients yielding <1 vs =1, <2 vs =2.56 vs >2.56, and <5 x 106 CD34+ cells/kg. While we have used VAMP (infusional chemotherapy without Cy) in the past, a direct comparison between C-VAMP and VAMP was not possible because all VAMP-treated patients underwent bone marrow harvests. We conclude that the cumulative dose of Cy infused during C-VAMP chemotherapy does not adversely affect subsequent CD34+ cell collection for autotransplantation. Intensification of infusional induction chemotherapy in myeloma by the addition of weekly Cy is an appropriate step in the sequential therapy of myeloma.

P453

Intermediate dose melphalan (70mg/m2) and G-CSF in patients with refractory myeloma is a safe and effective therapy which enables peripheral stem cell harvesting for transplantation

We investigated efficacy of and tolerance to intermediate dose melphalan (IDM; 70mg/m2) and G-CSF (5mg/kg) as therapy and for mobilisation of peripheral blood stem cells (PBSC) in nine patients (median age 54, range 47-59) with resistant or relapsing myeloma who were referred to our centre over the past two years. The toxicity of IDM consisted mainly of bone marrow suppression with a median time for white cell count recovery > 1 x 109/l of 19 days (range 16-21) and for platelet recovery > 20 x 109/l of 16.5 days (range 13-35). At three months post PBSCT a major response in the paraprotein at three months post PBSCT was observed in 5/9 (56%) patients and a significant myeloma response (20% or greater reduction in paraprotein) was observed in 3/9(33%) patients. The toxicity of IDM consisted mainly of bone marrow suppression with a median time for white cell count recovery > 1 x 109/l of 19 days (range 16-21) and for platelet recovery > 20 x 109/l of 16.5 days (range 13-35). A significant myeloma response (20% or greater reduction in paraprotein) was seen in all patients. Major response (at least 50% reduction in paraprotein) was observed in 3/9(33%) patients. Successful PBSC harvesting was achieved in 7/9 (80%) patients after a median of 22 days (range 19-28). Two patients failed to mobilise PBSC.

The seven patients who mobilised stem cells subsequently underwent PBCT with Melphalan 200 mg/m2 conditioning. Engraftment was relatively rapid: neutrophil count > 0.5 x 109/l on day 18 (range 15-22); white cell count > 1 x 109/l on day 16 (range 13-18) and platelet count > 20 x 109/l on day 23 (range 13-35). At three months post PBSCT a major response in the paraprotein was achieved in all seven patients. We conclude that IDM is a safe and useful second line treatment for refractory or relapsing myeloma with an acceptable toxicity profile. The majority of patients mobilised adequate peripheral blood CD34+ cells for subsequent transplantation which resulted in a major response in the paraprotein at three months post transplant. Follow up of these patients continues.
CD34+ cell selection has no impact on the outcome of autologous blood stem cell transplantation (ABSCT) in multiple myeloma (MM)

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Intensive therapy supported by ABSCT does not cure MM. One attempt to prevent relapses has been through CD34+ cell selection. This paper describes the results of a non-randomised comparison between ABSCT with CD34+ cell selected (n=25; SEL group) and unselected (n=39; UNSEL group) grafts in 64 consecutive patients with MM transplanted between 4/93 and 12/98. As of 31.8.2001, the median follow-up time of the survivors is 67.5 months. Leukaphereses were performed using Fenwal CS-3000 Plus device (Baxter), and the CD34+ cell selection with CellPro Ceprate (Bothell) or Isolex 300i (Baxter) devices. The median CD34+ cell purity in the graft after selection was 89% for CellPro Ceprate and 98% for Isolex 300i. High-dose therapy was MEL 140 mg/m² + TBI 12 Gy (n=17) or MEL 200 mg/m² (n=47). The CD34+ cell doses, engraftment kinetics, days until discharge from hospital, and responses to high-dose therapy were similar for both the SEL and UNSEL groups. There was no difference in the median overall survival (75 and 78 months, resp.) or progression free survival (30 and 26 months, resp) between the SEL and UNSEL groups. We conclude that even taking into account the apparent biases of patient selection of the non-randomised comparison, CD34+ cell selection does not seem to have any impact on both short-term and long-term outcome after ABSCT for MM.

Erythropoietin in combination with lenograstim after cyclophosphamide improves peripheral blood progenitor cell yield in multiple myeloma patients

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Intensive treatment with autologous hematopoietic support has become the treatment of choice for multiple myeloma patients up to 65 years of age. Recent observations in vitro and in vivo have suggested that Erythropoietin, when used in addition to chemotherapy and G-CSF, enhances PBPC mobilization. In our study we decided to compare, in a randomized fashion, the effects, on hematopoietic recovery and PBPC mobilization, of administration of CTX (5g/m²) plus G-CSF at a dose of 10mg/Kg/d subcutaneously from day 2 to day 13 versus CTX 5g/m² plus G-CSF and EPO (150 IU/Kg) every other day from day 2 to day 13 in patients with multiple myeloma after 3 regimens of VAD and before Melphalan (200mg/m²).

Materials and Methods: Between December 1999 and June 2001, 22 patients with stage II-III Multiple Myeloma (MM) ranging in age from 48 to 64 years were enrolled. Comparisons between groups of patients were performed by Mann-Whitney U. non parametric tests.

Results: G-CSF-treated patients had median WBC and PMN counts of 72/mL and 31/mL respectively, whereas G-CSF + EPO treated patients had counts of 70.5/mL (p=0.62) and 30.5/mL (p=0.81) respectively, on the day of WBC nadir. The median number of CD34+ cells collected in the first leukapheresis was significantly higher in the EPO group (7.1 x 106 CD34+ cells/Kg) than in the G-CSF group alone (4.9 x 106 CD34+ cells/Kg; p=0.04). Numbers of CFU-GM (x 104/Kg) and MNC count (x 108/Kg) were also significantly higher in the EPO group (43.1 vs. 24.1; p=0.03), (150.5 vs. 79, p=0.04 respectively). The median number of leukaphereses to reach the target yield of 5 x 106 CD34+ cells/Kg was 1 in the EPO group versus 2 in the G-CSF group alone. (p=0.003)

The results obtained in terms of hematopoietic recovery in patients given transplants of G-CSF or G-CSF + EPO mobilized PBPC indicate that G-CSF + EPO treated patients had a significantly faster PLT recovery than G-CSF patients (median number of days for untransfused PLT to reach more than 50.000/L(11 days and 12 days respectively), with reduction of PLT transfusion requirements. (0.7 single donor units vs. 1.7 units in G-CSF patients).

High-dose idarubicin, busulphan and melphalan conditioning regimen for autograft in multiple myeloma

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Since 1997, 54 MM patients responsive to chemotherapy were autografted with Idarubicin-Busulphan-Melphalan (IDA: 42 mg/m²; BU: 16 mg/kg; MELP: 60 mg/m²) conditioning regimen. We update the results on 32 patients (22M/10F) transplanted after lst line therapy and homogeneously treated with DAV regimen (Dexametazone, Vincristine, Dacarbazine) × 3 cycles, followed by Cyclophosphamide 2.4 g/m² + Dexametazone + G-CSF to collect peripheral blood stem cells.

Median age was 53 ys (range 30-60). At diagnosis 4 patients were stage I, 5 stage II and 23 stage III, and the median interval from diagnosis to PBSCT was of 7 months (range 4-39). Recovery of PMN (>0.5x10⁹/l) and PLTS (> 20 x 10⁹/l) was observed after a median of 10 days (range 8-15) and 11 days (range 7-44) from transplant respectively. We observed an objective response (CR+PR) in 13/32 patients (41%) occurring after a median of 9 days (range 7-13). Thirty-one patients had fever, for a total of 20 documented infections (18 bacterial, 2 fungal). Most patients experienced severe oral mucositis, which resolved in association with haemopoietic reconstitution. Median time of hospitalization was of 32 days (range 28-78).One toxic death due to liver failure was reported. Interferon-alpha maintenance treatment was started in 25/32 patients after a median of 5 months (range 3-7).

Overall response rate at three months was 82%. At the time of this analysis twenty-five patients are alive after a median of 32 months (range 1-54), and 22 are progression-free after a median of 32 months (range 1-52). Nine patients progressed after a median of 16 months (range 5-47). The 4-year projected probability of overall survival and progression-free survival are of 67% and 63% respectively. A prospective multicenter randomized study of the EORTC-GIMEMA cooperative group is currently ongoing, to evaluate the efficacy of an Idarubicine-intensified Busulphan-Melphalan regimen compared to a standard Busulphan-Melphalan conditioning.

Intensive Treatment with High-Dose Melphalan as initial Therapy in Newly-Diagnosed Patients with Multiple Myeloma: Key to Attain Rapid Complete Remission (CR)


Myeloma patients responding faster to infusional induction chemotherapy have superior outcome (Powles et al. Blood 2000;96;3277a). Primary therapy with 140 mg/m² melphalan without hematopoietic or growth factor support results in high response rates and very long-term survival in some patients. It is feasible to collect peripheral blood stem cells in newly-diagnosed patients with myeloma so that autotransplantation could potentially be primary therapy (Powles et al. Blood 2001;98.6570a). We hypothesised that the use of 200 mg/m² melphalan (HDM200) as primary therapy followed by C-VAMP chemotherapy may be better than the standard sequence of treatment (C-VAMP therapy followed by HDM200-autotransplantation) by increasing the speed of response and getting patients into CR faster rather than by improving the response rate. Stem cells have been collected at presentation in 38 patients so far with 1 g/m² methylprednisolone on days 1-6 and 12-16 microg/kg G-CSF on days 3-6 followed by leukapheresis on days 6 and 7. 3 patients have received primary HDM200 with autotransplantation. The number of CD34+ cells
infused (collected) were 1.05 (1.05), 3.67 (5.32), and 0.61 (0.98) x 106/kg respectively. The post-autograft period was uneventful with neutrophil recovery to 0.5 x 109/L in 13-19 days and platelet recovery to 50 x 109/L in 16-18 days. The serum paraprotein levels of 57, 39 and 4g/L at the time of HDM200 changed to 0, 8 and 5 g/L respectively on day 35 post-autograft. The plan was to start post-transplant consolidation with C-VAMP chemotherapy and day 42 (4 courses in patients attaining CR with HDM200). C-VAMP was started on time in 2 with detectable paraprotein after HDM200 cleared it promptly after the first cycle of C-VAMP. All 3 patients have now received 4 courses of C-VAMP chemotherapy with no unusual side effects, and adequate hematologic recovery to baseline post-transplant levels has been seen after each course. All 3 patients have attained CR with normal marrow and have started interferon-alpha2b maintenance therapy. As more patients are treated, it will be seen if this encouragingly high response rate persists and if the remissions are sustained. Whether this early intensive chemotherapy in new patients with myeloma is associated with better long-term outcome and perhaps a reduced risk of myelodysplasia to be seen.

P458
Early mobilization, MEL 100, autologous transplantation, and VAD remission maintenance for very high-risk multiple myeloma. Pilot study
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Approximately 1/3 of relatively young (below 60) multiple myeloma patients are diagnosed in either very advanced disease or with disease not responding to standard VAD debulking treatment. In these patients, full recovery usually progresses very fast and they have no chance to benefit from standard VAD x 3, cyclophosphamide mobilization, high dose melphalan (200 mg/m2) chemotherapy, and autologous hematopoietic transplantation because they die earlier. Palumbo et al. (Blood 1999,94,1248) reported a protocol for elderly patients that utilized reduced conditioning (melphalan 100 mg/m2 x 3 at 2 mo intervals each time supported by autologous hematopoietic rescue: MEL 100). We have used and modified this protocol with the aim of developing an effective method of inducing remission in aforementioned subgroup of myeloma patients. Currently, new patients with stage III of disease, who do not respond to one VAD course are then treated with one or two cycles of cyclophosphamide (2-4 g/m2) and have their stem cells collected from the peripheral blood. Subsequently, they are treated with three-four doses of melphalan 100 mg/m2 at 8-12 week intervals each time supported by infusion of peripheral blood stem cells. So far 12 patients entered the protocol and 9 finished it. Four responded with complete remission and remaining with very good partial remission. The compliance was very good, better with each consecutive course. Initial patients who have been extensively pretreated without response with various chemotherapies (including up to 6 VAD courses) had the problem of relatively early myeloma relapse after such treatment. We have attempted to use interferon-alpha, VAD, and thalidomide to prolong remission with mixed results. Subsequently, VAD instead of being used as debulking treatment prior transplantation was introduced as remission maintenance after transplantation with reasoning that it may be more effective in residual than in advanced disease. This method when developed may offer a new treatment alternative for a subgroup of very high risk multiple myeloma patients.

P459
Therapy of multiple myeloma (Mm) with high-dose melphalan followed by maintenance therapy (Mt) with interferon alpha (Ifn) or sequential Mt Ifn and dexamethasone (Dex) - Interim analysis of the randomized trial

212 patients were enrolled in the trial “4W” from April 1996 to August 2001. Previously untreated multiple MM patients underwent 4 courses of VAD chemotherapy; priming with cyclophosphamide 5 g/m2 and high-dose chemotherapy with melphalan 200mg/m2. 161 pts. underwent transplantation and 151 pts. were randomized into the two arms of maintenance therapy (MT): IFN or sequential MT (IFN for 3 months followed, after 4 weeks pause, by 40 mg DEX in days 1-4,10-13,20-23 and after 4 weeks IFN starts again for thenext 3 months). Overall responses were 71 % after VAD and 79 % after PBSC with only 15 % of CR confirmed with immunofixation after transplantation. Early transplant related mortality was 2.48 % (4/161) and almost 26 % of pts. who did not achieve randomisation procedures. 74 pts. and 77 pts. were randomised into IFN group, IFN/DEX group respectively. Total of 54 relapses and total of 21 death already occurred in these randomised patients. Results of interim analysis of the trial “4W” will be presented.

P460
Potential of serum free light chains for managing patients with multiple myeloma and amyloidosis
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At present, monoclonal immunoglobulin markers present in multiple myeloma and light chain amyloidosis are assessed by serum and urine electrophoresis. These techniques are relatively insensitive and poorly quantitative compared with other immunooassays for tumour markers. We have recently shown that immunooassays for serum free light chains can be used in the diagnosis of patients with these diseases. Thus, 80% of patients with nonsecretory myeloma and 97% of patients with amyloidosis had quantifiable abnormalities of serum free light chains. This compared with 0% and 52% respectively, using electrophoretic techniques. These results also have important implications for monitoring patients. A patient with oligosecretory myeloma was assessed prior to bone marrow transplantation and illustrates the main utility of the assays. (A) Serum and urine concentrations of flc in this patient were undetectable by electrophoresis so no quantitative data was available, whilst fcc concentrations by immunooassay were elevated 20 fold and of clear use for disease monitoring. (B) During relapse, bone marrow biopsy showed only 5% plasma cells while X-ray and MRI scans were normal. However, the escalating flc concentrations were convincing evidence of tumour relapse and this observation allowed initiation of chemotherapy. (C) Over this period the flc increase indicated a tumour doubling time of 16 months. This provided prognostic information. Quantification of serum flc may have other advantages. (1) In patients producing both intact immunoglobulin and fcc monoclonal proteins, the short half-life of flc (1-4 hours) is more suited to monitoring tumour therapy than IgG (half-life of 21-25 days). It should be possible to assess responses to individual courses of chemotherapy. (2) Serum fcc concentrations in some patients increase from 10-20mg/L to 100,000mg/L. This large range should allow improved assessment of changes in tumour mass, before and after bone marrow transplantation, compared with the modest changes in immunoglobulin concentrations. (3) Changes in the concentrations of the non-tumour light chain reflect responses of normal plasma cells to therapy and changes in renal
function. This information might help with decisions on the effect of chemotherapy on normal marrow activity and renal function management.

P461
Early chemosensitivity to VAD predicts a favourable outcome after autologous stem cell transplantation in multiple myeloma
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Objective: Abnormal karyotypes or increase of serum beta 2 microglobulin and C-reactive protein or IgA isotype were identified as unfavourable prognostic factors in large series of multiple myeloma (MM) patients treated with ASCT. Since pretreatment with alkylating agents correlated with poor mobilization of PBSC, VAD is commonly used as induction therapy. We investigate if the response to VAD chemotherapy can predict further outcome in a population of MM patients candidate to high-dose therapy.

Methods: Forty-four consecutive MM patients younger than 65 years were treated with high-dose therapy consisting of 4 VAD cycles, collection of PBSC mobilized by 7g/m2 cyclophosphamide (CY) + G-CSF, myeloablative treatment with 12mg/Kg busulfan plus 120mg/m2 melphalan. All the patients had a measurable M-component (MC) in serum or urine at diagnosis. We considered as early chemosensitivity (ES) a reduction greater than 50% of the MC evaluated after 2 VAD cycles and we correlated ES with the response to ASCT and with the progression-free and overall survival.

Results: Out of the 44 patients, 24 (54%) showed ES and the other 20 (46%) cases showed early resistance (ER) or progression after 2 VAD cycles. All the patients were treated with other two VAD cycles and with CY: 2 out of the 20 resistant patients achieved CR2 and we correlated ES with the response to ASCT, and with the progression-free and overall survival.

The difference between the two groups was significative (p<0.001) at every step of the treatment. The median follow-up of the total population was 52 months. No significative difference in the median PFS and OS was shown between EA and ER patients.

Conclusion: Patients who had an early chemosensitivity after 2 VAD were more likely to obtain a CR after ASCT than chemotherapy resistant cases. This observation could help to optimize the design of high-dose therapy protocols, since the ER patients could be identified just after two courses of VAD therapy and shifted to a different chemotherapy regimen.

P462
"Discontinuous complete remission": a new endpoint to evaluate the success of therapy in keeping myeloma patients disease-free for extended periods of time
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In most patients autografted for hematologic malignancies, relapse results in death in a short time. Myeloma is unique - while disease-free for extended periods of time, relapse results in death in a short time. Myeloma is unique - while response and autotransplantation in myeloma has led to CR rates ranging from 50-75%, relapse is however inevitable even in these CR patients and with salvage therapy, these patients are able to attain a second CR and a normal quality of life. The aim of this analysis was to identify prognostic criteria at the time of relapse which predicts for a better survival and also help identify a group of patients who would benefit from further intensive treatment at relapse. 150 newly diagnosed secretory myeloma patients went into CR after (94 IgG, 30 IgA, 26 light chain; 30-79 y, median 55; 71% stage III) who presented with CR2 lasting 2 mo to 5.6+ y, and 3 patients achieved CR3. Total DCR duration was 3 mo to 13.7+ y. 72 patients died 6 mo to 11.3 y after initial therapy, and 78 are alive at 1.2-14.4 y. Age >55, albumin 25 g/L and beta2M >3 mg/L at presentation were associated with shorter CR1 and DCR, and lower survival in univariate analysis. Based on albumin and beta2M, 2 distinct groups emerged. The median CR1/DCR/OS in patients with albumin > 25 and beta2M<3 is 5.7/3.9/9y compared to 3.3/3.9/5y in those with albumin 25 or beta2M>3 (P=0.009/0.010/0.001). Close correlation between CR1 and survival (r2=0.74) was seen, correlation between DCR length and survival was stronger (r2=0.97). Outcome of this group of patients, especially the 44% with favorable albumin-beta2M combination, is exceptionally good with median CR1 length ranging from others to tandem transplantation (4.2 y/4.2+ y, mean=5.6). With availability of agents like thalidomide, one would expect DCR and survival durations to improve further without necessarily prolonging CR1 durations. DCR is a meaningful endpoint in evaluating patients with myeloma; especially useful to gauge impact of salvage therapy on overall outcome.

P463
Relapse following complete remission: Outcome predictors in patients with secretory myeloma

The increasing use of infusional chemotherapy to maximum response and autotransplantation in myeloma has led to CR rates ranging from 50-75%. Relapse is however inevitable even in these CR patients and with salvage therapy, these patients are able to attain a second CR and lead a normal quality of life. The aim of this analysis was to identify prognostic criteria at the time of relapse which predicts for a better survival and also help identify a group of patients who would benefit from further intensive therapy at relapse. 150 newly diagnosed secretory myeloma patients went into CR after (94 IgG, 30 IgA, 26 light chain; 30-79 y, median 55; 71% stage III) who presented with CR2 lasting 2 mo to 5.6+ y, and 3 patients achieved CR3. Total DCR duration was 3 mo to 13.7+ y. 72 patients died 6 mo to 11.3 y after initial therapy, and 78 are alive at 1.2-14.4 y. Age >55, albumin 25 g/L and beta2M >3 mg/L at presentation were associated with shorter CR1 and DCR, and lower survival in univariate analysis. Based on albumin and beta2M, 2 distinct groups emerged. The median CR1/DCR/OS in patients with albumin > 25 and beta2M<3 is 5.7/3.9/9y compared to 3.3/3.9/5y in those with albumin 25 or beta2M>3 (P=0.009/0.010/0.001). Close correlation between CR1 and survival (r2=0.74) was seen, correlation between DCR length and survival was stronger (r2=0.97). Outcome of this group of patients, especially the 44% with favorable albumin-beta2M combination, is exceptionally good with median CR1 length ranging from others to tandem transplantation (4.2 y/4.2+ y, mean=5.6). With availability of agents like thalidomide, one would expect DCR and survival durations to improve further without necessarily prolonging CR1 durations. DCR is a meaningful endpoint in evaluating patients with myeloma; especially useful to gauge impact of salvage therapy on overall outcome.

\[ \text{Median CR1/DCR/OS} = \begin{cases} 5.7/3.9/9y & \text{if albumin > 25 and beta2M<3} \\ 3.3/3.9/5y & \text{if albumin 25 or beta2M>3} \end{cases} \]
bad PS, then the median OS is 3.7 y, 2.1 y and 1.3 y respectively (P=0.001). 5-y probability of OS in the good risk category was 46% (95% CI, 26-64%). These data show that at the time of relapse good performance status and age ≤ 55 y predict for a significantly better outcome. As CR is a surrogate marker of quality of life, these patients should be eligible for intensive chemotherapy at the time of relapse.

P464
Implication of impaired renal function at the time of initiation of infusional chemotherapy including cyclophosphamide (C-VAMP) in patients with newly-diagnosed myeloma

Presentation creatinine value in myeloma is used for staging and assessment of prognosis. We reviewed outcome of 51 patients with creatinine >200 micromol/L at start of infusional chemotherapy with VAMP/C-VAMP (n=9/42). 11 patients died early (1-11 wks, median 3). 9/42 receiving C-VAMP died vs 2/9 getting VAMP (P=0.6). Of remaining 40, 7 attained CR, 23 PR, and 10 did not respond to 2-8 cycles(median 5). On an intent-to-treat basis, overall response rate to induction was 59%. Creatinine declined with therapy in 84% of evaluable patients, and change in creatinine between cycles 1 to 2 was -75% to +137%(median -38%). Extent of change in creatinine (>200 vs others at time of 2nd cycle/25% decline/50% decline) was not predictive of eventual survival suggesting that intensive therapy should be continued even if renal function does not improve after 1 cycle. 40 patients were eligible for high-dose therapy (HDT). 31(78%) had HDT, most with 200 mg/m2 melphalan+autograft, a median of 4.5 mo after starting induction. At HDT, 16 patients had creatinine >200(including 5 on dialysis and 1 about to start). 3/9-3 on

Allogeneic transplantation for multiple myeloma - relapses as isolated solitary plasmacytoma may occur despite molecular remission
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Allogeneic transplantation for multiple myeloma may be curative for young patients but its role remains controversial due to a reported high TRM in some series. Since 1991 we have performed 25 allogeneic transplants for multiple myeloma from fully matched sibling donors using either bone marrow (n=13) or G-CSF mobilised PBSC (n=12). The median age of the patients is 48.2 years (range 34-58 years) and 13 patients were in 1st plateau phase whilst 12 had more advanced or refractory disease. Conditioning was with TBI (12 Gy in 6 fractions) and melphalan 110 mg/m2. Of the 18 evaluable patients at 3 months 13 had achieved CR and the mean time to attainment of CR post-transplant was 2.5 months. The patients who were CR when assessed at 3 months were commenced on a short course of alpha-interferon (3 MGU x 3/week) and 4 subsequently achieved CR with this approach after a median of 82 days (range 42-252). One patient who failed to respond to IFN went on to achieve CR after several doses of DLI therapy thus giving an overall CR rate of 72%. 7 patients have died of transplant related causes (5 infection, 1 GVHD and 1 cerebral haemorrhage) giving a TRM rate of 24%. 7 patients have relapsed at a median of 4.7 years post-transplant (range 1.38-7.7 years) including 2 of the patients who had required IFN therapy to achieve CR. In 5 of these cases relapse has been as an isolated solitary plasmacytoma with no evidence of involvement of the bone marrow by trephine biopsy or by molecular analysis. All patients with localised relapse have been treated with local radiotherapy +/- DLI and are currently disease free despite 1 of these patients having further treatment for a 2nd solitary plasmacytoma. There have been 2 deaths from relapsed disease and 1 patient continues to receive DLI therapy for persistent myeloma. The overall survival at 8 years for all patients is currently 69% and the EFS is 26%. These results suggest that allogeneic transplantation for myeloma using TBI/Melphalan can be carried out with an acceptable TRM and a high CR rate. However late relapses as a solitary plasmacytoma may be a frequent finding and may represent foci of disease not eradicated by the conditioning. The

Differential regulation of dendritic cell function by the immunomodulatory drug thalidomide: implications for multiple myeloma therapy
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Thalidomide was shown to be a potent immunomodulating agent with a broad spectrum of effects. Beside its efficiency in the treatment of erythema nodosum leprosum, it has potential therapeutic applications that span a wide spectrum of diseases, including malignancies, especially multiple myeloma. Recently, it has been shown that thalidomide represents an important therapeutic tool for refractory multiple myeloma patients, even after high-dose chemotherapy. However, the mechanism of action of this drug is unclear. Because of their central role in the induction of primary immune responses, we investigated the effects of thalidomide on monocyte-derived dendritic cells. We show that thalidomide did not block the differentiation of monocytes into dendritic cells, and induced little or no modifications of surface markers. However, thalidomide exerted a dose-dependent modulation of the stimulatory capacity of dendritic cells and their cytokine secretion profile. At a low dose (10 µg/ml), thalidomide little modified the allostimulatory capacity of dendritic cells, whereas a higher dose (20 µg/ml) up-regulated this capacity (P < .01), and increased IL-12 production (P = .001). Moreover, mature dendritic cells generated in the presence of low doses of thalidomide, were poor stimulators of Th1-type responses (P = .01), but a higher dose of thalidomide was able to strengthen Th1 responses (P = .03). The observation that multiple myeloma patients receiving the highest thalidomide doses, are likely to be the best responders further supports our results where the higher dose of thalidomide was associated with the highest IL-12p70 level. The effects of thalidomide on the secretion of IL-12p70 by mature Mo-DC and strengthening of Th1 response by naïve T cells, might account, at least in part, for the antitumor effects of this drug. Collectively, this is the first study on the effects of thalidomide on DC giving new insights into the action of this enigmatic drug. Our study provide framework for the development and testing of dendritic cells/thalidomide-based immunotherapeutic strategies in multiple myeloma and other cancer patients under stringent biological and cellular monitoring.
use of MRI scanning and top-up radiotherapy to involved areas prior to allogeneic transplantation may be useful in preventing these relapses.

P467

Autografting followed by nonmyeloablative allografting (NST) for multiple myeloma

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Allogeneic stem cell transplantation is a potential curative therapeutic option for multiple myeloma patients. However, high treatment related mortality for allografting has been reported for these patients. Immune-mediated graft vs myeloma effect is under study as therapeutic tool for producing remissions in multiple myeloma patients. On the other hand, previous experiences have demonstrated that the curative potential of allografting is affected by the remission state in which the procedure is carried out. For these reasons, we designed a strategy to reduce disease in myeloma patients using autografting and then induce Graft-versus-Myeloma using nonmyeloablative allografting. We report here the outcome data of this combined procedure. A total of 13 patients were treated. Median age was 48 (range 37-62). At diagnosis all patients had Stage III disease. Patients received high-dose melphalan (HD-M) under the age of 65 years, and TBI 200 cGy (Flu-TBI). After a median of three months, 5 patients received an immunosuppressive regimen consisting of fludarabine 30 mg/m2/d x 3 days with cyclophosphamide 300 mg/m2/d x 3 days (Flu-Cy); 8 patients were conditioned with fludarabine 30 mg/m2/d x 3 days followed by TBI 200 cGy (Flu-TBI). At allografting 2 patients were in complete remission, 9 patients in partial response and 2 patients had progressive disease. At a median follow-up of 6 months (range 1-15) full chimerism was achieved from 1/5 patients that received Flu-Cy protocol and in 4/8 patients conditioned with Flu-TBI. Sustained mixed chimerism was observed in 1/5 patient of the Flu-Cy group and 4/8 patients that received Flu-TBI. Seven patients received donor lymphocyte infusum, five patients for chemotherapy refractory disease and two for progression of disease. Four patients maintained the remission achieved before NST. Two chemotherapy refractory patients had Grade 3 aGVHD followed by remarkable graft-versus-myeloma responses. Disease remission was obtained in another full-chimeric patient. No patient died of procedure related mortality; one patient died for progression at 215 days from transplant. These data demonstrate that this novel combined approach reduced acute toxicities of conventional allografting even in high-risk myeloma patients.

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Nonmyeloablative conditioning and allogeneic stem cell transplantation as consolidation therapy after high-dose melphalan and autologous stem cell transplantation for multiple myeloma

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High-dose melphalan (HD-M) followed by autologous stem cell transplantation is standard therapy for patients with multiple Myeloma (MM) under the age of 65 years. But this therapy is not curative. Allogeneic stem cell transplantation after conventional high-dose chemotherapy represents a curative approach due to the graft-versus-myeloma (GvM) effect, but is associated with high transplant-related mortality and therefore only recommendable to a small number of patients. Dose reduced conditioning regimens, sparing toxicity by conserving the GvM effect, are increasingly performed. We treated 9 patients, 6 males and 3 females, median age 47 years (39-59) all suffering from MM stage II-III with HD-M (200mg/m2) and autologous peripheral blood stem cell support. Five pts. were in complete remission (CR), 2 in partial remission (PR), one in stable (SD) and one in progressive disease (PD). Non-hematologic toxicity did not exceed grade II and hematological toxicity was grade III-IV. No treatment related death occurred after a median follow-up of 3 months (1-10). We allografted with peripheral blood stem cells of an HLA-identical sibling donor (n=6) or a matched unrelated donor (MUD) (n=3). Conditioning regime consisted in fludarabine (3x30 mg/m2) and TBI (2 Gy). Prophylaxis for graft-versus-host disease (GVHD) consisted in mycophenolate mofetil (MMF) and cyclosporine A (CsA) for family donors or MMF and tacrolimus for MUD. Five pts. were allografted, two in PR and two in PD at the time of allografting. Median nadir for leukocytes was 1.200/µl (500-1600) and only one pt. required platelet support. Renal toxicity was not seen, mild hepatic toxicity was seen in two pts. Three pts. (all MUD) presented acute GVHD of the skin (grade II), only one requiring and responding to therapy (steroids and PUVA). Despite suffering aGVHD of the skin and gut (overall grade IV) one pt. died on day 99 of PD. Chronic GVHD developed in 3 pts., limited in one and extensive in two. Eight patients are alive after a median follow up of 8 months (3-10) after allografting. Remissions are stable in six pts., while two had progressive disease, which responded transiently to tapering immunosuppression. These data show that allografting after non-myeloablative chemotherapy is a safe and feasible option for patients with MM after reducing tumor burden with HD-M and autologous stem cell support. Larger number of patients are needed to evaluate this therapy as consolidation for MM.

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In MM allogeneic transplantation is associated with an exceedingly high TRM. To reduce mortality and improve disease response we have developed a protocol with G-CSF-primed PBSC transplantation in MM. We report here the results in 30 patients allografted from their HLA-identical siblings. There were 23 males and 7 females. Their median age was 49 y, (range 31-55). Disease characteristics were the following: IgG=17, IgA=6, IgD=1, BJ=5, non-secr=1; stage I=3, stage II=3, stage III=23, plasma-cell leukemia=1; time to transplant = 8 months (3-107). At the time of allograft, 13 patients were refractory and 2 in progression. Only 4 were in CR and 11 in PR. All were conditioned with non-BMI containing regimens, busulfan and melphalan in most cases (N=25), and received donor PBSC collected after priming with G-CSF (N=26) or GM-CSF+G-CSF (N=4). The graft contained a median of 7.9 (4.4-24.1) x 10^6/kg CD34+ and 2.3 (0.9-7.0) x 10^6/kg CD3+ cells. GVHD prophylaxis was CSA-MTX in all. The patients engrafted 0.5 x 10^9/L PMN and 50 x 10^9/L PLT on (median) day 12 (9-17) and 13 (11-22), respectively. aGVHD grade II occurred in 16 patients (53%), and was severe (III-IV) in only 5 (16%). Actuarial incidence of cGVHD was 65%. Actuarial TRM at 100 days was 16%. Of the 27 evaluable patients, 22 (81%) were in CR following transplantation, and only one relapsed. On PCR analysis of IgH-gene rearrangement (patient-specific probes), nine of the 10 patients studied proved negative at least once following the allograft. In the single case with disease recurrence PCR remained positive until relapse. Interestingly, one patient became PCR negative only one year after transplantation with the emergence of cGVHD. Overall survival is 60% and PFS 67% at 6 years. aGVHD was associated with a higher TRM, cGVHD with a better PFS (Cox regression analysis). In MM the use of allogeneic PBSC and chemotherapy-based conditioning results in high remission and low relapse rate. This may result from graft-versus-myeloma (GVM) effect. We are working at a program aimed at minimizing the unwanted GVM effects without relieving the GVM.
Oligoclonal reconstitution after autologous PBPC in myeloma patients - Implications for assessment of remission status


Current criteria for CR require negative immunofixation (IF) in addition to negative electrophoresis (EP) of serum and urine. Several studies have shown that achieving CR post-transplant by these criteria has prognostic significance. We have used these criteria to evaluate response in an EBMT phase III multicentre study comparing CD34+ selected and unselected PBPC. All laboratory data were centrally reviewed and where possible copies of EP and IF strips were examined. 111 patients were transplanted and response was evaluable in 103. Oligoclonal bands were detected by serum EP in 20 pts and by IF in 46, appearing at a median of 3 months post-transplant and persisting for up to 2 years. 75% were IgGk or IgGl. In 28 pts at least one band of the same isotype as the original paraprotein was present. 10 of these pts were clearly not in CR, with a peak of original mobility on EP, and/or >5% BM plasma cell; thus the band of original isotype on IF represented the original clone. In the remaining 18 patients EP was negative with <5% BM plasma cells and it was impossible to know whether or not the band of original isotype was derived from the original clone. These pts were classified as ?CR. On follow-up it was possible to classify some as definite CR or PR. In 4 patients the band of original isotype disappeared (PR); in 5 the original isotype persisted after all other bands disappeared (PR). In 9 patients, however, the pattern of oligoclonal bands with one or more of original isotype persisted during the period of follow-up (still ?CR). The final analysis of best response post-transplant was CR 33 pts; ?CR 9; PR 57; 4 progressed within 100 days. Median PFS was 35 months in CR pts and 25 months in PR pts (p=0.08). PFS for ?CR pts was intermediate. Analysis of factors predictive for PFS showed that isotype, beta2microglobulin at diagnosis and remission status post-transplant were significant. Arm of study, CRP, Durie-Salmon stage at diagnosis and pre-transplant response status were not significant. On multivariate analysis remission status remained significantly correlated with outcome, with a relative risk of relapse/progression of 2.8 for PR patients compared with CR patients (p = 0.08). Conclusion: Oligoclonal bands are frequently observed post-HDT in myeloma patients and may complicate the analysis of remission status. As OR is associated with improved outcome, careful follow-up is important in order to classify remission status accurately.

Additional abstracts to this topic

Impact of the number of infused CD34+ cells on the long-term outcome of myeloma patients treated with high-dose melphalan and autotransplantation


74 newly-diagnosed myeloma patients (30-73 y, median 56; 51 M; 41 IgG, 17 IgA, 16 other; 58% stage III) treated between 11/94 and 12/00 were studied to determine the effect of the CD34+ cell quantity infused at the time of autograft on disease outcome. Patients received 2-8 (median 5) cycles of C-VAMP infusional chemotherapy (cyclophosphamide, vincristine, doxorubicin, mephal-HD200) with an autograft. Whenever possible, a portion of the harvested stem cells were stored for future use. IFN-alpha2b was administered after hematologic recovery. The quantity of CD34+ cells (106/kg) collected and infused was 0.2-11.6 (median 2.3) and 0.1-5.8 (median 1.4) respectively. At the time of the transplant, 9 patients (12%) had disease that was unresponsive to C-VAMP and 63 had sensitive disease (12 CR,51 PR). Overall response rate to autograft was 92%; 6 patients did not respond or died too early to be evaluable, 44 were in CR, and 24 in PR. The quantity of CD34+ cells infused did not affect treatment-related mortality (TRM). Recovery of neutrophils to 0.5 x 109/l (P=0.01/ 0.08) and platelets to 25 x 109/l (P=0.0008/ P=0.0001) and 50 (P<0.0001/0.0001) was significantly faster with CD34 infused > 1 x 106/kg/ > 2 x 106/kg respectively. The 4-y relapse free survival/EFS/OS was 69/31/73% in those who received <=1 x 106 CD34 vs 43/54/85% in those who received > 1 x 106/kg (P=0.03/0.07/0.4) and 64/35/78% with <=2 vs 26/71/95% with >2 (P=0.02/0.05/0.2) respectively. Because of effective salvage therapy, it was not surprising that differences in relapse and EFS did not translate into OS differences. Albumin >3 g/dL, beta2-M<mg/L, and chemosensitive disease were associated with better outcome in univariate analysis. The only factor affecting OS and EFS independently in Cox analysis was chemosensitive disease. There was a trend towards higher relapse (RR 3.7, P=0.09) in patients receiving <=2 x 106 CD34cells/kg. This observation could indicate that either CD34+ cell yields are higher in patients with better-risk disease and are merely a surrogate marker of favorable prognosis, or that higher CD34+ cells affect disease outcome favorably by some unidentified mechanism such as faster immune reconstitution.

Long-term follow-up of patients transplanted with CD 34+ selected versus unselected stem cells for plasma cell disorders

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After an observation time > 2 years 19 patients ( 8 female, 11 male) with plasma cell disorders were analysed after a single autologous blood stem cell transplantation (ABCT) with or without CD 34+ selected stem cells. Patients age ranged from 44 – 62 years (median age: 52). 18 pts suffered from Multiple Myeloma (progressive stage I: 2, stage II: 2, stage III: 14) and one from AL- Amyloidosis. 10 (group 1) patients (CR:1, PR:9) were transplanted with CD 34+ selected stem cells (7x Ceptrax, 3x Isolox) and 9 (group 2) patients (CR:2, PR:6, PD:1) with unselected stem cells after high dose chemotherapy with 200 mg/m2 Melphalan. G-CSF (total 30 MioU) was given from day -5 until WBC recovery. The hematological recovery for PMN > 0.5 x10^9/l was achieved significantly faster in group 1 (8 days) than in group 2 (12 days). The platelet recovery > 50 G/l occured later in group 1 (day 16) than in group 2 (day 13). A higher number of severe side effects or serious infectious complications (2x mucositis grade III/IV, 1x enteritis grade III, 4x septicemia, 4x HSV infection, 1x CMV reactivation) were diagnosed in group 1 compared to group 2 ( no grade III/IV side effect, 3x FUO, 2x HSV infection) within 100 days after transplantation. Late complications (x-day 100) did not differ in both groups (3x bacterial infection, 1x herpes virusinfection and 1x RSV bronchitis in group 1 vs. 2x bacterial infection and 3x herpes virusinfection in group 2). 6 patients achieved CR and 13 patients PR after transplantation. Interferon alpha (3xMioU/week) was administered subcutaneously to maintain remission. In group 1 6/10 patients relapsed after a median time of 16 months (13-30), 2 of them died 40 and 54 months after ABCT. In group 2 5/9 patients relapsed after a median time of 16 months (7-46), 4 of them died 8, 19, 25 and 26 months post ABCT.

In conclusion ABCT with CD34+ selected stem cells in plasma cell disorders led to a higher incidence of early but not of late transplant related morbidity in this small group of patients.
Paradoxical increased complete remission (CR) rate in patients with chromosome 13 abnormalities in new patients treated with C-VAMP to maximum response


Previous studies looking at chromosome 13 deletion and outcome data have been confined to programmes in which three or less courses of insufflational chemotherapy are given. Our policy is to give insufflational chemotherapy with C-VAMP (cyclophosphamide, vincristine, doxorubicin and methylprednisolone) to a maximum response or plus an additional consolidative course. CR in myeloma patients is now regarded as the pivotal treatment decision point for possibly obtaining "operational cure" (Powles et al, Blood 2000:96:2215a) and chromosome 13 abnormalities link strongly to survival. Between 12/97 and 5/01, interphase fluorescence in situ hybridisation (FISH) using three different probes for 13q14 (RB-1, D13S319, D13S25) was done on 37 patients. The median age of the patients was 55 y (29-79), 13 female, 24 male; IgG-27, IgA-6, Bence-Jones-4; kappa-22, lambda-9; stage IIIA-B73%; bone lesions >2 in 65%; median serum creatinine 97 micromol/L (50-496); median serum beta2M 3.6 mg/L(1.8-31.2); median bone marrow plasma cell percentage 35% (7-94). Deletions of 13q14 were found in 15/37 (41%) of patients. All except one patient received infusional chemotherapy with C-VAMP to a median of 4 courses (1-6). The maximum response to C-VAMP was 6 CR, 23 partial responses (PR) and 8 non-responders who died due to sepsis. One patient received induction with upfront high dose melphalan followed by C-VAMP and is in CR. 31 patients have received high-dose therapy till now and the total CR rate is 17/37 (46%). Of the 15 patients who had deletion of 13q14, 6 (40%) attained CR post induction chemotherapy compared to 1/22 (4.5%) amongst those who did not (P=0.01; Fisher's exact Test), suggesting that the CR rate was significantly higher in those patients who had deletion of 13q14. With a median follow up of 14.2 mo, 3/15 patients have already relapsed amongst those who had deletion 13q14 (post-CVAMP, 2 CR and 1 NR) compared to none in the group without deletion 13q14 (P=0.06; Fisher's exact Test). This data shows that like B-Acute Lymphoblastic Leukaemia, chromosome 13 myeloma disease although very responsive to initial treatment relapses aggressively even after single high-dose therapy. This may be the group of patients who need additional intensive therapy or other innovative treatment immediately after high-dose.

Response to induction chemotherapy is not essential to obtain survival benefit from high-dose melphalan and autologous transplantation for myeloma


The standard approach to symptomatic myeloma at the RMH is C-VAMP infusional chemotherapy (IC) for 1 cycle beyond maximum response, PBSCT harvest with G-CSF and 200 mg/m2 melphalan (HDM200) with a single PBSCT autograft followed by interferon maintenance. The sequence is followed irrespective of response to C-VAMP. We studied 220 patients treated as described above to see if response to induction therapy affected outcome. Recipients of non-IC and marrow grafts were excluded. The age of 37 patients (n=3). 154 patients (70%) are alive 1 -100 mo (median 33) post-HDM200. Good response to induction therapy predicted for a significantly higher likelihood of CR after HDM200 (P<0.0001). Outcome of patients in CR after HDM200 was independent of response to induction. Of the 42 NR to IC , 17 had CR post-HDM200. The 5-y probability (%) of relapse/EFS/OS for these patients (n=17) was 52/48/79 compared to 74/16/47 for the non-CR patients (n=25), P=0.13/0.003/0.01 respectively. The 17/42(40%)NR to IC attained CR post-HDM200; 74/138(54%)PR to IC attained CR post-HDM200; 39/40 (1 died) CR to IC patients continued in CR post-HDM200. The probability of relapse/EFS/OS for these CR patients (17 vs 74 vs 39) was comparable, P=0.86/0.81/0.69 respectively. In Cox analysis, relapse was lower with post-HDM200 CR (RR 0.52; P=0.006), EFS was higher with post-HDM200 CR(RR 2.52; P=0.0001) and beta2-M 2.5 mg/L (RR 1.55; P=0.04), and OS was higher with post-HDM200 CR (RR 2.98; P<0.0001) and beta2-microglobulin 2.5 mg/L (RR 1.87; P=0.03). We conclude that lack of response to induction therapy does not automatically predict poor long-term outcome since a substantial proportion of these patients attain CR after HDM200 and benefit from transplant. This finding has major implications for determining suitability of myeloma patients for an autograft.

Oral fludarabine for the conditioning regimen of sub-myeloablative BMT in multiple myeloma (MM)

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Fludarabine phosphate (F-AMP)(Scherer) is in a wide use for immune-suppression as part of sub/non-myeloablative BMT regimens. The drug is currently given intravenously as 25-30mg/m2 for 3-6 days. It has been shown that the efficacy of oral F-AMP in the treatment of CLL and low grade NHL patients does not differ from the IV formulation with 60% oral bioavailability and a quite similar safety profile. Methods: We used oral F-AMP 48mg/m2 x 5 days in combination with melphalan 140mg/m2 as a conditioning regimen prior to BMT from HLA matched related donors in MM. Four patients (F-3, M-1) age 52-64 years were treated for responding (2 pts)or resistant (1 pt) disease that was progressing after an autologous BMT, and for primary resistant disease with deletion of chromosome 13 (1 pt). Unmanipulated PBSCT graft (>5x10 million CD34+ cells/kg) was infused >24 hours after the completion of therapy. Cyclosporin-A and MTX were used for GVHD prevention. Results: The main transplant related toxicity was mucositis (grade III-1pt, grade II-2pts) with no other >grade II complications. The time to ANC engraftment was 9-13 days with 6-10 days of absolute neutropenia. One patient had the entire therapy in an ambulatory set-up and was hospitalized on day +4 for supportive care, and the other three patients were treated semi-ambulatory until neutropenia, as for their request. With a median follow-up of 127 days (82-152) the four patients are alive and well with 100% donor chimerism. Three patients have a controlled acute GVHD of grade I-II (GIT-2pts, skin-1 pt) that is being treated ambulatory, and are in CR of the disease. One patient with no GVHD and good PR post transplant (transplanted with resistant progressive disease) is due to DLI therapy. Conclusions: The use of oral F-AMP is feasible and effective for immune-suppression prior to sub-myeloablative BMT in MM. The use of the oral formulation can facilitate an ambulatory transplant and should be studied in additional indications and combinations.

Autologous transplantation in multiple myeloma. Is age a prognostic factor?


The aim of this study was to evaluate the impact of age in survival and transplant related mortality in patients with multiple myeloma receiving an autologous transplantation in Argentina. Between February 1991 and April 2001, 258 patients (pts) entered the study, with a median age of 53 years old (28-71).Seventy nine % of them received Melphalan as conditioning regimen and 92% were mobilized with chemotherapy plus G-CSF. For this
evaluation, the patients population was divided in 2 groups: <60 years old (197 pts) and >= 60 years old (61 pts). Patients characteristics are the following: Median age was 51 and 63 years old, disease status at time of transplantation was 59 (30%) and 10 (16%) in CR for both groups, treatment related mortality was 3% for both groups. At 4 years the probability of event-free survival was 33% and 35% (p=NS) and the overall survival was 52% and 44% respectively (p=NS).

These results shows that age >= 60 years is not an adverse prognostic factor for survival and toxicity for patients with multiple myeloma who receive an autologous transplantation.

**Treatment of massive plasmacytoma with high-dose melphalan and autologous peripheral blood stem cell transplantation followed by radical radiotherapy**

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Massive plasmacytomas of the chest wall (>10cm in diameter) are associated with a high incidence of treatment failure following local radiotherapy alone or VAD followed by local radiotherapy.

We report 3 patients with massive plasmacytoma who were treated with initial VAD chemotherapy consolidated by high dose melphalan (HDM, 200 mg/m2), autologous PBSC and radical radiotherapy. Three patients aged (50-62 years) presented with chest pain. CT of the chest wall revealed a massive plasmacytoma with a maximum diameter of 11, 12 and 13.5cm respectively with associated rib destruction. Bone marrow involvement was demonstrated in one case only with a low-level bone marrow plasma cell infiltrate (7%). Initial treatment with 4 cycles of VAD was used to debulk the tumour followed by PBSC mobilisation with cyclophosphamide (3g/m2) and G-CSF. In one patient the initial response to VAD was poor and this patient proceeded to radiotherapy (30 Gy in 10 fractions) prior to HDM. The other 2 patients received HDM following VAD chemotherapy and finally radiotherapy (30-40Gy) to the residual mass. All patients responded and 2 remain in complete remission at 2.5 and 3.5 years although 1 patient has a large residual calcified mass.

The remaining patient achieved CR but relapsed at 8 months as a generalised immunoblastic lymphoma. As previous studies have shown a high risk of treatment failure in massive plasmacytomas this approach offers the potential for long-term disease free survival.

**Intensive sequential therapy comprehensive of autologous stem cell transplant in multiple myeloma: a single center experience**

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Between January 1994 and April 2001, 26 patients with a median age of 51 years and untreated multiple myeloma were enrolled in an intensive sequential therapy consisting of 2 courses of VMD regimen (VCR, mitoxantrone and dexamethasone for 4 days) followed of three consecutive non-cross-resistant regimens: a) cyclophosphamide (CY) 7 g/mq followed by G-CSF 5 mcg/Kg per day and leukapheresis upon recovery from white blood cell nadir; b) EDHAP with G-CSF and beam protocol with G-CSF was used as the third regimen. Peripheral blood stem cells were collected after each regimen. The high dose therapy with Melphalan 140 mg/mq +/- fractioned TBI 1200 cGy followed by infusion of PBSC collections was subsequently offered to our pts. In few patients, who previously received radiation therapy the preparative regimen was Busulfan 16 mg/Kg and melphalan 60 mg/Kg. The selection criteria included the presence of symptoms, age < a 60 years, adequate cardiopulmonary function, no HLA sibling donor. Renal disfunction or evidence of amyloidosis at diagnosis were not a contraindication. Twenty pts had stage III and 6 pts stage II. The median CD34+ cells collected was 15x106 /Kg (range 2.2-46).

Five pts achieved complete remission and the others pts achieved partial remission before transplant. The median CD34+ cell dose infused at the time of autografting was 6.5x106/Kg (range 2.2-21.2 ). The neutrophil (>500 micron/L) and the platelets recovery (>20.000 micron/L) occurred a median of 12 days and 16 days after ASCT, respectively. The interval between of induction therapy and ASCT was 5.5 months. No treatment-related deaths were observed. At a median of 3 months, 14 pts received IFN therapy as maintenance. To date, 15 pts are alive, of whom 7 in complete remission. Six relapsed pts underwent a second autografting. At a median of 4.5 years, the overall survival is 57%.

**Thalidomide overcomes VAD chemoresistance and allows PBSC collection in 3 multiple myeloma patients**

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Objective: Thalidomide (Thal) showed a 30-50% of partial responses when it was used as salvage treatment in several series of resistant or relapsing multiple myeloma (MM) patients, who had been heavily pretreated in the majority of the cases. We investigated the activity of Thal in 3 newly diagnosed MM patients unresponsive to VAD induction therapy and included in a program of mobilization of PBSC and high-dose treatment.

Methods: Three previously untreated stage III A MM patients of respectively 50, 59 and 63 years old, who showed a resistant or progressive disease after 2 VAD cycles, started on 2 further VAD in association with 200 mg Thal, then were treated with Thal alone at escalating doses to the maximum tolerated dose until they showed the greatest response. At this time PBSC were collected and an ASCT was sheduled.

Results: The maximum tolerated dose was 100 mg for patient n.1 and 400 mg for patient n.2 and n.3. Side-effects were WHO grade III peripheral neurotoxicity for patient n.1 and WHO grade II in association with 200 mg Thal, then were treated with Thal alone at escalating doses to the maximum tolerated dose until they showed the greatest response. At this time PBSC were collected and an ASCT was sheduled.

Conclusions: Thal obtained a reduction > 50% of M-component (MC) and plasma cell (PC) marrow infiltration in 3 newly diagnosed MM patients who had a primary resistance to VAD and allowed to mobilize a number of CD34+ PBSC adequate for an autotransplantation.

**5. Autoimmune Disease**

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**Total body irradiation (TBI) and high-dose chemotherapy combined with T-cell depleted marrow grafts in multiple sclerosis (MS)**


Ablation of the immune system followed by autologous stem cell rescue is currently explored as therapy for patients with multiple sclerosis (MS). We used rigorously depleton of T-cells in the recipient by employing antithymocyte globulin (ATG) and TBI as...
the preparative components, as well as using marrow grafts (rather than using peripheral blood) and CD34 selection. Patients with lastly relapsing progressive form of MS and with a score between 5 and 7 on the expanded disability scale (EDSS) were included. We report on 8 successive patients who were followed for 9-32 months post transplant (median: 18.5 mo). Time to neutrophil recovery varied from 12 to 38 days (median: 27 days). Platelet recovery was observed between 13 to 31 days (median: 25 days). Significant toxicity (WHO grade 2 or more) during the first three months after stem cell transplantation (SCT) included mucositis (gr 2, n=6); hepatic dysfunction (gr 2, n=1; gr 3, n=1); neutropenic fever (gr 2, n=2; gr 3, n=3); skin toxicity (exanthema gr 2, n=4; gr 3, n=1), and neurologic problems like spasms (gr 2, n=3; gr 3, n=1) have also been observed. In 5 of 8 patients who were all IgG-Epstein-Barr virus (EBV) positive prior to transplant, EBV reactivation was quantitatively monitored in the patients plasma samples using a validated real-time PCR test at weekly intervals. After transplantation, all 5 patients showed at least one, sometimes repeated EBV-PCR reactivations. In 3 patients, EBV-DNA was detected at high levels (peak values: 6,250 - 240,000 copies/mL). In patients with EBV reactivation and plasma EBV-DNA levels exceeding 1,000 copies/mL, no signs of EBV-related lymphoproliferative disease (EBV-LPD) were found. Effects of this form of therapy were evaluated by EDSS and follow-up exam of the cerebrum. Evaluation of the EDSS scores at current follow-up indicates progression of disability in 4, stabilisation in 2, and improvement in 2 patients. MRI scans showed a significant decrease in gadolinium positive lesions following the SCT procedure in all patients. Thus, autologous SCT with rigorous T-cell depletions might be beneficial for MS patients with acceptable toxicity. EBV reactivation should be monitored carefully in this setting.

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Autologous stem cell transplantation for refractory myasthenia gravis

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Objectives: Severe refractory autoimmune disease can be treated with high dose immunosuppression followed by autologous blood stem cell transplantation (ASCT). We report on a 50 years old female patient who has been suffering from myasthenia gravis (MG) since January 1992. Despite a thymectomy in 1993 her clinical symptoms deteriorated. In the following years she was refractory to a maximum dose of pyridostigmine, steroids, azathioprine and weekly immunoadsorption therapy. Therefore, she was referred for an autologous stem cell transplantation.

Methods: Peripheral blood stem cells were mobilized with cyclophosphamide (Cy) at 150 mg / kg body weight (b.w.) and antithymocyte globulin (ATG) at 50 mg / kg (b.w.). Since she had a myasthenical crisis on day 3 of the conditioning therapy with respiratory insufficiency requiring mechanical ventilation for 7 days, the planned total dose of both Cy and ATG could not be administered. The infused autologous stem cell number was 9.21 x 10^6 CD34+ cells / kg b.w. The ANC count rose above 0.5 x 10^9 / l on day +6 and the patient showed trilinear engraftment. From day +6 on the patient was independent from red blood cell or platelet transfusions and without clinical signs of MG. Afterwards the patient was monitored for clinical activity, acetylcholine receptor antibodies, B and T cell count and activity. Results: Quantitative MG - score improved from 20 to 7 within 2 months after transplantation and remained stable for the current observation time of 6 months. Steroid therapy and immunoadsorption were stopped on day +5 after ASCT and the patient is currently under a reduced dose of pyridostigmine.

Conclusion: Autologous stem cell transplantation with immunoaffective conditioning therapy may be a promising therapeutic approach in refractory myasthenia gravis. Long - term improvement, however, still needs to be ascertained.

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Treatment of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) with high-dose immunosuppressive therapy (HDIT) using cyclophosphamide with autologous hematopoietic stem cell rescue


Background: Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is considered to be an autoimmune disorder. Current treatment regimens leave 4% to 30% of patients with CIDP with moderate or severe disability. Conventional dose immunosuppressive therapy has produced very limited success in halting disease progression in treatment-resistant patients. It has been proposed that high-dose immunosuppressive therapy using cyclophosphamide with autologous hematopoietic stem cell rescue may be an effective treatment for severe autoimmune diseases.

Methods: We have designed a study to test in patients with severe autoimmune disorder the toxicity and efficacy of a myeloablative regimen of cyclophosphamide 50 mg/kg x 4. Autologous hematopoietic stem cells were mobilized with cyclophosphamide 4 g/m2 and G-CSF 5 µg/kg. A total of >5x106 CD34+ cells/kg were stored before proceeding to conditioning therapy.

Case report: The 36-year-old male patient suffered from classic progressive CIDP starting on 1988 with progressive weakness of the upper and lower limbs. Previous treatment included corticosteroids, intravenous immunoglobulins and interferon-beta 1a with the partial control of the progression of the disease. During the year before HDIT the patient showed a worsening of the disease despite the high-dose immunoglobulins. HSC mobilization required 2 apheresis procedures to collect and store >5x106 CD34+ cells/kg. The conditioning regimen has been well tolerated and caused only g.i. side effects < grade II. No life-threatening regimen-related toxicity or infection have been encountered in the post-transplant period. Engraftment was rapid and the ANC was <500/µl for 9 days, platelet counts < 20000/µl for 7 days. With follow-up of 24 months there has been no evidence of disease progression. The patient experienced a sustained clinical improvement with a decrease of motor disability. Motor nerve conduction studies measured 24 months after treatment showed slight improvement.

Conclusions: With follow-up of 24 months, clinically important improvement was observed in one patient with CIDP after HDIT using cyclophosphamide with autologous hematopoietic stem cell rescue. HDIT should be considered in CIDP patients with progressive disease under conventional treatments.

P474

Unrelated allogeneic stem cell transplant with reduced intensity conditioning in a patient with antiphospholipid syndrome and Evans syndrome

A. Wahlin (Umea, S)

The patient is a female born in 1944, with DAT-positive hemolytic anemia from 1998. A small population of monoclonal B-cells was repeatedly seen in the marrow. She had high titer of cardiolipin antibody and lupus anticoagulant, long APT-time and falsely positive serological tests for syphilis, but ANF and rheuma factor were negative. There was no response to steroids, azathioprine, cyclophosphamide or rituximab. In 2000, severe autoimmune thrombocytopenia with ureteral bleeding caused bilateral obstruction and need of hemodialysis for two months. Mobilization of stem cells with GCSF and cyclophosphamide failed. No family donor was available. Due to continuing thrombocytopenic bleedings, peripheral stem cell transplantation with an unrelated
matching donor was performed in June 2001, after conditioning with fludarabine 180 mg/msq, cyclophosphamide 60 mg/kg, ATG 40 mg/kg. Neutrophil recovery was seen two weeks after transplantation, she had acute GVHD grade 1, and she was treated for relapsing CMV after two months. Due to persistence of thrombocytopenia, anemia, and mixed chimerism she was given one donor lymphocyte infusion after four months and her T, B and myeloid cells are 100% donor-derived one month later. She is in good condition five months post-transplant and does not need transfusions of RBC or platelets, although the platelet count is still subnormal. The cardiolipin antibody has disappeared and the bleeding time is normal.

**P475**

**Successful allogeneic bone marrow transplant (BMT) in patient with Alps-like disease**


The decreased fuction of Fas or mutations of Fas gene leads to a clinical picture characterized by non-malignant lymphoproliferation with autoimmune manifestations. Two successful allogeneic BMT have been reported in patients affected by Fas deficit so far. We describe the third case cured from ALPS-like pattern by allogeneic BMT. The 12 years old patient was referred to our Centr. The 12 years old patient was referred to our Centr. The firstborn brother presented diffuse lymphadenopathy, hepatosplenomegaly and pancytopenia at 6 years of age, previously expired at 13 years of age with severe thrombocytopenia and autoimmune disorders. Analysis of Fas function was normal in the mother, slightly decreased in the father and strongly decreased in the sister. Expansion of double-negative T cells was absent. Sequencing of the Fas gene did not detect any mutation. The family history and the Fas function analysis suggested a genetically mediated autoimmune disease involving the Fas function. As the only HLA identical sister was suspected to be affected with the same disorder, the HLA phenotypically identical mother (matched for HLA-A,-B,-C, DRB1-B4, DQA1-B1 loci and mismatched for 1 allele on locus DPB1) was selected as the bone marrow donor. The patient was prepared to BMT with BU 14 + CY 200, CsA and sMTX were given as GVHD prophylaxis. Allogeneic engraftment was documented on day +20. She developed grade II skin aGVHD, resolved with steroid therapy. At +10 months post-BMT the clinical conditions were good with 100% of donor engraftment, without signs of cGVHD and with normal blood count. The immunosuppressive therapy was completely stopped at 1 year after BMT. Fourteen months after BMT she is in good clinical conditions and cured of her genetically inherited disease. In conclusion this case provides confirmation that the only radical treatment for patients with ALPS or ALPS-like diseases is represented by allogeneic BMT.

**P476**

**Improvement of inflammatory bowl disease after allogeneic stem cell transplantation**

M. Ditschkowski, H. Einsele, U. Schaefer, A. Elmaagachi (Essen, Tuebingen, D)

After allogeneic marrow transplantation remissions of several autoimmune diseases were described. We retrospectively analysed the course of coexistent inflammatory bowel disease (IBD) in 6 patients who underwent allogeneic stem cell transplantation for acute (n=1) or chronic (n=5) myeloid leukemia. Between July 1994 and November 2000, four patients with Crohn’s disease and two patients with idiopathic ulcerative colitis and AML or CML were transplanted with bone marrow (n=4) or peripheral blood stem cells (n=2) from a HLA-identical (n=4) and HLA-partial identical (n=1) sibling donor or unrelated HLA-matched donor (n=1). Before transplant, three patients received an immunosuppressive medication for the IBD. Two of them received low doses of steroid and 5-ASA, another patient received 5-ASA alone. None of the patients was found to have an active IBD at transplantation. After a mean follow up period of 57 months after transplantation all patients are alive, in complete remission of CML or AML and without recurrence of IBD. Three patients suffered from temporary cytogenetic relapse of CML but did not develop any signs of intestinal inflammation again. One patient only revealed clinical signs of his idiopathic ulcerative colitis 11 months after transplant. In most disease related mortality of this high did not prove a relapse of ulcerative colitis and diarrhea diminished under supportive therapy. This patient remained free of any signs of IBD up to date with a mean follow up of 39 after bone marrow transplantation. Five of six patients remained free of IBD over a follow up period of 107 months (mean 57 months). These observations may imply that an underlying host immune dysregulation might play a central role in the perpetuation of IBD. It may be corrected by allogeneic hematopoietic stem cell transplantation.

**P477**

**P-ANCA associated vasculitis (M. Wegener): successful HLA-identical BMT**


P-ANCA associated vasculitis is a rare but fulminant disease in paediatric patients. The introduction of immunosuppressive treatment with e.g. cyclophosphamide and prednisolone has dramatically increased the remission rate. However, the rate of relapses and mortality remains high. We describe an 8 year old girl with a generalised form of Wegener’s Granulomatosis with eye, renal, heart and lung involvement requiring mechanical ventilation due to pulmonary haemorrhage and ARDS. Other clinical manifestations were thrombocytopenia and increasing hepatosplenomegaly with intrahepatic cholestasis. The treatment consisted of prednisolone, cyclophosphamide and one course of methotrexate. After first signs of remission, disease progression and toxicity related problems BMT from the HLA-identical brother was considered. The girl underwent allogeneic BMT 19 months after diagnosis. To reduce toxicity, the conditioning regimen consisted of fludarabine, 2 Gy TBI and 2.5 Gy TLI. GvHD and rejection prophylaxis consisted of CsA and MMF, and 3.6 x10^6 CD34 cells and 6.4 x10^7 CD3 cells per kg of the recipient were transfused. G-CSF was given from day +20 till day +30 and myeloid engraftment with ANC>0.5 G/L was achieved on day +22. Chimerism analysis was performed on FACS-sorted cells by FISH and showed mixed donor chimerism from day +20 on. Full donor chimerism in all cell lines was first seen on day +78 and continues with a follow up of 12 months. P-ANCA titer is constantly negative since day +78. Transplant related toxicity was mild and transient. Infectious complications were short febrile neutropenia, herpes simplex and EBV reactivation. The later post transplant course was complicated by increasing cirrhosis of the liver and persistent transfusion depending thrombocytopenia. Since splenectomy 8 months after transplantation the patient has been transfusion independent. Limited de novo chronic skin GVHD was treated with oral prednisolone. The girl has been attending school for 3 months with only mild restriction of life quality. Based on this experience, we conclude that allogeneic BMT with reduced intensity conditioning regimen may be a curative treatment option for refractory autoimmune disease.
Autologous stem cell transplantation (ASCT) in four children with refractory JCA and SLE


ASCT has been proposed as a new therapeutic option for patients with severe autoimmune disease refractory to conventional treatment. Here, we report three children with a severe form of systemic JCA and one patient with severe systemic lupus erythematoses treated with ASCT in a phase I study. Patients: Three patients (age: 5, 9, 14 yrs) who developed severe systemic JCA with high spiking fever, rashes, hepatomegaly, polyarthritis, morning stiffness, ESR > 100 mm/h, CRP > 100 mg/l were refractory to NSAIDs, MTX, cyclophosphamide, steroids etanercept after 2.5, 13 and 6 yrs. 1 patient (16 y-old) with SLE had a disease duration of 2.5 yrs with arthritis, carditis, pericarditis, hyperpotonos, reduced pulmonary capacity, ANA: 1: 5120, anti-ds DNA 485, anti-ss DNA > 200, anti-cardiolipin IgM 13.4, C4 0.09 g/l, lupus anticoagulants positive was refractory to steroids, MTX, IVIG, CsA and cyclophosphamide. This patient acquired on day + 45 EBV infection with LPD which was treated successfully with ganciclovir, cidofovir and rituximab. Stem cell harvest: After a priming dose of cyclophosphamide (2 g/m²) and mobilization with G-CSF (10 µg/kg/day) peripheral blood stem cells were collected using a Cobe separator. In a Clinimacs device, CD34-positive selection was performed yielding a final CD34+ -cell amount of 4.2 – 6.5 x 10^6/kg contaminated with zero 1.0 GPT/l: days –7 to –2; ATG (5 -10 mg/kg): days –6 to –2; methylprednisolone (1g/m²): days –4 to –2; ATG (5 -10 mg/kg): days –6 to –2; methylprednisolone (1g/m²): days –4 to –2. On day 0, the frozen CD34+ cells were thawed and infused. Results: Rapid engraftment of neutrophils > 1.0 GPT/l: days +10 to +13; platelets > 20 GPT/l: days +6 to +14; lymphocytes > 1.0 GPT/l: days +46 to +66. Patients were discharged from hospital on day + 24 to +53, respectively and remained free from active JCA and SLE with no immunosuppressive medication for 9, 10, 20 and 20 months, respectively.

Autologous stem cell transplantation for refractory autoimmune cytopenias

J. Passweg, M. Rabusin, M. Musso, Y. Beguin, G. Ehninger, V. Koza, I. Lisukov, A. Marmont, P. Philippe, P. Quartier, J. Vavrinec, J. Vormoor, L. Jost, A. Tyndall, A. Gratwohl for the Autoimmune Disease Working Party of the EBMT

Autologous Stem Cell Transplantation (ASCT) has been explored as a treatment option in severe uncontrolled autoimmune disease. We report on outcome in 17 patients with hematologic autoimmune cytopenias reported to the autoimmune disease working party of the EBMT by 13 centers. Patients had idiopathic thrombocytopenic purpura (10), pure red cell aplasia (4), autoimmune hemolytic anemia (2), and Evan’s syndrome (1). Median age was 31 (4-45) years, 8 were female. Median disease duration prior to transplant was 93 months (12-236). Stem cells were from bone marrow (14) or from marrow (2). Peripheral stem cells were mobilized using either growth factors alone (7) or G-CSF in combination with cyclophosphamide (6). Pretransplant conditioning regimens included cyclophosphamide alone (n=3), cyclophosphamide with other drugs or ATG, (n=9), Melphalan (2), or were Fludarabine based (2). Stem cells were either unpurged (n=4) or purged of immune cells using varying methods of depletion (n=12). Median follow-up of surviving patients is 30 (5-53 months). Three patients died within 100 days posttransplant, 2 of hemorrhage and infectious complications, and 1 with progressive hemolysis. Nine patients showed a response to treatment, 5 complete remissions (3 ITP, 1 Evans, 1 PRCA) sustained in 4 patients. Of note, one of these complete remissions occurred after mobilization only. ASCT may induce a sustained remission in a fraction of patients with severe autoimmune cytopenias of long duration.

Additional abstracts to this topic

The dynamic of immunological reconstitution in multiple sclerosis patients after autologous peripheral stem cells transplantation

A. Novik, V. Meinlitchenko, S. Voloshin, G. Bisaga, N. Kalinina, V. Nikitin, N. Osipova (St.Petersburg, RUS)

Introduction: Peripheral stem cells transplantation (PSCT) is a new perspective method in the treatment of severe autoimmune diseases. PSCT allows to achieve a stabilization in 85% patients during 5 or more years. Goals: The determination of the peculiarities of immunological reconstitution in MS patients after high dose immunosuppressive therapy (HDIT) with autologous peripheral stem cells support. Materials & methods: The immune monitoring (IM) was carried out in 5 MS patients after HDIT with autologous stem cells support. In four cases the PSCT were made according to EBMT protocol. One patient underwent nonmyeloablative HDIT with CSF support (Granocyte). There were four females and one male, median age was 38 +/- 14 y.o. The EDSS scale varied from 2.0 to 8. Results: Clinical improvement in two of five patients result in decrease of EDSS level by 0.5 and 1.5 respectively. In three other patients the stabilization of MS was observed. In patients with positive clinical results the significant decrease of initially increased autoimmune activity markers (CD4) was found out with future slight increase and stabilization on normal level. Relative increase of protective subpopulations (CD8, CD16) was determined as well. Such immunological profile was preserved during all the period of MS remission (follow up period varied from 3 months to 2 years). Conclusions: Along with clinical improvement immunological changes pointing to decrease of autoimmune processes activity in
MS patients after HDIT with peripheral stem cells support are observed.

6. Donor Issues

P481

Mobilization and harvest of peripheral blood stem cells from 128 healthy donors. A single center experience
G. Kumlien, G. Bergström, A. Shanwell, H. Hägglund (Stockholm, S)
Background: Between January 1995 and August 2001, mobilisation and harvest of peripheral blood stem cells for allogeneic transplantation was performed in 92 related and 36 unrelated healthy donors. Four donors were harvested on two separate occasions after renewed G-CSF mobilisation.
Method: Retrospective analysis of 128 medical reports.
Results: Median age was 40 years (range 14 -71), median weight was 75 kg (range 50-130) and the male/female ratio was 1.5 (76/52). In a majority of donors (96%, 123/128) antecubital veins were used as access to the circulation. In 4% of donors antecubital veins were unsuitable, and a femoral catheter was therefore employed.
In each apheresis procedure, two blood volumes (Cobe Spectra: median 10.1 L, range 6.8-14.6 L) or 10.0 L of whole blood (CS3000) were processed. Median 2 (range 1-3) apheresis procedures were performed/donation. During G-CSF mobilisation, 79% (101/128) donors reported mild to moderate side effects. After G-CSF mobilisation, median WBC was 42.9 x 10^9/L (range 17.8-78.3 x 10^9/L). During apheresis, 59% (76/128) of the donors reported mild symptoms such as typical citrate mediated circumoral paresthesias and 16% (21/128) experienced other types of moderate symptoms. After the first apheresis 9% (9/99) of the donors had a serum potassium level below 3.0 mmol/L (min 2.7 mmol/L).
During 1999 there was a change of cell separator from Baxter CS3000 to Cobe Spectra (AutoPBCS program) with the intent to minimize platelet loss and to gain better control of the citrate administration. Thrombocytopenia (defined as platelet count <100 x 10^9/L) occurred in 44% (17/39) of the donors harvested with CS3000 and in 5% (3/66) of the donors harvested with Cobe Spectra. Moderate citrate related side effects was reported in 49% (29/59) of the donors harvested with CS3000 and in 66% (45/68) of the donors harvested with Cobe Spectra. There was no difference between the two cell separators regarding yield of CD34+ cells: median 5.48 and 5.59 x 10^6 CD34+/kg body weight of the donor for CS3000 and Cobe Spectra respectively.
Conclusion: No serious side effects were reported during G-CSF mobilisation or apheresis.
A lower frequency of thrombocytopenia (p=0.001) was seen after apheresis with Cobe Spectra AutoPBS than with CS3000 with no difference regarding yield of CD34+ cells.

P482

Peripheral blood stem cells (Pbsc) mobilization and collection from healthy donors receiving granulocyte colony-stimulating factor (G-CSf) - A single-center experience about safety aspects
From May 1996 to November 2001, a total of 48 healthy peripheral blood stem cell donors (5 of them underwent 2 mobilizations), 27 male and 21 female, median age 46 years (16-63), were mobilized by a mean dose of G-CSF 9.7+2.2 mg/kg/day. A prophylaxis with paracetamol and heparin low-dose was administered to prevent side effects from G-CSF. A total of 133 aphereses were performed with Fresenius cell separator using the AS 104 or AS 204 systems. No donor needed CVC. A median of 2 procedures (range 1-4) and of 13.5 L (range 9-18) were performed. WBC increased to a maximum of 80.0 x10^9/L (mean 47.8+11.6). After the aphereses, platelet count decreased to a minimum of 42.0 x10^9/L (mean 104.4+40.6) and returning to baseline values at median day 6 (range 2-35) from the last procedure. Reinfusion of autologous platelet-rich plasma was necessary in 23/133 procedures (17.2%) because of donor platelet-count <80 x 10^9/L. Mean number of MNC and CD34+ cells harvested were 999.3+344.6 x10^6/kg and 12.5+5.7 x10^6/kg, respectively. The target dose of 5.0 x10^6/kg CD34+ cells was obtained in 58.5% of cases after a single aphereses. Target CD34+ cells was reached in all cases. The most frequent G-CSF related side-effect was bone pain (8/53 WHO grade 1, 10/53 WHO grade 2 and 1/53 WHO grade 3). Paraesthesia occurred in 36/133 (27%) aphereses (32 mild, 4 severe). 3 donors developed headache, 1 lymphopaenia (<2cm) and 1 donor showed a mild increase in ALT levels. After a median follow-up of 19.7 months (1-73.6), no donor developed long-term adverse effects. In conclusion, G-CSF mobilization and harvesting of allogeneic PBPC was feasible and well tolerated in all donors without major short-term or long-term adverse effects.

P483

Safety issues of hematopoietic progenitor cell (HPC) collection from healthy donors: transient thrombocytopenia in older age and multiple aphereses
C. Vadicola, A. Tsompsonakou, C. Smias, P. Kalogiannidis, E. Yannaki, D. Sotropoulos, G. Dourvas, N. Eleftheriadis, A. Fassas, A. Anagnostopoulos (Thessaloniki, GR)
We retrospectively analyzed possible correlations between CD34+ cell count in HPC collections from healthy individuals with the following variables: age, sex, body weight, medical history, bone marrow (BM) cellularity, medications, dose/ duration of rhG-CSF administration, number of aphereses, peripheral blood (PB) CD34+ cell numbers and WBC count. We also present complications and side effects of rhG-CSF administration and apheresis in this cohort. Included in the analysis were 65 donors with a median age of 29 years (10-76); there were 57 PB and 8 BM harvests. Donors were subjected to detailed laboratory examinations, including: complete blood count, serum biochemistry, tests for hepatitis B and C viruses, HIV and herpesviruses, electrocardiogram, chest x-ray and assessment of BM smear. rhG-CSF was administered at a median dose of 10 mg/kg/day for a median of 6 days (2-12); the median number of aphereses was 3 (2-6). The median CD34+ cell count was 5.32 x10^6/kg (0.18-18.1); significantly (p=0.04) more CD34+ cells were collected with increasing number of aphereses. CD34+ cell mobilization pattern was the same, regardless of age; however, in older donors more aphereses were required for adequate harvests. On multivariate analysis, the number of aphereses was the only variable significantly associated with CD34+ cell count (p=0.018). Generally, the collection procedure was well tolerated, the main complications being reversible thrombocytopenia (41.5%), hypocalcemia (12.3%), bone pain (1.5%), decrease in hemoglobin level (26.1%), liver aminotransferase elevation (7.6%) and headache (3.0%). On multivariate analysis, thrombocytopenia was associated with increasing number of aphereses (p=0.03), while on multivariate analysis with more advanced age (p=0.012). With a median follow-up of 33 months (9-59), there were no late side effects attributable to rhG-CSF. Nevertheless, firm conclusions about potential long-term toxicities of this method can not be drawn at this moment.
Hemostatic changes in the course of stem cell mobilization and apheresis in patients undergoing PBSCT
R. Ostersch, A. Schmitt-Thommesen, A. Ganser, M. von Depka, B. Hertenstein (Hannover, D)

Introduction: Stem cell mobilisation and collection are well tolerated and adverse effects are rare. However, recent data suggested that haemostatic changes may occur in some cases prompting us to assess prospectively the influence of stem cell mobilisation and apheresis on the coagulation system.

Patients and Methods: We investigated 30 patients undergoing autologous PBSC collection (50 yrs., range 30-68), 24 healthy donors of PBSC (35, 22-64) and 21 healthy platelet donors (38, 23-63). Patients suffered from: NHL (n=8), HD (n=3), CML (n=1), AML (n=5), ALL (n=2), multiple myeloma (n=6) and solid tumours (n=5). We performed a detailed panel of coagulation analyses including FV G1619A mutation, prothrombin G20210A mutation, and the MTHFR C677T variant. Results: One patient was prothrombin G20210A heterozygous, one healthy donor and two platelet donors were FV G1619A heterozygous. In patients undergoing autologous PBSC FT, FD and DD were higher compared to healthy donors (median FD 201%, range 78-400 versus 172%, 76-887; p<0.003; median FD 150%, 78-200 versus 109%, 68-165; p<0.001; median DD 1275 mg/l, 86-9366 mg/l versus 194 mg/l, 65-389, p<0.001). Significantly lower levels of AT and PC were observed in all groups during mobilisation. Groups were not significantly different. Fibrinogen was significantly higher after mobilisation (patients: 3.3 g/L, range 1.1-8.9 versus 4.1 g/L, 2.5-7, p<0.001; healthy donors: 2.6 g/L, range 2.1-3.8 versus 3.2 g/L, 2.5-4.0, p<0.003). Fil and FV levels were lower after apheresis in patients (FII 99% range 63-125 versus 89%, 50-141, p<0.02; FV:141%, 82-200 versus 115%, 67-183, p=0.005) and healthy donors (FII: 93%, range 69-115, versus 78%, 47-104, p>0.001; FV:124%, 68-172 versus 107%, 67-163, p=0.024).

Discussion: Our data show that patients with autologous stem cell collection show significantly different haemostatic parameters (e.g. FV, D-dimers, FVIII) compared to donors of PBSCs or platelets. Similarly altered haemostatic parameters in patients and healthy donors can be found after mobilisation as well as after apheresis: fibrinogen levels rise, whereas activities of AT and PC drop during mobilisation. During apheresis activities of AT and PC drop further, fibrinogen levels remain increased. The levels of FII and FV were lower after apheresis. As a consequence a prothrombotic tendency may result, especially during mobilisation.

Proposal of a scoring system for CD34+ cell mobilization in donors
M. Fernandez-Jimenez, R. Arrieta, F. Hernandez-Navarro (Madrid, E)

Background and objective: Allogeneic transplantation of cytokine-mobilized peripheral blood stem cells (PBSC) is now being increasingly performed. Progenitors are generally mobilized for collection from healthy donors using rhG-CSF. However, approximately 10-20% of normal donors exhibit poor mobilization. The aim of this study was to identify factors that could predict poor mobilization.

Donors and methods: We evaluated data from 49 normal donors (28 male, 21 female) who underwent mobilization with G-CSF at a dose of 10 mcg/kg/day for four days, followed by leukapheresis on day five with a continuous-flow blood cell separator Spectra. Factors previously involved in donor mobilization such as age, sex, WBC and CD34+ cell count pre-mobilization (CD34 pre-G-CSF) were analyzed for their correlation with the number of CD34+ cells/kg donor weight collected.

Results: Median number of CD34+ cells collected in the first apheresis was 4.47 x10e6/kg (range 0.82-18). Mean number of CD34+ cells collected in the first apheresis was significantly lower (p=0.03) in donors >35 years of age. A CD34 pre-G-CSF < 2.5 /mcl correlated with lower CD34+ cell harvest (p=0.02). There was a small but significant difference in WBC count at the time of mobilization between donors who yielded >= 2.5 x10e6 CD34+cells/kg and those who failed to reach that dose of CD34+cells/kg (6.95 x10e9/l vs. 5.5 x10e9/l; p=0.041). No difference was found between male and female donors. On the basis of these data, we defined a scoring system to predict mobilization potential. A value of 1 point was given to a WBC count < 6 x10e9/l, and 1.5 points were given to a CD34 pre-G-CSF <2.5/mcl as well as to an age >35 years. Donors presenting at mobilization with a score >= 3 points had a significantly lower cell harvest.

Conclusion: According to our scoring system, donors with a score > 3 points at time of mobilization associate with poor yield of PBSC (<2.5 x10e6 CD34+cells/kg) and a more frequent requirement for two apheresis. In this subset of donors, mobilizing protocols including G-CSF at higher dosage can be of value.
P487
Search efficiency for blood stem cell donors over a 15 year period

In an attempt to identify the causes which are responsible for the failure to find a stem cell donor (other than an HLA-identical sibling) and/or to reach transplantation we analysed the search process in our centre over the last 15 years, divided over three time periods.

As shown in table 1 the chance of finding a suitable matched unrelated donor (mud) after histocompatability testing increased over time, as did the number of donors found within the core or extended family. Besides a better success rate, also the time it takes to find a suitable donor decreased (table 2). It is to be preferred to include the core/extended family in the initial search process because it takes less time to find a donor within the family.

Unfortunately the number of patients receiving a bone marrow transplant did not increase with time. This is due to multiple causes such as increasing age of patients and substantially higher number of poor risk patients within the last period. Patients from minority groups were not included in this study because of the known difficulty in finding a suitable donor for these patients.

In conclusion it can be stated that over this period of 15 years the success rate of identifying a suitable donor has increased and additionally the time needed for a donor search has decreased. Possible explanations may be that nowadays searches are being conducted more efficiently and the fact that the total number of potential donors in the BMDW (Bone Marrow Donors Worldwide) has increased. However more efficient search strategies including cord blood units have to be developed.

Table 1: Percent of donors found compared to the number of searches within 3 consecutive periods.

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<tr>
<td>Number of donors found</td>
<td>1,000</td>
<td>1,500</td>
<td>2,000</td>
</tr>
<tr>
<td>Number of searches</td>
<td>10,000</td>
<td>15,000</td>
<td>20,000</td>
</tr>
<tr>
<td>Complete search</td>
<td>25%</td>
<td>30%</td>
<td>35%</td>
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<tr>
<td>Core and extended family</td>
<td>40%</td>
<td>45%</td>
<td>50%</td>
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<tr>
<td>Total donors found</td>
<td>30%</td>
<td>45%</td>
<td>60%</td>
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P488
Stem cell donor availability: mailings, or how to maintain an "up-to-date" database
C. Rutt, D. Baier (Tuebingen, D)

Recruiting unrelated stem cell donors is the first step to build up an efficient donor center. Building up a repository of donor specimens reduces the need to redraw a new probe from a donor and so helps to reduce the time to achieve a more detailed HLA-typing result if required.

But most important is the simple fact that the unrelated donor must be contactable, therefore the DKMS traces the addresses of its over 825,000 donors by yearly sending out a "mailing". This does not only update our address-database, the donors are also informed about activities of the donor center and they are reminded of their status as unrelated stem cell donor. Furthermore the mailing is designed that the donors do not feel as a number but as member of the “DKMS family”. The mailing is not only an "up-to-date" database

Table 2: The median time it takes to find a suitable or mud donor and family donor.

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<tr>
<td>Median time of finding a mud donor</td>
<td>5 months</td>
<td>8 months</td>
<td>10 months</td>
</tr>
<tr>
<td>Median time of finding a family donor</td>
<td>2 months</td>
<td>3 months</td>
<td>3 months</td>
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P489
'Back Up Donor' help for unrelated stem cell donors and patients in one step
A. Gawellek, G. Rall, C. Rutt, D. Baier (Tuebingen, D)

Goal of the "Back Up Donor" strategy is to provide an alternative donor for the search community in case that a donor has completed a stem cell donation and is reserved for potential further donations for the same patient in a timeframe of actually 2 years.

First intention is to provide a donor as long as possible for "his" patient afterwards the 2 year suspension. This would enable us to avoid second or further stem cell donations if required.

Second intention is to protect the donor from requests for multiple patients. It could be observed several times that post-donation donors are of increased interest for the search community and were requested for confirmatory testing (CT) multiple times. Providing alternative donors would take off the burden for a single donor.

Third intention is to fill up the gap in HL-A-phenotypes when a donor is suspended from further donations within 2 years post-donation and to increase the number of high resolution typed donors in our donor center.

To find a "Back Up Donor" we perform up to 100 DR medium resolution or up to 5 DR high resolution typings on our own behalf for each donor post donation. Own searches and typings are repeated till success as long as a reasonable chance remains to find a "Back Up Donor".

End of 2001 over 9000 donors will be HLA-DR typed on medium or high resolution to provide a "Back Up Donor" for more than 560 donors.

P490
Strategies for prospective HLA-DR typing: how to ease the search process for an unrelated stem cell donor
A. Stahr, A. Gawellek, A. Grathwohl, J. Keller, U. Schuler, C. Rutt, D. Baier (Tuebingen, Dresden, D)

The DKMS (Deutsche Knochenspendenregister) performed since 1994 more than 170,000 additional (prospective) HLA-DR typings on its over 825,000 stem cell donors without any patient initiated search. This accounts for 44% of all DR-typed donors (over 382,000). Efficiency is shown by the fact that more than the half of the over 3,200 stem cell donations resulted from prospective DR typed donors.

Therefore the DKMS can provide the search centers with a wide spectrum of nearly 150,000 different HLA-AB-DR typed donors.

Without providing further typed donors it is difficult to find a DR matched donor if the initial search for a patient returns several donors. Providing a higher number and diversity of DR typed donors does not only shorten the time for an donor search it also helps to reduce the costs for patients.

Donor center initiated prospective DR typing was always performed at DNA-level on an intermediate resolution level allowing us to narrow down the possible alleles from a donor by using the NMDP-Allele-Codes. This enables the search center to know in advance if there is a chance to find a specially required allele by high resolution DR typing if the donor and patient share the same serology.
The DKMS favors the donor center initiated (prospective) DR typing of selected donors already in the database above the complete (HLA-A, B & DR) typing at time of recruitment. The logic behind the donor selection uses demographic data and the observed genotype frequency ranking.

P491
The outcome of the Czech patients with ALL/AML/CML referred to the Czech National Marrow Donor Registry (CNMDR) in 1993-1999 - A retrospective analysis
P. Jindra, V. Koza, H. Pitrakova, P. Sedlacek, J. Voglova, E. Faber, E. Krahalulova (Pilsen, Prague, Hradec Krafove, Olomouc, Brno, CZ)

The unrelated stem cell transplantation (Tx) is considered the only curative option for CML and some ALL/AML patients (pts) without sibling donor. This assumption should be proven in the random, unselected population of pts referred to unrelated donor (URD) registry for searching an URD. Is there any benefit for those successfully searched (i.e. proceeding to URD Tx) compared to the unsuccessful ones? We thus retrospectively analyzed the outcome of Czech pts with ALL/AML/CML for whom CNMDR performed URD search during period of 1993-1999. Of 215 searched pts sufficient follow-up data were obtained from 142 pts (66%) – 50 ALL, 34 AML and 58 CML with proportion of 30, 26 and 41% respectively, being Tx with URD. The medians of follow-up from search request were 26m for ALL, 38m for AML and 31m for CML and were not significantly different between groups of Tx and non-Tx pts (p=0,32-0,91) for each dg. Except for the younger age in the Tx pts with AML and CML (23vs37y, p=0,002 and 27vs35, p=0,014 respectively) the clinical characteristics among Tx and non-Tx pts within each dg were not significantly different (i.e. time to search, phase of disease at search etc.) The median time from search request to URD Tx was 4 months for ALL (range 2-6) and AML (3-18) and 6 months (3-30) for CML respectively. As of 01/01/2001 98 pts (69 %) were dead. The Kaplan-Meier probabilities of 5 years OS from search request are as follows: As expected, the majority of non-Tx pts died of leukaemia (96 % of deaths), on the other hand the TRM was leading mortality cause in group of Tx pts (80 %). Our results show the survival advantage if the searched pts proceed to URD Tx at for ALL and AML, while for CML the longer follow-up due to the nature of disease is obviously needed. The indirect evidence of correct URDtx indication by the Czech transplant centres as well as the effectiveness of CNMDR search operations for the pts referred to CNMDR are also demonstrated.

P492
Case report: detection of occult B-cell lymphoma in a stem cell donor by bone marrow examination

Transmission of donor cell neoplasia to recipient of allogeneic stem cell transplantation has been reported for two cases. Berg et al. described recently manifestation of donor cell type non-Hodgkin's lymphoma three years after transplantation and Niederwieser published transmission of acute myeloid leukemia in 1990. Inclusion of bone marrow puncture in evaluation procedure of stem cell donors is not generally recommended. We have evaluated a 65-year-old male sibling for stem cell donation for his HLA-identical sister suffering from chronic myeloid leukemia. The man's history did not give any hint for a hematological malignancy. B-signs were not reported. The spleen size measured by ultrasound was 102*37mm. Routine parameters of clinical chemistry, automated and microscopic blood cell examination gave normal results. Diagnostic bone marrow examination was done despite is not required in routine donor evaluation, neither by literature nor by NMDP or DKMS. Microscopic picture of marrow smear was normal, however by FACS-analysis a mature B-cell population with co-expression of CD19 and CD23 monoclonal for light chains was detected. Four weeks later a slight lymphocytosis of 45% was seen in peripheral blood and immunohistological bone marrow examination confirmed slight infiltration of the marrow by chronic lymphocytic leukemia. The man was withdrawn from donation. We conclude that there is a potential risk of transmission of donor cell neoplasia in early stages, especially when routine marrow examination is skipped and that marrow puncture should be considered in donor evaluation, at least in elder people.

P493
The reliability of serological HLA-A,B homozygosity in Czech National Marrow Donor Registry
P. Jindra, V. Koza, H. Pitrakova, J. Navratilova, M. Karas, K. Cerna (Pilsen, CZ)

Objective: Although DNA-based HLA class II typing generally replaced serology in bone marrow donor registries, it is assumed that the serological typing for class I is still valuable and effective in this setting due to the lower error rate than for class II. However, there exists a concern regarding its reliability especially for highly "homozygous" samples and high error rate in these samples was convincingly demonstrated by us (EFI conference 2000) and others (e.g. Lorentzen et al. 1997). We thus tried to determine the accuracy of serological HLA-A,B homozygosity in the Czech National Marrow Donor Registry (CNMDR).

Methods and Results: 120 consecutive Caucasian (West Slavonic) volunteer donors who entered the CNMDR in 1999/2000 and were assigned serologically as both HLA-A and -B homozygous (regardless of DR locus, i.e. 2-loci homozygous) were retrospectively DNA-rtyped by PCR-SSP (Genovision, Olerup SSP™). As expected and reflecting the haplotype frequencies, majority of examined samples were originally A1B8 and A3B7 (10%). After DNA retyping, we found that altogether 37 (31%) of these putative HLA-A and B homozygotes had turned out to be heterozygous. 6 of them (16% of errors) for A, 24 (65%) for B and 7 (19%) for both loci. Moreover, 2 of them also with incorrectly assigned "homozygous" allele. There was no clear pattern of discrepancies although identified "blanks" within the same cross-reactive group as seeming homozygous antigens accounted for 17 (49%) of missed antigens. Although representing 29 % (i.e. 35) of evaluated samples, the A1B8 constituted only 5% (2 errors) of all the false homozygous. The error rate for A1B8 serologic homozygotes was thus 6% (2 errors from 35 samples) but for others 41% (35 errors from 85 samples). Conclusions: HLA-A and -B serological homozygosity in CNMDR is encumbered with relatively high error rate (false homozygous rate of 31 %) confirming the overestimation of homozygosity in serology. This is even more significant in "non A1B8" samples (41 % error rate). These facts could be forcibly utilized in the most effective Class I DNA retyping strategy in CNMDR and also could have implications in the searching for the "true" (genotypically) homozygous patients.

Additional abstracts to this topic

Results of preliminary search of unrelated donors implicate the organization of an efficient national marrow donor registry: One year experiences of a Russian transplant center
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As successful organ or marrow transplantation correlates with the degree of HLA-compatibility between patient and donor, registries have been developed to facilitate matching. However,
geographical and national specificity of HLA-typ led to establishing national donor marrow registries in the eighties and early nineties. More than 2000 donors exist in the St. Petersburg marrow donor registry. It started its work within the last year by performing some donor actions in the north west of Russia. Since September, 2000, the joint venture German Russian BMT department at Centre of Haematology in St. Petersburg has started the unrelated donor marrow transplant program. Up to summertime 2001, seven patients were transplanted with stem cells from unrelated donors. In this period more than 100 preliminary donor searches have been performed. After HLA-DR Typing of HLA-matched donors no donors were determined in St. Petersburg marrow registry. So it was necessary to extend searches through "Bone marrow donor world" in cooperation with German partners. For 104 patients with leukemia, severe aplastic anaemia and lymphomas a search was performed. No HLA-ABDR identical donor were determined for 51% (53) of the patients. For an additional 35% not more than 10 donors were registered in "Bone marrow donor world wide". At this moment the data from participants from 49 bone marrow donor registries of 37 countries with 7,424,398 donors are included in this international registry.

In comparison: for more than 75% of the German patients HLA-ABDR identical donors were found in preliminary searches. We analysed the number of HLA-ABDR broad identical donors founded for our cohort of patients, and moreover, HLA-AB identical donors. In addition, we found special HLA-typs for those patients who didn't determinate HLA-ABDR identical donors. In conclusion, a further development of national marrow registry is an recent challenge for establishing an unrelated marrow donor transplant program in Russia.  

Timing of donor lymphocyte apheresis for immunotherapy after allogeneic blood stem cell transplantation  
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Objectives: Donor lymphocyte infusions (DLI) are an effective therapeutic tool for leukemic relapse after allogeneic stem cell transplantation. Since May 1997 lymphocyte aphereses from 49 stem cell donors (related and unrelated) were performed using a COBE Spectra cell separator to obtain a lymphocyte concentrate for patients with high risk factors for or with leukemic relapse after allogeneic stem cell transplantation. Lymphocyte aphereses before and after G-CSF stimulated stem cell harvest were compared.

Methods and Results: Performing a lymphocyte apheresis before stem cell harvest has no influence on the outcome of the stem cell product. However there are evidences that G-CSF could lead to a functional defect of the lymphocytes, so that lymphocytes should be collected without G-CSF stimulation before stem cell harvest. With one lymphocyte apheresis procedure 7 x 10^7 CD3+ cells/kg body weight of a 70 kg recipient could be obtained, making cryopreservation of several portions possible for consecutive immunotherapy. Viability of lymphocytes after apheresis was 98% and after cryopreservation 88% with a NC-recovery of 97% after thawing up.

Conclusion: For high risk patients especially in case of T-cell depleted stem cell transplants or after reduced conditioning where T-cell depletion is not possible, we suggest to begin DLI before stem cell harvest.

P494  
Combination of tissue plasminogen activator (rt-PA) and low dose heparin with or without defibrotide (DF) for the treatment of clinically suspected hepatic veno-occlusive disease (HVOD) following allogeneic hematopoietic stem cell transplant (AH SCT)  
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70 patients (M:40, F:30; median age:35yr, range:14-36) with clinically suspected HVOD following AH SCT (marrow:26, PBSC: 35, both:1, cord blood:1) were treated with rt-PA(10mg/d) and low dose heparin(1500U bolus, 100U/kg/d) with (N=51, M:27, F:24, median age:37 yr., range:14-56) or without DF(12-20mg/kg/d, n=19, M:13, F:6; median age:37 yr. range:21-54). Transplants were done for Ac. Leukaemia (n=38), Chr. leukaemia (n=21) or other disease (n=11) after conditioning with (n=53) or without (n=17) TBI and CyA with (n=69) or without Mtx (n=1) as GVHD prophylaxis. HVOD was suspected at a median of 11 d (range: 3-44d). Patients not receiving DF were planned to receive 2 days of rt-PA but patients in DF group received rt-PA as long as there was response or side effects. HVOD patients not receiving defibrotide had significantly higher bilirubin levels (107 vs 51, p<0.001) and higher urea (11.7 vs 7.2, p=0.003). Median days of therapy with rt-PA (2 d vs 10d, p<0.001) and heparin (9d vs 9d, p=0.27) was respectively in defibrotide and other group. Duration of therapy with defibrotide was 20d. Amongst patients receiving defibrotide 30 patients attained complete response, 5 showed stabilization and 16 had no response while in the other group 6 had complete response, 3 had stabilization and 10 had no response (p=0.13) but complete response rate was higher with defibrotide (30/51 vs 6/19, p=0.043). There was no difference in the time to ANC (23d each p=0.48) or platelet recovery to 50 (69d vs 82d, p=0.63). There was no difference in the incidence of bleeding episodes in two groups (11/51 vs 7/19, p=0.19). There was no difference in the incidence of AGVHD in two groups (44/51 vs 17/19, p=0.72). Maximum level of bilirubin was significantly lower in defibrotide group (110 vs 168, p=0.001). Day 100 mortality was similar in two groups (28/51 vs 11/19, p=0.82) and so was OS. Currently 16/51 in defibrotide group and 3/19 in the other group are alive with a follow-up of 1-76 months. In conclusion, combination of defibrotide with extended therapy with rt-PA and heparin is associated with higher complete response rate for HVOD without increased complications. This drug needs evaluation in formal randomized trials.

P495  
Late-onset VOD may benefit from rt-TPA therapy daily monitored with colorflow sonography: a case report  
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VOD is a clinical syndrome of multifactorial origin and still unproved treatment, in which the day of onset seems critical for diagnostic criteria: Seattle's criteria reduce the onset time from d=30 to d=20. However, later cases have been occasionally described. We report a late-onset serious VOD and its management. A 32 y.o. woman with IgA Multiple Myeloma in stage IIIa was admitted for MUD allo-Tx from a very well matched donor (13/14 Ag). Baseline liver status was unremarkable (HAV, HBV, HCV negative and liver tests normal). Conditioning was Busulphan 16 mg/Kg, L-PAM 140 mg/mq and Thymoglobuline for 2 days. GVHD prophylaxis was standard: CSPA and short term MTX. VOD prophylaxis with heparin 10000 U/day i.v. from admission to discharge is routinely used in our Unit. She was discharged on...
Effect of CD34+ cell doses on long-term overall and disease-free survival after allogeneic blood or marrow stem cell transplantation for hematologic malignancies: more is better


We have observed that infusion of low CD34+ cell numbers results in higher non-relapse mortality (NRM) and poorer survival after allogeneic blood or marrow stem cell transplantation in patients with hematologic malignancies (Singhal et al. Bone Marrow Transplant 2000;26:489-96). We now look at the same patient group with 2 more years of follow-up if the differences detected initially have been sustained at 5 y. 39 patients with hematologic malignancies were allografted from HLA-identical sibling using blood stem cells (BSCT; n=20) or marrow (BMT; n=19) after standard myeloablative regimens. Patients with acute leukemia in first remission and chronic myeloid leukemia in first chronic phase were considered standard-risk, and the rest were considered high-risk. The median CD34+ cell dose (3.7 vs 1.5 x 10^6/kg; P=0.002) was significantly higher with BSCT: 13 patients (6 BMT, 7 BSCT) died of toxicity at 15-733 d (median 57). Cox analysis, disease risk status and the CD34+ cell dose were the only factors found to significantly affect NRM, DFS and OS independently. CD34+ cell dose 3x10^6/kg was associated with lower NRM (RR 0.04, P=0.006), and higher OS (RR 25.3, P=0.004) and DFS (RR 26.2,P=0.003). Standard-risk disease was associated with lower NRM (RR 0.1; P=0.006), and higher OS (RR 7.5,P=0.001) and DFS (RR 6.3,P=0.001). There were significant differences (P=0.02 to P=0.004) in the actuarial 5-y outcome measures based upon the risk status and the CD34+ cell dose. These 2 variables can be combined to produce 3 groups whose outcomes are highly significantly (P<0.0001) different: Good (standard-risk disease and CD34>=3), intermediate (standard-risk disease and CD34 <3 or high-risk disease and CD34=3), and poor (high-risk disease and CD34 <3). The 5-y NRM, DFS and OS for good risk disease was 0%, 100% and 100%; intermediate risk 22%, 63%, 74%; poor risk 82%, 8% and 8%. We conclude that the number of CD34+ cells infused has a profound effect on the outcome of allogeneic transplantation through an impact on NRM. Infusion of 3 x 10^6 CD34+ cells/kg almost eliminates NRM in low-risk patients and decreases it substantially in high-risk patients. Since it is virtually impossible to obtain such a CD34+ cell dose for adult recipients from marrow, blood is likely to be the only viable source of large quantities of stem cells from most donors.
P499
Pre and post-transplant factors affecting platelet engraftment in children undergoing hematopoietic stem cell transplantation

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Introduction: Few studies in the last decade addressed the issue of mechanisms impairing platelets (PTL) engraftment after transplant (T). This monocenter retrospective study aimed at evaluating the role of factors potentially affecting the attainment of stable PTL counts.

Patients and methods: Ninety-one children (58 males, 33 females, median age at BMT 8 years) underwent AlloT (60) or AutoT (31) mostly for malignant diseases in 1st or 2nd CR (72.5%) between 1997 and 2000. Forty-eight patients (53%) received irradiation-based conditioning regimen. A median of 6.3 x 10^6/Kg CD34+ cells was infused (6.8 for AlloT; 3.6 for AutoT and 5.5 for AutoPBT, respectively).

Baseline variables, such as age, sex, type of transplant, HLA-compatibility, disease, TBI, CD34+ cells infused, and time-dependent variables, such as infectious diseases other than CMV, GVHD, hemorrhagic cystitis (HC), thrombotic thrombocytopenic purpura (TTP), potentially affecting PTL engraftment (defined as the first of the 5 consecutive days with PTL > 50 x 10^9/L without transfusional support), were included in the univariate and multivariate analysis.

Results: Overall eighty patients (88%) reached full PTL engraftment (median time: 27 days, range: 8-219), without significant difference between Allo and AutoT. Only 1/11 patients who never reached PTL engraftment died because of hemorraghic disease. By multivariate analysis CD34+ cells less than 5 x 10^6 (p=0.015) and the occurrence of TTP and HC (p=0.052 and 0.048) were significantly associated with delayed PTL engraftment in the allogeneic setting; AML/disease, TBI, CD34+ cells infused, and disease-free survival (DFS) were 73% and 41%, respectively. Multivariate analysis showed that the likelihood of DFS was decreased among patients transplanted in active disease (response to last cytoreduction less than very good partial remission) (p = 0.0004), females (p = 0.003), and patients recovering platelets later than a median of 11 days (p = 0.019).

Age, CD34+ cell dose, and time to leukocyte recovery had no influence on the OS and DFS. Improvement of these results will require more effective pre-conditioning cytoreduction and answering why slower platelet recovery affects disease-free survival.

Disease-free survival by time to platelets recovery

P500
Time to platelets recovery post autotransplantation predicts for survival and remission duration. A report of 100 procedures from Warsaw


Between June 1997 and March 2001, 100 patients mostly with malignant lymphoma (71%) who relapsed or failed initial treatment or with multiple myeloma (17%) received high-dose chemotherapy and mobilised autologous peripheral blood mononuclear cells. CD34+ cells were mobilised with a combination of chemotherapy and G-CSF. Predominant myeloablative regimens were BuCyV, Mel200 or BEAM. Five patients (5%) died from the regimen related toxicity. The 2-year actuarial probabilities of survival (OS) and disease-free survival (DFS) were 73% and 41%, respectively. Multivariate analysis showed that the likelihood of DFS was decreased among patients transplanted in active disease (response to last cytoreduction less than very good partial remission) (p = 0.0004), females (p = 0.003), and patients recovering platelets later than a median of 11 days (p = 0.019). Age, CD34+ cell dose, and time to leukocyte recovery had no influence on the OS and DFS. Improvement of these results will require more effective pre-conditioning cytoreduction and answering why slower platelet recovery affects disease-free survival.

P501
ABO-incompatibility as a risk factor for hematopoietic stem cell transplantation

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During last years ABO-incompatibility was reevaluated as a risk factor of hematopoietic stem cell transplantation (HSCT). While ABO-barrier does not influence the survival and the incidence of relapses after HSCT, an increased number of immunohematological complications and transfusion problems can be expected.

Material and methods: The study included 63 patients (pts), who undergoing HLA-matched HSCT. 32 pairs donor-pts were ABO matched (gr.I), 31 pairs were ABO mismatched ( 19 - major and 2-bidirectional - gr.II, 10 - minor - gr. III). The ratio of MUD/sibling transplants was 7/56, PBSC/ BMT – 17/46. The procedure of plasma removal was performed by marrow centrifugation for all cases with minor and bidirectional ABO-incompatibility and red cells were removed by sedimentation with 10% Polycrullin or 6% HES for the cases of major and bi-directional ABO-incompatibility. Three groups had similar conditioning regimens and GVHD prophylaxis.

Results: We did not observe any cases of acute hemolysis after BMT or PBSC infusion. The onset time of erythropoiesis differed between gr.I and gr. II (the mean delay of 1% reticulocyte level was 10+ days). In gr.II there was a 1,5-fold increase of requirement of RBC transfusions compared to gr.I. We observed the delayed onset of immune hemolysis in 4 cases (2 pts in gr. II and 2 – in gr.II) that were successfully treated with i.v. fluids.
An early increase of C-reactive protein is an independent risk factor for the occurrence of major complications and day-100 transplant-related mortality after allogeneic bone marrow transplantation

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We closely monitored levels of C-reactive protein (CRP) in a consecutive cohort of 96 adult patients (age 15-50 years) who had an allogeneic bone marrow transplantation (BMT) in our unit. Major transplant-related complications (MTC) occurred in 32% of cases. As MTC were included: hepatic veno-occlusive disease, pneumonia, severe endothelial leakage syndrome and >II acute GVHD. Transplant-related mortality (TRM), defined as toxic death before day 100 post-BMT was 13.5%. Mean CRP levels during each 5-day episode of the first 25 days after transplant were higher (p=0.05 – > p<0.001) in case of MTC. Univariate analysis revealed several risk factors associated with MTC: bad risk disease category (p=0.04), no T-cell depletion (p=0.018), bacteremia (p=0.029), mean CRP-levels between days 0 and 5 post-transplant (CRPd0-5) > 50 mg/L (p=0.002) and mean CRP-levels between days 6 and 10 post-transplant (CRPd6-10) > 100 mg/L (p<0.001). Risk factors associated with TRM: bad risk category (p=0.011), bacteremia (p=0.049), CRPd0-5 >50 mg/L (p=0.001) and CRPd6-10 >100 mg/L (p=0.001). In a stepwise logistic regression model, only CRP-levels (for MTC and TRM) (p<0.001) and donor-type (for TRM) (p=0.02) remained independent risk factors. CRPd6-10 >100 mg/L was highly predictive (estimated probability) for MTC: 73% versus 17% and for TRM: 36.5% versus 1% (identical sibling donor) and 88% versus 12.5% (other donor). We conclude that the degree of inflammatory reaction, as reflected by CRP levels, during the first 5-10 days after BMT identifies patients at risk of MTC and TRM. Our data are useful to select patients for clinical trials involving pre-emptive anti-inflammatory treatment.

This work was supported by grant from the Scientific Fund Willy Gepts AZ-VUB.

P504

Routine prophylactic platelet transfusions are not necessary for patients in clinical stable condition after autologous peripheral stem cell transplantation (ASCT)

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It is standard practice to give prophylactic platelet transfusion to prevent bleeding after ASCT at least when platelet count is < 10/nl. Long-term experience with an only therapeutic platelet transfusion strategy has proven to be safe in clinically stable patients (pts) with aplastic anemia and myelodysplasia (Sagemeister M, Blood 1999). We are currently performing a prospective study to see whether this strategy is safe and cost effective in pts after ASCT.

Patients: 16 pts consecutively admitted to our unit for high dose therapy followed by ASCT were included in this study. Median age was 50 years (18 - 66). The diagnoses were high grade NHL, multiple myeloma, ALL and ovarian cancer. The conditioning regimes were according to standard protocols.

Transfusion protocol: In clinically stable pts (fever < 38.5, no local infectious site, no sepsis syndrome) no prophylactic platelet transfusion was performed regardless of the morning platelet count. These pts received an only therapeutic platelet transfusion when bleeding WHO grade > I was documented. In clinically unstable pts a prophylactic platelet transfusion to prevent hemorrhagic complications was administered when the morning platelet count was < 10/nl. Results: Total days of thrombocytopenia < 20/nl and < 10/nl were 92 and 42 respectively. Clinically relevant bleeding (WHO grade > I) was not observed in 9 out of 16 therapy courses. Bleeding WHO grade II was documented in 7 out of 16 courses. There were no bleeding complications WHO grade III or IV and no treatment related death. The median number of platelet transfusions (for MTC and TRM) (p<0.001) and donor-type (for TRM) (p=0.02) remained independent risk factors. CRPd6-10 >100 mg/L was highly predictive (estimated probability) for MTC: 73% versus 17% and for TRM: 36.5% versus 1% (identical sibling donor) and 88% versus 12.5% (other donor). We conclude that the degree of inflammatory reaction, as reflected by CRP levels, during the first 5-10 days after BMT identifies patients at risk of MTC and TRM. Our data are useful to select patients for clinical trials involving pre-emptive anti-inflammatory treatment.

This work was supported by grant from the Scientific Fund Willy Gepts AZ-VUB.
Economic evaluation alongside a randomized trial comparing two schedules of granocyte(R) after autologous peripheral stem cell transplantation (AP SCT) to a third group without granocyte


The aim of this trial was to test the potential benefit of the use of G-CSF after APBSCT and to compare 2 schedules of administration. The clinical results were presented in EBMT 2001.

Methods: Children or adults with hematological malignancies or solid tumors planned to receive a consolidation with high dose chemotherapy +/- TBI and APBSCT were randomized to receive 150 microg/m^2/d of Granocyte(R) since day 1 post SCT (G1) or since day 5 (G5) or no Granocyte(R) (G0). The prospective economic evaluation was performed from the hospital view point, and studied direct medical costs. Cost factors were measured in physical units for each patient (collected in trial CRFs'). The following sources of unit cost data were used: prices before negotiation for drugs (G-CSF, antibiotics...), official French prices established by direct government regulation for blood products, the prices established by the French Social Security for laboratory and diagnostic examinations. The cost of a day of inpatient stay included costs of labor, consumable supplies, hotel, depreciation of equipment and overheads and was calculated assuming an optimal utilization of rooms. Sensitivity analysis tested the robustness of the results in relation to change in unit costs.

Results: 52 children and 188 adults were randomized in 23 French transplantation units between 09/1998 and 11/1999. Duration between SCT and recovery of PNN > 0.5 10^9/l was significantly reduced by GS-CSF administration in adults and children. There was no difference of thrombopenia duration, transfusion support, and non hematological toxicity between the 3 arms. Among adults, the duration of hospitalization after SCT was significantly reduced with G-CSF, with no difference according to the beginning of G-CSF. There was no difference of hospitalization duration in children. G-CSF represented 4 and 6% of the total cost in G5 and G1 arms in children and 10 and 12% in adults. Hospitalization represented 67, 64 and 65% of the total cost in G0, G5 and G1 arms in children and 52, 47 and 43% in adults. There was no significant difference of cost between the 3 arms in adults and children. This result was robust to changes in unit costs of G-CSF antibiotics and day of inpatient stay.

Conclusion: The administration of G-CSF after APBSCT did not increase total costs. We recommend the use of G-CSF since day 5 post AP SCT.

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P506

Similar benefit of day +5 low dose G-CSF versus day +1 standard dose G-CSF for autologous stem cell engraftment after myeloablative conditioning regimens


G-CSF is widely used for the promotion of engraftment after peripheral blood stem cell transplantation (PBSCT). The substantial cost of hematopoietic growth factors has spurred attempts to optimize the dosing schedule in order to reduce the expense without impairing engraftment.

We have retrospectively compared two dosing schedules using (a) standard dose filgrastim 480ug starting at day +1 after autologous PBSCT versus (b) low dose lenograstim 130ug starting at day +5 after autologous PBSCT in a case series of 24 and 14 lymphoma patients, respectively. Disease distribution and stem cell graft size were comparable in both groups.

In the standard dose group the median time to engraftment was 10 days, in the low dose group 11 days. The median febrile days in the standard dose group versus low dose group were 2 days and 2.5 days, respectively. The use of platelet units (7 vs. 4) and red blood cell units (4 vs. 2.5) were slightly in favor of the low dose schedule. The median days between stem cell transplantation and hospital discharge (14 vs. 15) were not significantly different between the two groups.

Since the use of hematopoietic growth factors is an important factor of expense in peripheral blood stem cell transplantation protocols, reducing the dose of G-CSF without impairing engraftment may significantly improve the cost-effectiveness of the procedure. To confirm these data a randomized trial is currently conducted.
veins thrombosis was not registered. Finally the employment of CVC allowed us to complete adequate PBSC collection in all the cases without interruption of the procedure. The 20 patients, who execute transplant, did not experience any kind of complications (no bleedings, infections). This experience concerning a new ecoguided approach of CVC insertion have shown a very low percentage of failure in reaching central vein, the absence of pneumothorax, of thrombosis and of significant bleedings.

P508

International survey of the intestinal bacterial decontamination practices in stem cell transplantation in the year 2001
S. Corbacioglu, T. Guengoer, C. Peters (Zurich, CH; Vienna, A)

Introduction: Intestinal bacterial decontamination (IBD) is often used during stem cell transplantation. The rational is the selective reduction or complete elimination of the intestinal microflora to diminish the risk for sepsemia and acute graft-versus-host disease (GVHD). IBD is a widely but not universally accepted practice.

Methods: In order to shed some light on this issue we contacted two hundred centers of the EBMT membership list from over 25 countries worldwide and collected data from 86 transplant centers between April and June of 2001 (response rate: 43%).

Results: The majority of the responding centers were large transplant centers reflecting the standard of care in 2001. Overall 73% of the centers use IBD. The reasons were equally distributed between infection (43%) only and infection and GVHD (45%). Twenty-one different drugs were used overall. The most frequently used drugs were from four pharmacological groups namely the quinolones, imidazoles, metronidazoles and polyenes. The most frequently used combinations in adults were quinolones and imidazoles (82%), followed by quinolones alone (27%) and quinolones together with metronidazole (18%). In the pediatric centers the polyeone/aminoglycoside combination was used as frequently (31%) as quinolones together with polyenes, followed by the quinolones/imidazoles and quinolones/metronidazole (19% each). The majority of centers reported to stop IBD with neutrophil engraftment (55%). The reported incidence of vancomycin resistant enterococci (VRE) was low even in those centers that used vancomycin as part of their regimen.

Conclusions: A large variability concerning the overall use, the drug combinations, duration and indications was observed between transplant centers worldwide. But a distinct pattern of drug combinations, duration and indications was observed in each). The majority of centers reported to stop IBD with neutrophil engraftment (55%). The reported incidence of vancomycin resistant enterococci (VRE) was low even in those centers that used vancomycin as part of their regimen.

P509

Prospective evaluation of antiemetic therapy with 5-HT3 antagonist (+/- steroids) following high-dose chemotherapy (HDC) and stem cell transplantation (SCT)
R. Carrion, P. Balsalobre, D. Serrano, I. Buño, E. Moreno-López, A. Gómez-Pineda, S. Carrasco, J.L. Diaz-Martín (Madrid, E)

HDC includes combinations of highly emetogenic antineoplastic agents given on consecutive days, often together with total-body irradiation (TBI). Emetic control is a specific problem in patients (pts) under HDC following SCT where 5-HT3 antagonists with/without steroids have demonstrated their efficacy. Purpose: Pts under HDC/SCT were evaluated during the six day period following initiation of HDC or TBI from March-98 to November-01. Endpoints: number of emetic episodes (EE) and grade of nausea (4 point categorical scale). Methods: 90 pts were treated with 5-HT3 antagonists (Granisetron (GRN) 3 mg bid, (OND) Ondansetron 24 mg/d and Tropisetron (TRP) 5 mg/d) with/without dexamethasone (DXM) 16 mg/d (GRN 12 pts; OND 16 pts; TRP 9 pts; GRN+DXM 4 pts; OND+DXM 19 pts; TRP+DXM 30 pts).

Pediatric pts and pts under non-myeloablative conditioning were excluded. Results: M/F: 33/57. Median age: 46 (range 15-69). Primary disease: leukemia 28, myeloma 13, lymphoma 16, Hodgkin’s disease 9, breast cancer 22 and sarcoma 2. Allo/Auto: 167/734. Conditioning regimen: BuCy 23, BuMel 13, CTX-TBI 4, BEAM 16, STAMP-V 22, Bu 5, CBV 4 and Other 3. Daily Complete Response (EE=0 + mild/no nausea): 78% (1st day), 59% (2nd), 40% (3rd), 34% (4th), 33% (5th) and 34% (6th). Global Complete Response (GCR) (EE=0 + mild/no nausea during assessment period) was observed in 17 pts (19%). No significant association was observed between GCR and age, primary disease and antiemetic scheme with/without steroids. Significant association was observed between sex and GCR rate (7% for women and 39% for men, p=0.0001). This association was maintained after adjusting for STAMP-V protocol for breast cancer (11% for women, p=0.008). GCR and conditioning regimen also showed significant association (p=0.001). The strongest emetogenic regimes were CBV, STAM-V, CTX-TBI and BuCy (GCR: 0%). The mildest emetogenic regimes were BEAM (56%) and BuMel (48%). Conclusions: Current antiemetic treatments based on 5-HT3 antagonists +/- steroids still have a low emetic control rate (19%) for pts under HDC/SCT. In our cohort, this was significant for women, for pts under conditioning regimens as CBV, STAM-V,CTX-TBI and BuCy and during the last three days of conditioning period. Our data showed a limited utility of steroids in emesis control, therefore further clinical trials are needed.

P510

Incidence and outcome of intensive care treatment after hematopoietic stem cell transplantation
M. Kiehl, H. Kolb, E. Roemer, H. Ostermann (Idar-Oberstein, Munich, D)

It is still a matter of debate if patients with (multi)organ failure requiring intensive care treatment after allogeneic stem cell transplantation (SCT) will profit from ICU treatment. To get information on incidence and outcome of ICU treatment in these patients we retrospectively analyse the data of all patients receiving an allogeneic transplant at the BMT centres Munich and Idar-Oberstein 1998 – 2000 for haematologic malignancies. 371 patients (153 female, 218 male) were available for analysis. Out of these 173 patients receives a transplant from a related and 198 from an unrelated donor. Underlying diseases were acute myeloid or lymphoblastic leukaemia, chronic myeloid leukaemia, lymphoma or Hodgkin’s disease in 78%, 14%, 5%, and 3%, respectively. Out of these 371 patients 52 (14.02%) required ICU treatment for sepsis or septic shock (50%), acute respiratory failure (23.3%), respiratory failure due to pneumonia (13.3%), acute renal failure (12.4%) or other reasons (1%). Only two of the patients with acute respiratory failure requiring intubation and mechanical ventilation survived and were discharged to the ward. Whereas all other patients requiring more than one technical assist, e.g. invasive ventilation, dialysis, vasoactive drug support died during treatment. Comparing these data with data from neutropenic patients after standard chemotherapy requiring intensive care it is conspicuous that ICU treatment in neutropenic patients improve over time whereas outcome in BMT patients is still the same as in the 1980’s. Therefore, it is evident that supreme efforts are needed to improve intensive care in this specific group of high risk patients.

P511

Successful treatment of severe hemorrhagic cystitis after allogeneic bone marrow transplantation with bladder irrigation with formalin and cauteterization
A. Ibrahim, E. Fadel, L. Abs, A. Mugharbil, M. Kaskas (Beirut, LBN)

Hemorrhagic cystitis (HC) is an important cause of morbidity and occasional mortality in patients (pts) undergoing bone marrow transplantation (BMT). HC is essentially due to toxic reaction to
chemotherapy with cyclophosphamide (CTX). HC is associated to BK virus but the role of BK virus remains contentious. Although mild cases of HC often respond to supportive treatment including hydration, blood and platelet transfusion, severe HC may require cyclophosphamide to remove all clots followed usually by chemical irrigation of the bladder and cauteryination. Silver nitrate, alum, prostaglandins, phenol and formalin are used for bladder irrigation. Among 23 pts who underwent allogeneic BMT in our Unit between 7/1997 and 10/2001, 3 pts developed severe HC. Allogeneic BMT was performed for CML in 2 cases and AML in one case. Patients were 21, 34 and 44 y.o. respectively. Conditioning regimen associated busulfan (16mg/kg) and CTX (120mg/kg with hydration and Mesna). Graft-versus-host disease prophylaxis combined ciclosporin A and methotrexate (days 1, 3, 6 and 11). Severe HC occurred on days 1, 67 and 54 respectively. Intensive bladder irrigation with normal saline, blood and platelet transfusion were done with no improvement. A cystoscopy was performed under general anesthesia for the 3 pts on days 30, 89 and 62 respectively. Cystoscopy showed hemorrhagic mucosa of the bladder with clots. After removing all clots, a 0.1% formalin solution was instilled and repeated at 0.4% concentration followed by cauteryination in the same session. HC ceased on days 34, 94 and 66 respectively. No relapse of HC was noted in the 3 pts.

P512
Promising use of defibrotide in bone marrow transplant associated thrombotic thrombocytopenic purpura (TTP)


Introduction: Despite several therapeutic approaches, the prognosis of BMT-associated TTP remains dismal with high incidence of mortality, variable according to the type of transplantation and the severity of the complication. The current retrospective study suggests a possible positive effect of defibrotide (DFT), an anti-thrombotic and thrombolytic agent, if used early.

Patients and methods: 12 patients (6 males; median age: 33 years, range: 27-45) underwent related (5) or unrelated (7) BMT, for ALL (2), interstitial pneumonia (3), CNS involvement (1). Conditioning regimen associated busulfan (16mg/kg) and CTX (120mg/kg with hydration and Mesna). Graft-versus-host disease prophylaxis combined ciclosporin A and methotrexate (days 1, 3, 6 and 11). Severe HC occurred on days 1, 67 and 11. Intensive bladder irrigation with normal saline, blood and platelet transfusion were done with no improvement. A cystoscopy was performed under general anesthesia for the 3 pts on days 30, 89 and 62 respectively. Cystoscopy showed hemorrhagic mucosa of the bladder with clots. After removing all clots, a 0.1% formalin solution was instilled and repeated at 0.4% concentration followed by cauteryination in the same session. HC ceased on days 34, 94 and 66 respectively. No relapse of HC was noted in the 3 pts.

P513
Immune reconstitution after conventional - and high-dose chemotherapy +/- amifostine in patients with germ cell cancer: a prospective randomized single center study

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Purpose: To assess the influence of amifostine on immune-reconstitution after conventional-dose (CDCT) and high-dose chemotherapy (HDCT) followed by autologous peripheral blood progenitor cell (PBPC) rescue in patients with germ cell cancer. Patients and Methods: Forty-four patients were treated with one cycle of 175 mg/m2 paclitaxel, 6 g/m2 ifosfamide, 100 mg/m2 cisplatin (TIP) plus 5 µg/kg/day G-CSF and one course of HDCT with 1,5 g/m2 carboplatin, 2,4g/m2 etoposide, 450 mg/m2 thiopeta (CET) plus PBPC rescue and 5 µg/kg/day G-CSF. All patients were randomized to receive an absolute dose of 500 mg amifostine (group A, n=20) on each day of chemotherapy or no amifostine (group B, n=20). Immune-status was measured prior each cycle of CDCT, prior HDCT, after hematologic engraftment from CET as well as 6 weeks and 3 months after PBPC rescue. Immune reconstitution was determined by the assessment of CD3, CD4, CD8, CD4, CD8, CD16, CD19, CD45R0, CD45RA and CD56 positive lymphocyte subpopulations and the CD4/CD8 ratio.

Results: Between the two study arms no statistically significant differences could be observed concerning reconstitution of lymphocyte subpopulations. Throughout treatment with TIP lymphocyte counts and their subpopulations remained low without severe clinical complications. Delayed reconstitution of the CD4+ cell compartment after CET could be observed in both study arms but did not result in any severe or atypical infections.

Conclusion: In our study treatment amifostine did not significantly influence the immune-reconstitution. Furthermore, low numbers of total lymphocytes during TIP and delayed reconstitution of CD4+ cells and other lymphocyte subpopulations after HDCT and PBPC rescue had no clinical relevance for patients with germ cell cancer.

P514
The effect of modulation of glutathione cellular content on busulphan-induced cytotoxicity in hematopoietic cells in vitro and in vivo

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Busulphan is an essential component in conditioning regimen prior to stem cell transplantation (SCT). Busulphan has a narrow therapeutic window and under- or overdosing may have a fatal outcome for the patient. Pharmacodynamic studies in patients have established a relation between exposure to drug expressed as area under the plasma concentration-time curve (AUC) and effect and/or adverse effects, such as veno-occlusive disease (VOD). Exhausation of glutathione (GSH) contributes to VOD in animal models and intracellular level of GSH has been shown to affect busulphan-induced toxicity in hepatocytes. We investigated the pharmacodynamic relation between exposure to busulphan, and its in vitro toxicity to CD34+ hematopoietic progenitors from healthy volunteers using clonogenic assay. We found that busulphan inhibited colony formation of CD34+ cells in an AUC-dependent manner, and that this relation was linear. Myeloid progenitors were more sensitive than erythroid progenitors, expressed as inhibition of colony formation by 50% (10.6 ± 2.9 µg/ml) in myeloid and 26.3 ± 5.3 in erythroid progenitors) and 100% (68.7 ± 7.5 µg/ml in myeloid and 140.3 ± 35.7 in erythroid progenitors). The observed exposure inhibiting colony growth in vitro corresponds to the total AUC obtained in patients treated with busulphan (1 mg/kg/day) for four days. Secondly, we studied the effect of modulation of GSH cellular levels on busulphan-induced toxicity in CD34+ cells from healthy volunteers in vitro, and in vitro.
murine bone marrow cells from Balb/c in vivo. The intracellular concentration of GSH was increased or decreased by treatment with N-acetylcysteine or buthionine sulfoximine, respectively. Neither in vitro nor in vivo treatment with GSH modulators affected the histopathological toxicity of busulphan. These results indicate that treatment with N-acetylcysteine would not interfere with the myelotoxic effect of busulphan. Thus, N-acetylcysteine is a potential candidate drug for VOD prophylaxis during busulphan-based conditioning regimen and our findings are of high clinical relevance.

8. Cellular Therapy

PS15

Flow cytometric detection of nucleated red blood cells (NRBC) in cord blood (CB) modifies Cd34+ cell quantification


Objectives. Cord blood represents an alternate source of stem cells for transplantation. NRBC are a physiological subset of CB cell population. Although it is crucial to have an accurate estimate of CD34+ cell number, commonly estimated using CD34/CD45 labelling, NRBC could compromise white blood cell (WBC) count and interfere with CD34+ cell quantification. The aim of this study was to determine the role of NRBC count in CD34+ enumeration using a simple and rapid flow cytometric method (Tsui T et al., 1999).

Methods. 110 CB were analysed for total nucleated cells (TNC), NRBC, CD34/CD45 cells by flow cytometry. NRBC were also determined conventionally by manual microscopy of Wright-Giemsa stained blood films where NRBC were counted separately and expressed as a proportion of a total of 300 WBC. Percentage of CD34+ cells corrected by NRBC count (CD34-c) were determined as followed: CD34-c = CD34+/CD45+ x 100 / TNC - NRBC. Regression analysis curve and coefficient of determination (r2) measurement were used for comparison between flow cytometry and manual microscopic results. A paired t-test was applied to determine statistically significant differences.

Results. Mean number of CD34+ cells and NRBC were 0.337% (range 0.08%-2.27%) and 6.68% (range 0.86%-39.34%) respectively. Comparison between flow cytometric and microscopic NRBC count showed a regression of y = 0.767x + 0.71 and a coefficient of determination of r2 = 0.76. No significant difference could be evidenced between the two methods. When corrected with NRBC count, mean number of CD34+c cells was 0.39% (range 0.09-3.63, p=0.004 compared with %CD34+). This significant difference was even stronger when comparing mean of total CD34+ cells with CD34+c cells: 5.28 ± 106 versus 5.89 ± 106 respectively (p=0.0001). When applying an arbitrary cut off of 10% NRBC (n=24 CB), mean number of total CD34+ and CD34+c were 10 106 (range 2.29-28.35) and 12.35 106 (range 2.7-33.37), p<0.0001.

Conclusion. Determination of NRBC using a rapid flow cytometric method might represent a new strategy for CD34+ quantification providing satisfactory quality assurance controls of CB products.

PS16

Flow cytometric analysis of dendritic cell subsets in peripheral blood of breast cancer patients after circulating progenitor cell priming with high-dose cyclophosphamide + G-CSF

B. Rovati, L. Cucca, E. Collovà, S. Ferrari, C. Porta, M. Danova (Pavia, I)

Dendritic cells (DCs), a subset of bone marrow-derived leukocytes, are the most efficient antigen-presenting cells that initiate and direct the immune response. DCs can be detected into the peripheral blood with flow cytometry (FCM) and subsetted in two subpopulations, named DC1 and DC2, with different functions. However, a direct measurement of DCs has been complicated by the lack of a suitable specific marker. Recently, three novel markers specific for distinct DC populations have been identified: 1) BDCA-1 expressed on CD11c-high / CD123-low myeloid DCs, which represent the major subset of myeloid DCs in human blood; 2) BDCA-2 that specifically identifies CD11c-neg / CD123-high lymphoid blood DCs; 3) BDCA-3 specific for a small population of CD11c-low / CD123-neg myeloid DCs. As a part of our ongoing studies on the effects of rituximab, circulating progenitor cell (CPC) mobilization regimens on blood DCs in cancer patients, we have utilized these markers using a multi-color FCM assay with an EPICS XL equipment. Lysed whole blood from 12 breast cancer patients undergone high-dose (HD) cyclophosphamide (CTX) + G-CSF CD34+ cell mobilization for autografting was studied. The mean percentages (± SD) of BDCA-1, BDCA-2 and BDCA-3 at the time of CD34+ cell peak (on days +7 - +11 after CTX) were: 0.11 ± 0.03, 0.05 ± 0.03 and 0.01 ± 0.005, respectively. In the studied patients' comparative data were obtained by calculating the percentage of circulating DCs when gated as negative for cell specific lineage markers (CD3, CD11b, CD14, CD16, CD19, CD20, CD34, CD56), positive for HLA-DR and identified as DC1 and DC2 according to the expression of CD11c and CD123, respectively. The mean percentage of BDCA-1 + BDCA-2 + BDCA-3 was 0.167 ± 0.05 and it was similar to that of DC1 + DC2 (0.137 ± 0.08) (p = n.s.), indicating that the two type of analysis identified the same global DC population. Both methods revealed values lower than those obtained from the steady - state peripheral blood of 20 healthy donors (% DCs = 0.95 ± 0.05), confirming our previous findings of an impaired mobilization of DCs into the blood after HD CTX + G-CSF in breast cancer patients. Further approaches of optimized CD34+ mobilization to be rapid, sensitive and could have immediate application for an efficient monitoring of the mobilization of specific DC subpopulations into the blood after stem cell priming for autografting.

PS17

Dendritic cells (DC) for NK/Lak activation: rationale for multicellular immunotherapy in neuroblastoma (Nb)


Natural killer cell (NK)/Lymphokine-activated NK (LAK) cell based-immunotherapy could be beneficial against MHC class I negative tumor residual disease such as neuroblastoma (NB), provided IL-2 or surrogate non toxic NK cell stimulatory factors could sustain NK cell activation and survival in vivo. Here we show that human monocyte-derived-dendritic cells (MD-DC) promote potent NK/LAK effector functions and support the implementation of DC/NK-based immunotherapy for purging the graft and/or controlling minimal residual disease after autologous stem cell transplantation. Such cells are planned to be administered with non manipulated PBSC after high dose chemotherapy in high risk neuroblastoma patients.

S119
PS18

Generation of potent Th1 responses from patients with lymphoid malignancies after differentiation of B lymphocytes into dendritic-like cells


Dendritic cells (DCs) are a group of potent antigen presenting cells (APC) specialized for initiating T cell immune responses. They originate from the bone marrow, and upon stimulation with bacterial products, cytokines, or CD40 ligation, they acquire the ability to migrate to the secondary lymphoid organs. In vitro DCs can be generated from human CD34+ bone marrow cells and CD14+ peripheral blood monocytes after culture with different cytokine combinations. Since most leukemic cells and tumors in general are devoid of APC capacities, various strategies have been used to increase their recognition and confer the capacity of antigen presentation on them. Because of our interest in the design of vaccine immunotherapy protocols for the adjuvant treatment of patients with lymphoid malignancies (LM), we chose to explore the capacity of human acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and plasma cell leukemia (PCL) to differentiate into cells with APC and DC features. We have tested 10 patients derived from that such approach is feasible. Leukemic cells could be induced in the presence of IL-4 and CD40L to exhibit a DC morphology with phenotype of mature DC-like cells. They could also induce a potent proliferative response in naive CD4+ T cells. In addition, they expressed chemokine receptor CCR-7 and CD62L, and could drive T cells towards a Th1 response with secretion of IFN-g. Our strategy leading to increase LM cells immunogenicity cannot have potential clinical applications and LM appear to be attracting candidates for adjuvant vaccination and adoptive immunotherapy.

PS19

Donor lymphocyte infusions after allogeneic stem cell transplantation: 119 lymphohocyte donations characteristics from 73 donors

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Donor Lymphocyte Infusions (DLI) have shown their efficacy for eradication of malignant diseases in relapse after allogeneic stem cell transplantations. However, very little is known about the biological impact of lymphocyte donation on donors, We retrospectively analysed the 119 donations among 73 donors who underwent apheresis for lymphocyte collection, in the same technical conditions, between 1996 to 2001, in our center. There were 43 males and 30 females, median age at harvest was 41 years (16-41), and 22 donors underwent multiple (<2) donations. On average 1.7 1010 (± 0.05) total mononuclear cells (MNC), 9 109 (± 0.3) total CD3+, 1.2 109 (± 0.1) total CD56+, 1.8 109 (± 0.14) total CD19+ cells were collected per apheresis. The process of apheresis, as expected, has an immediate impact on total absolute WBC counts: 6.51 G/l (± 0.15) before vs 5.5 G/l (± 0.1) after, and total absolute lymphocyte counts: 1.8 G/l (± 0.15) vs 1.4 G/l (± 0.05) (p<10-6 for both). The median delay between stem cell donation and lymphocyte donation was 2.33 (± 0.33) years and the type of stem cells donation (62 BM vs 11 PBSC) had no influence on total MNC or lymphocyte subsets harvested. We compared cohorts of single versus multiple donations, and significantly more total MNC were harvested per apheresis in single donations (1.8 (± 0.05) 1010 cells) than in the multiple donations (1.6 (± 0.08) 1010 cells) (p=0.03). Moreover the CD3+ compartment seemed to be uniquely altered with 1 1010 (± 0.05) total CD3+ cells collected for single donations vs 0.8 1010 (± 0.05) for multiple donations (p=0.01); whilst CD56+ and CD19+ compartments were not affected. We further compared data from first vs second apheresis, and no difference in any parameters was shown, suggesting that the previous differences were related to >2nd apheresis per donor parameters. No opportunistic infection was observed among all donors. In conclusion, multiple apheresis of donors for DLI significantly impoverishes MNC and CD3+ contents of the products, and can lead to important lymphopenias. However no significant infection was reported.

PS20

Novel inhibitors of multidrug resistance - structural and future applications

H. Galski, A. Nagler (Tel Hashomer, IL)

Of both clinical and mechanistic relevance, numerous structurally unrelated chemical compounds that inhibit the function of P-glycoprotein (Pgp) have been identified. These chemicals from different pharmacological classes, termed chemosensitizers, reverse multidrug resistance (MDR) of malignant cells to various cytotoxic agents. Previous binding studies of various chemosensitizers suggest that chemosensitizers block cytotoxic drug efflux by acting as competitive or non-competitive inhibitors, perhaps by binding to similar drug substrate binding sites that cause allosteric changes resulting in inhibition of cytotoxic drug binding or transport. However conflicting evidence exists with regard to whether chemosensitizers of different classes function similarly as inhibitors of drug efflux, where and how many binding sites on Pgp are involved and whether chemosensitizers share binding sites with cytotoxic drug substrates. We have recently discovered novel, structurally unrelated, Pgp inhibitors (KT-5720 and D-2015) that can reverse multidrug resistance of lymphoma (LM1/MDR) and carcinoma (KB-V1) cell lines over expressing the human Pgp. These modulators chemosensitize the multidrug resistant cells to various cytotoxic agents and also increase the accumulation of the Pgp-specific substrate, Rhodamine123. To understand their mode of action and their interactions with Pgp, we have performed competition experiments between these modulators and the classic Pgp inhibitor verapamil. Competition experiments between verapamil and KT-5720 have shown that the two modulators, in conjunction, exhibit complex interactions. While apparently exhibiting additive effects at low concentrations of verapamil, the effects of verapamil at higher concentrations (0.75 micro Molar and higher), in conjunction with KT-5720, are synergistic. This suggests the involvement of multiple binding sites of verapamil to Pgp, possibly low- and high- affinity binding sites. KT-5720 competes on the high affinity sites only. In contrast, combining the activity of verapamil with D-2015, a synergistic effect is achieved. Moreover the results of these experiments suggest the existence of separate and multiple binding sites on Pgp for verapamil and D-2015. Both KT-5720 and D-2015 can sensitize multidrug resistant lymphoma to chemotherapy agents in animal models. These MDR inhibitors are much less toxic than verapamil in vivo and are further characterized towards future clinical application.

PS21

Ex vivo progenitor generation from cord blood CD34+ cells to use in clinical protocols aiming to reduce neutropenia

S. Querol, M. Piquet, J. Garcia (Barcelona, E)

Introduction: A typical CB unit contains a mean of 3.51x106 CD34+ cells and 1.28x106 CFUs. With these quantities, 78% of patients engraft with a median time of 29 days (bc8 data). Engraftment could be accelerated by infusion of higher quantities of progenitor cells. Expansion of progenitor cells ex vivo that can reduces the neutropenia after co-infusion with an unmanipulated aliquot.

Objective: To define the culture conditions to amplify CB progenitor cells, measured by CD34 expression and clonogenic activity (CFU), intended for clinical use.

Material and methods: CD34+ cells were selected using a miniMACS system. After apheresis of donors for DLI significantly impoverishes MNC and CD3+ contents of the products, and can lead to important lymphopenias. However no significant infection was reported. Ex vivo progenitor generation from cord blood CD34+ cells to use in clinical protocols aiming to reduce neutropenia

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Objective: To define the culture conditions to amplify CB progenitor cells, measured by CD34 expression and clonogenic activity (CFU), intended for clinical use.

Material and methods: CD34+ cells were selected using a miniMACS system. After selection cell were cultured in serum-free medium (CellGro(R)) at low cell concentration (25x104 cells/ml). Culture was fed every 3 days with medium and cytokines, and cell medium (CellGro(R)) at low cell concentration (25x104 cells/ml).
flask surface. Cytokines used for expansion were: SCF, FLT3L, IL6, TPO (SF6T) at 50 ng/ml. At day 6, effect of Epo(E) and GCSF(G) at 2 Ul/ml and 10 ng/ml was analysed. Results: Nucleated cell (NC), CD34, and CFU expansion is shown in figure. Use of EG results in a higher expansion rate of NC and CFU, but similar of CD34. In contrast, viability of CD34+ cells were higher using EG (day-10: 80% vs 60% by 7-AAD analysis). Cells generated express multilineage antigens (CD61, CD15, CD11b, GlyA) in both conditions. But, presence of G further increases CFU-GM colonies, resulting in a higher CD15 generation after 14 days. Moreover, use of E promotes a substantial differentiation of cells expressing GlyA, and further expansion of BFU-E colonies. Expansion of CD61+ cells showed a similar rate with or without EG. Progenitor cells generated were cryopreserved to test their tolerance. CD34+ cell number decreases up to 60% immediately after thawing, indicating an increased sensitivity to cryopreservation. But viable CD34+ cells have comparable clonogenic activity than fresh progenitors.

Conclusions: cryopreserved during 2-2 weeks increasing amounts of progenitors. After 10 days, the benefit to maintain the culture is low and viability decrease. The use of EG after 6-days, in addition to the basic combination SF6T, increases cell viability and promotes a higher CFU expansion. Using this protocol, a generation of 2-additional logs of progenitor cells can be achieved.

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P522
Alloimmunization after transfusion of RhD-incompatible platelet concentrates
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Background: RhD-alloimmunization in patients with various malignant and non-malignant diseases after transfusion of RhD-positive platelet concentrates has been analyzed.

Methods: 96 RhD-negative patients were included in our study. Antibody screening using the sensitive gel test (DiaMed, Cressier, Switzerland) was negative prior to inclusion in the study and none of them received RhD-positive packed red cells throughout the entire observation period. Buffy coat derived platelet concentrates were prepared from four different whole blood donors. Residual red blood cells were estimated using the Bürker-Türk chamber.

Results: 10 (10.4%) patients (5 males, 5 females) developed an anti-D antibody following 2.9±0.9 RhD-incompatible platelet transfusions. Those patients without antibody formation received significantly more incompatible units (4.9±0.85 vs 0.6±0.05; p=0.0005). Antibody formation was observed in each patient group studied. 10% (7/70) of the haematological patient group developed an anti-D, the mean period of time until identification of the antibody was 97 (11-393) days. In the group of the oncological patients 14% (2/14) were immunized, in the group of the non-malignant diseased patients 6% (1/12). The mean period of time until detection of the antibody was 112, 388 and 12 days, respectively.

The mean contamination of the single donor platelet concentrates and the pooled concentrates with red blood cells was 0.008x10^9 and 0.13x10^9 per unit, respectively (p<0.01). Considering the amount of erythrocytes in whole blood as few as about 100 microliter erythrocytes have been transfused with each single platelet unit. Interestingly, 5 out of 10 patients developed an anti-D following a single incompatible platelet transfusion.

Conclusions: The risk of alloimmunization following RhD-positive platelet transfusions appears to be small in the patient groups studied. However, anti-D formation seems to be independent of the number of transfused erythrocytes. Even in immunosuppressed patients antibody formation was recognized.

The administration of an anti-D prophylaxis in young female should be once more considered.

Additional abstracts to this topic

Extracorporeal photochemotherapy (ECP) in pediatrics: safe and high selective MNC collection using Cobe Spectra Auto PBSC in treatment of acute or chronic GvHD

Over the last 5 years, we performed 569 leukoapheresis with Cobe-Spectra Cell Separator Auto PBSC(c 6.1) utilizing the original dual stage channel device, automatic procedure and a low extracorporeal volume in 33 children (mean age7years) who underwent ECP for acute or chronic GvHD, resistant immuno suppressive therapy.

In patients with acute hemolysis in children weighing <15 Kg, in patients (>15 Kg) with Hb basal value < 9g/dl, blood cell priming was used: collected no more than 7 days before, cross-matched red cells was irradiated, leukodepleted, washed, HTC equal to the patient.

In our protocol, total patient’s blood volume processed was 1.5, MNC collection’s cycles were 4-5 and final harvest volume, was no more than 40-50 ml.Fluids balance was maintained, during the procedure, with little plasma collections, to contrast the ACD overload infused. The BFR was no more than < 15-30 ml/min, adapted to the patient’s weight and flow. ACD HA anticoagulant was used and citrate rates were decreased from 1:13,5 to 1:14 ,after processing 500ml blood volume processed: 2 ml of Calcium Gluconate were prophilattically, infused for every blood volume ,to prevent hypocalcemic symptoms. Procedure time was no more than 2 h-2.30’, always was well tolerated and no significant variations parameters were observed in patient before and after ECP.

The majority of our patients had a central venous line: usually dual lumen catheters, more rarely single lumen-catheters with additional, minimum, 20G size peripheral catetars. The MNC collected for ECP was mean 1.6±109, MNC efficiency was present 80.5%, HTC < 2%, purity MNC collection was mean 92 %, and very low platelet contamination was observed MNC cells diluted at 300ml final constant volume with polysaline solution, 8-MOP were added, ex vivo, a final concentration 200 ng/ml and transferred in special UVA permeable plastic bags for UVA, Matic Irradiation system, at 2.3/cm2. Finally, the treated MNC, were reinfused, in 1 h or more in very small children. Complete blood count in each harvest, sterility control samples and cyto-flowmeter parameters involving T and B leucocyte subsets at start protocol and monthly in each patient, were evaluated. Our protocol consisted, normally, of 24 total procedures: two procedures, performed weekly, on consecutive days, during the first month, at 2 week intervals between the 2nd and 3rd month, then monthly until requested by individual clinical status.

Mitoxantrone/cytarabine/fluoradribine (FLAM regimen) combined with donor stem cells as salvage chemo-immunotherapy for relapsed leukemia after allogeneic transplant
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Relapse of leukemia despite allogeneic transplant is often perceived as being untreatable. We have attempted to improve the outcome of such patients by incorporating a chemotherapeutic regimen which should be effective against the leukemia but does not impair the state of chimerism already induced by the allogeneic transplant, thus allowing a platform for further treatment with donor lymphocytes. Thus we have chosen the FLAM regimen, as reported by Koller, et al (Cancer, 1999 (86)11: 2246-51), designed to maximize pharmacological interactions of the three agents without excess toxicity, and added donor G-CSF mobilized stem cells infused 36 hrs after chemotherapy. We present two patients who were restored to full chimerism after relapsing post allogeneic transplant. The first patient was a 19 year old with T cell ALL/lymphoma, who was previously treated with M-BOACD, BFM-ALL 90 and 96, as well as cranial axis and
mediastral irradiation. After these repeated relapses, he received busulfan/cytarabine as conditioning, then 24.5x10^6 CD34+ cells/kg of G-CSF mobilized PBSC from his HLA matched brother were infused. He maintained 100% donor status by VNT for two months, then relapsed, resulting in a 20% donor/80% blast chimera, with concomitant CNS involvement. He was re-induced with the FLAM salvage regimen (Mitoxantrone - 15 mg/ m^2 x 4, Fludarabine - 30 mg/m^2 x 3, Cytosar - 1 g/m^2 x 3) along with G-CSF mobilized PBSC from the same donor. He remained 100% donor for three months, with molecular assessment done weekly, with bone marrow free of ALL at the time of death from progressive CNS disease. The second patient was a 46 year old woman who was diagnosed with AML-M4. She underwent PBSC while in second relapse 1.5 years after diagnosis. Conditioning was with busulfan/fludarabine/ATG followed by 5.47x10^6 G-CSF mobilized CD34+cells/Kg from an HLA-matched unrelated donor. She became 100% donor, and maintained this state for 10 months, after which she relapsed into a state of 20% donor/80% blasts. At this point she received the FLAM protocol followed by an infusion of G-CSF mobilized PBSC from the same donor. She then became 100% donor by VNTR.

Our experience suggests that the FLAM protocol followed by infusion of stem cells may represent an effective treatment modality for patients who relapse after allogeneic transplantation.

**CD34+/CD41a+, CD34+/AC133+, CD34+/CD33- examination in cryopreserved peripheral stem cells concentrates suitable for improving the recovery prediction of hematopoiesis after transplantation**


**Objectives:** A total target progenitor cell yield from leukapheresis is important for transplantation – the number of infused progenitor cells correlates with the rate of hematopoiesis reconstitution which affects the safety and cost of transplants. Some works suggest that estimation of CD34+ cells infused may not be the best indicator for long term reconstitution, for platelet engraftment especially. We wanted to enable improvement of recovery prediction and examine the CD34+/CD41a+, CD34+/AC133+ and CD34+/CD33- cells in yielded concentrates.

**Methods:** We began the examination in fresh leukapheresed products, however it was technically difficult (necessary examinations in weekend time) and possible only in patients with high concentration of CD34+ cells (about 10% of our patients), because the percentage of CD41a+ to small (about 1 – 5 % of CD34+ fraction). That is why we used the method of CD34+ isolation and examination of CD41a+, AC133+ and CD33- cells in this cell fraction. It was used MiniMACS purification system (Miltenyi Biotech, Germany) and cells were labelled using CD34 MicroBeads. A special puffer was used. The isolated fraction had 70 – 90% of CD34+ cells and the CD41a+, AC133+, CD33- percentage was determined by flow cytometric analysis (Coulter Electronic, Hialeah, FL,USA). We examined 34 cryopreserved stem cell concentrates in 25 patients with malignant lymphomas and myelomas.

**Results:** The estimated percentage of CD34+/CD41a+; 3.21% (range 13.4 – 1.1), CD34+/AC133+; 95.09% (range 98.5 – 80.5), CD34+/CD33-; 3.42 (range 15.2 – 1.07). The method is relatively simple, reliable, not much time consuming. It is necessary to have good experience with the method of cell isolation and flow cytometry. The method is prepared and first results are provided to our colleagues for correlation of transfused cell amount with the rate of hematopoiesis reconstitution (see the further work from our faculty hospital).

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**P523**

**BCR-ABL real-time quantitative RT-PCR and STR hematopoietic chimerism monitoring of tyrosine kinase inhibitor (STI571/Imatinib) treated Ph+ leukemia patients in relapse after hematopoietic stem cell transplantation**


Imatinib (STI571), specific inhibitor of Bcr-Abl tyrosine kinase is currently on clinical trials on interferon resistant/intolerant CML and Ph+ ALL, were molecularly serially investigated (median follow up: 7 mths, range 3-15 mths). In the CML group, 6/9 were in hematologic relapse and chronic phase, 2/9 blastic phase and 1/9 in molecular relapse. In the ALL group, 2/3 were in hematologic relapse and 1/3 was in molecular relapse. Results: Bcr-Abl transcript level decrease was very rapid in most patients. Patients were evaluated each three mths and divided in 4 categories of ALL were molecularly serially investigated (median follow up: 7 mths, range 3-15 mths). In the CML group, 6/9 were in hematologic relapse and chronic phase, 2/9 blastic phase and 1/9 in molecular relapse. In the ALL group, 2/3 were in hematologic relapse and 1/3 was in molecular relapse. A complete response was a Bcr-Abl transcript level below the PCR sensitivity level. A major response was a superior to 1 log (>10x) decrease. A partial response was an inferior to 1 log (<10x) but superior to twice (>2x) decrease. A “no response” was an inferior to 2x increase or decrease. An escape was a superior to 2x increase. After three months of treatment, 5 patients were in complete molecular response, 4 were in major response, 2 in partial response and 1 did not respond. After 6 months, 7 patients were evaluable. 2/7 were in complete response, 3/7 in major response, 1/7 in partial response and 1/3 escaped (ALL). Molecular date were correlated to cytogenetic data. At time of relapse, 7 patients had mixed chimerism or complete host peripheral hematopoiesis. All of them reverted to full donor chimerism or at least to mixed chimerism.

**Conclusion:** Molecular monitoring is a powerful tool to demonstrate imatinib dramatic efficiency in post-SCT relapse of Ph+ leukemia patients.

**P524**

**The value of monitoring CML and ALL patients harboring the bcr-abl translocation by serial real-time quantitative PCR after allogeneic stem cell transplantation**

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**Objectives:** The bcr-abl translocation characterizes the malignant cell clone in almost all cases of chronic myeloid leukemia (CML) and in about 20% of acute lymphoblastic leukemia (ALL). Posttransplant long term surveillance in these patients requires sensitive, reliable and meaningful methods to detect potential relapse early when intervention is most successful. Here we developed a real-time quantitative reverse transcriptase PCR (RQ-PCR) assay based on TaqMan technology to quantify the
bcr-abl translocation and determined its value in the follow-up of CML and ALL patients after transplantation.

Methods: 39 patients with CML in first chronic phase or advanced disease and 2 patients with ALL received allogeneic bone marrow or peripheral blood stem cell transplantation from related or unrelated donors after conditioning therapy with a high dose busulfan-based regimen or a low intensity protocol comprising fludarabine, BCNU, and melphalan. Serial peripheral blood and bone marrow samples taken at various time points after transplantation were analysed by RQ-PCR.

Results: The RQ-PCR assay detected up to 1 bcr-abl positive cell in a background of 10^6 normal cells. It was fast to perform and highly reproducible. Correction for the varying RNA quality encountered in routine clinical samples by normalization with the housekeeping gene GAPDH (bcr-abl/GAPDH ratio) proved mandatory for meaningful quantitative results. In patients eventually relapsing, increasing bcr-abl/GAPDH ratios were observed, which preceded hematologic relapse up to 630 days. Response to treatment (e.g. by donor lymphocyte infusion) could be monitored by decreasing bcr-abl/GAPDH values.

Conclusion: Serial RQ-PCR of bcr-abl should be the preferred method for the surveillance of CML and bcr-abl positive ALL patients after allogeneic transplantation. This method allows decisions based on the amount of bcr-abl transcripts and in particular on their dynamic behavior over time. Serial RQ-PCR provides a sensitive, fast, reproducible and easy-to-perform way for early detection of potential relapse with built-in control for RNA quality, ideal for routine clinical use.

P525

Minimal residual disease (MRD) monitoring with qualitative RT-PCR in chronic myeloid leukemia (CML) patients submitted to allogeneic stem cell transplantation


The t(9;22)(q34;q11) translocation resulting in the Ph chromosome and in the BCR-ABL rearrangement is strictly correlated with a CML diagnosis. The BCR-ABL rearrangement plays a crucial role in CML pathogenesis and its amplification through RT-PCR can be employed to monitor MRD in CML patients (pts) undergoing different therapeutic approaches. We report on 39 CML pts submitted to allogeneic transplantation at our Institution; 36 were in first chronic phase (CP) and 3 in an accelerated phase (AP). Twenty-four pts received stem cells from a sibling donor with a BUSCY conditioning regimen, the remaining 15 received stem cells from an unrelated donor with a CY+TBI regimen. The stem cell source was the marrow (31 cases) and peripheral blood stem cells (PBSC) (5 cases). Thirteen pts received the stem cells from a sex-mismatched donor and chimerism was checked with FISH using sex chromosome alpheid probes. Cut-off values were 0.791% in male recipients of a female donor, 0.477% in female recipient of a male donor. Six months post-transplant 12 pts had a RT-PCR positive assay. Seven of them, having received a sex-mismatched transplant, were also tested for chimerism. Two, both CP, were complete chimeras and 5 (3 CP and 2 AP) were mixed chimeras. Considering these last 5 pts, the 3 CP showed a low recipient cell percentage (0.4-2.7%), while the 2 AP a recipient cell percentage reaching 86%. Basing on RT-PCR data, we decided to suspend Cyclosporin A (CsA). Both the pts experienced acute GVHD that determined a progressive lowering of recipient cell percentage and the achievement of a negative RT-PCR assay. Five pts showed at least one positive RT-PCR 6-12 months post-transplant. Three of them never relapsed, one died with a negative test, the remaining, the one transplanted in AP and with a negative test after CsA withdrawn, experienced an extra-medullary relapse. Twenty-two pts have a follow-up superior to one year: 18 cases had always had negative RT-PCR assay, one had a positive test only once and 3 had at least two positive tests. Two of these last 3 pts still have a positive RT-PCR. Our data suggest that a qualitative RT-PCR positive test within the first 6-12 months post-transplant is unable to predict disease outcome. In our experience relapse occurred only in pts transplanted in AP. Moreover a RT-PCR positive assay more than one year does not always signify impending relapse. In these pts a quantitative RT-PCR analysis is absolutely mandatory.

P526

Molecular methods for detection of minimal residual disease in multiple myeloma: sensitivity and specificity of real-time quantitative and nested PCR

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Multiple myeloma is characterized by malignant plasma cell infiltration of bone marrow. Treatment with high-dose therapy results in a high rate of clinical remissions, but almost all patients ultimately relapse. Clinical staging and detection of relapse is limited in sensitivity. Therefore, we established molecular methods based on the highly clone-specific CDR regions of the immunoglobulin VH focus for sensitive and specific detection of residual myeloma cells after bone marrow transplantation.

Methods: VDJ rearrangements were identified using a set of VH primers and a JH primer. Clone specific rearrangements were detected by comparison with germline sequences. With the nested pcr approach first round amplification with the consensus primers was done followed by second amplification with myeloma specific primers.

The real-time quantitative pcr was performed using a myeloma-specific forward primer in combination with a JH consensus Taqman probe and reverse primer.

Results: Sensitivity was tested using dilutions of myeloma cell-lines into mononuclear cells. Nested PCR had a sensitivity of 10^-6 and Taq-man-pcr of 10^-5. Specificity was determined by testing different cell lines and patients probes. With accurate primer design and high annealing temperatures no unspecific results were observed. These results were confirmed by follow up of three patients after dosis reduced conditioning and allogeneic transplantation. One patient in clinical remission is still positive with nested-pcr, but tumor-load as measured by Taq-man pcr is lower than 0.001% myeloma cells.

Conclusion: Molecular methods are a very sensitive and specific tool for follow up of myeloma patients after allogeneic transplantation. By using the quantitative approach it is possible to see kinetics of bone marrow tumor-load which can be used to guide therapeutic decisions like DLI.

P527

Qualitative and quantitative molecular evaluation of MRD in indolent NHL treated with high-dose therapy and rituximab

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In order to determine if the Rituximab in association with high-dose therapy could modify the molecular status of patients with indolent NHL, qualitative and quantitative sensitive molecular assays (Genescan and Real Time PCR) were performed.

Thirty-two patients (2 chronic lymphocytic leukemias, 4 lymphoplasmocytic, 23 follicular and 3 mantle cell lymphomas) were enrolled in the study and monitored by the IgH, bcl1/JH and bcl2/JH rearrangement. Seventeen patients started CHOP /Rituximab/high-dose therapy as first line strategy and 14 at progression or relapse. Sixteen patients received 2 doses of the anti-CD20 monoclonal antibody before mobilization; 20 patients were transplanted and cases in the Rituximab arm further received 2 antibody doses after the PBSC. 31/32 patients survived with a median follow up of 32 months. The overall response rate was 75%, with higher CR rate (81% versus 69%) and lower progression/relapse rate (19% versus 31%) in the Rituximab arm.

A molecular marker was observed at diagnosis in 66% of patients, without significant differences between the two groups,
but PCR-negativity rate of harvests resulted higher in the R-HD group than in the HD group (87% versus 56%). In the control cohort 5 cases PCR-negative before treatment harvested contaminated cells; no patients in the R-HD arm, negative at diagnosis, harvested PCR-positive precursors. Quantitative assays showed that even PCR-positive harvests had a 1-3 log reduction of MRD, resulting in < 1 neoplastic cell among 1000 cells in 14 tested cases. Interestingly, in the rituximab arm MRD <10-3 was found in 89% of patients versus 43% of those who did not receive anti-CD20 antibody. 20 patients were autotransplanted: 79% of them were PCR-negative. In accordance with Genescan PCR, Real time PCR showed the reduction of contamination in harvests and it predicted the relapse in patients not having reduction of MRD after PBSCT. All positive patients were already PCR-positive at the start of HD therapy and received contaminated precursors. No patients receiving a PCR-negative graft resulted positive after PBSCT; only in the R-HD group 2 patients PCR-positive precociously after transplant achieved the molecular remission after 3 months. So, the present study confirms the important role of the Rituximab in the achievement of either clinical or molecular (qualitative and quantitative) remission in indolent NHL cases.

P528
Prognostic significance of minimal residual disease after hematopoietic stem cell transplantation
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In a single-center study, we investigated the level of minimal residual disease (MRD) in a cohort of 70 children with ALL (n=30), AML (n=27), MDS (n=10), or CML (n=3) after hematopoietic stem cell transplantation (HSCT). Twelve patients underwent autologous HSCT and 58 patients received allogeneic HSCT. The donor was HLA-identical related in 32 patients and HLA-matched unrelated in 26 patients. The Wilms' tumor gene (WT1) expression was used for the detection of MRD because WT1 gene is expressed in the majority of patients with leukemia. In contrast, WT1 gene is not expressed in normal peripheral blood mononuclear cells (PBMCs) and only weakly expressed in normal bone marrow (BM) cells. We performed a quantitative nested reverse transcriptase-polymerase chain reaction to examine the level of WT1 gene expression. In a dilution experiment using 10 fold dilutions of K562 cells in PBMCs of a healthy donor, one leukemic cell could be detected in a background of 100,000 normal PBMCs using WT1 gene expression. For the present study, we followed up MRD in both BM and PB after HSCT. Analysis of paired BM and PB samples revealed that the level of MRD in BM was on average 14 times higher and paralleled that in PB. All 42 patients (60%) with continuous normal WT1 expression levels in BM and continuous undetectable WT1 expression levels in PB remained in complete remission after HSCT. In contrast, all 26 patients (37%) who suffered from hematological relapse presented with high levels of WT1 gene expression in BM and PB (P < 0.001). In 14 patients, we observed a gradual or rapid increase of WT1 expression levels before hematological relapse. The increase of WT1 expression levels occurred at a median of 4 weeks (range 1 week to 4 months) before hematological relapse. In two patients (3%), we diagnosed a molecular relapse using WT1 gene expression. In both patients, molecular remission was achieved by withdrawal of cyclosporine and by donor lymphocyte transplantation, respectively. In conclusion, quantitative analysis of WT1 gene expression is a valuable tool for monitoring of MRD in patients with ALL, AML, CML, and MDS after HSCT. In addition, this approach is very useful for early diagnosis and treatment of molecular relapse after HSCT.

P529
Multicentric analysis of hematopoietic chimerism

Hematopoietic chimerism analysis has been emphasized in order to evaluate donor cell engraftment in allogeneic hematopoietic stem cell transplantation and to adapt immunosuppressive or cell therapy. Full donor or persistent mixed chimerism is essential for sustained engraftment and prevention of relapse. Precise analysis of chimerism, especially in multicentric trials, requires comparison of methodological approaches and data reports. The French working group on hematopoietic chimerism was first composed of 7 laboratories and performed comparison of technical protocols and quality control exchange. In 5 laboratories, chimerism was analyzed by competitive PCR with a panel of STRs on isolated cell populations followed by semi-quantitation using automated DNA sequencer and Genescan software (Applied Biosystems). One laboratory used non radioactive hybridization od PCR amplified VNTRs. Absolute chimerism quantification was performed by real time PCR amplification of sequence specific polymorphisms in 1 laboratory using Taqman probes and ABIprism 7700 Sequence Detection System (Applied Biosystems). Quality control exchange samples were composed of 4 mixes each containing a known ratio of recipient and donor's DNAs. As shown in figure, evaluation of chimerism was concordant between laboratories with a coefficient of variation not exceeding 25%. Our data stress the usefulness of quality control exchanges, and the necessity to pursue our work of protocol standardization including nuclear acid extraction and cell sorting protocols.
CR/CP and 90 in later stages. A majority (86%) of the patients had a HLA-A, -B and -DR matched donor. Conditioning consisted of total body irradiation and cyclophosphamide and GVHD prophylaxis of cyclosporine and methotrexate in a majority of the patients. All patients have an anti-thymocyte globulin as part of pre-transplant conditioning. Stem-cell source were peripheral blood in 46 cases and bone marrow in 139. After transplantation, G-CSF was given to 84% of the patients.

Results: In the multivariate risk-factor analysis for relapse we found that high-risk disease (RR 2.20, CI 1.25-3.90, p=0.006), acute GVHD 0-1 (RR 3.00, CI 1.30-6.96, p=0.012) and ALL diagnosis (RR 1.97, CI 1.16-3.95, p=0.012) were independent risk factors for relapse after HSCT with unrelated donors. The strongest anti-leukemia effect was found in patients with acute GVHD grade II, independent of chronic GVHD. Relapse incidence in patients with acute GVHD grade II were 18% compared to 46% in those with no or grade I (p=0.039). In patients with and without chronic GVHD, the incidences of relapse were 36% and 44% (p=0.03).

Conclusion: Risk factors for relapse after HSCT with unrelated donors were: Acute lymphoid leukemia, disease stage beyond CR1/CP1 and no or mild acute GVHD. The strongest anti-leukemia effect was seen in patients with acute GVHD grade II.

P531
Treatment of malignant diseases by up-regulation of anti-tumor responses and down-regulation of anti-host responses
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Alloreactive donor lymphocytes (T, not excluding NK and NKT cells) represent the major therapeutic component of the bone marrow transplant (BMT) procedure. We hypothesized that improved outcome may be accomplished by reduced intensity conditioning, focusing on induction of host-versus-graft tolerance following non-myeloablative stem cell transplantation (NST), followed by induction of graft-vs-tumor (GVT) effects by donor lymphocytes. Using a murine model of B cell leukemia/lymphoma (BCL1) and metastatic breast cancer (4T1) in BALB/c recipients, we could show that eradication of malignancy can be best accomplished by immune donor lymphocytes (IDL) under conditions where naive lymphocytes are ineffective. Interestingly, IDL resulted in reduced anti-host responses. Whereas survival of BALB/c mice inoculated with BCL1 was ~28 days, survival of mice receiving 2x10^5 IDL immune donor lymphocytes was 95% with only 1/20 mice developing leukemia. Similarly, whereas all recipients of 12 metastatic 4T1 cells injected intravenously or intraperitoneally developed extensive metastases, none of secondary recipients of 2x10^6 IDL showed evidence of pulmonary or hepatic metastases, respectively and all 12 of adoptive recipients of lung cells obtained from treated mice survived >250 days. Clonogenic assays of liver cells from recipients of syngeneic or allologenic lymphocytes confirmed GVT effects. By combining non-myeloablative conditioning for induction of BALB/c vs C57BL/6 transplantation tolerance based on deletion of alloreactive cells with Cytoxan 200mg/Kg and treatment with IDL 90% of mice were cured with no GVHD, as compared with no survival among 12 controls treated with naïve donor lymphocytes. Proof of principle was documented in a patient with relapsed CML resistant to BMT and DLI who responded to IDL against parental alloantigens and alpha-interferon, with continuous molecular remission (>9 years). More recently, we have pioneered in vivo immunization of donor lymphocytes with dendritic cells pulsed with tumor antigens. In conclusion, better and safer immunotherapy of cancer may involve NST and cell therapy with IDL.

P532
Graft versus leukemia effect in pediatric stem cell recipients
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Objectives: In paediatric patients with leukaemia disease treated with stem cell transplantation (SCT), the major cause of treatment failure and death is relapse of the leukaemia. A graft versus leukemia (GVL) effect in humans has been proposed, and acute and chronic graft-versus-host disease (GVHD) have been presented as favourable when evaluating leukaemia-free survival. The aim with the present study was to examine whether a GVL effect also is present in a paediatric setting and to relate it to the occurrence of GVHD.

Methods: A total of 181 children with leukaemia, who had undergone treatment with SCT at Huddinge Hospital, were retrospectively evaluated. Out of these, 112 children had ALL, 57 AML and 12 CML. The median age was 9 years (range: 0.5-17) and the median time of follow up was 7.5 years. The graft was obtained from an unrelated donor in 51 of the cases.

Results: The 5-year probability of survival was 52%, while the 5-year probability of relapse was 41%. Forty of the sixty relapses occurred within the first year post SCT. In a Cox regression model, acute GVHD grade II-IV and chronic GVHD were independent predictors of relapse, with a relative risk of 0.43 (95% CI 0.18-1.00) and 0.39 (95% CI 0.22-0.78), respectively. In terms of death, chronic GVHD remained an independent predictor with a relative risk of 0.42 (95% CI 0.22-0.78).

Figure: Patients with chronicGVHD had a significantly better survival than those without chronic GVHD, p = 0.013.

Conclusion: Both acute GVHD grade II-IV and chronic GVHD were shown to be independently associated with a lower risk of relapse post SCT. Moreover chronic GVHD remained an independent determinant for post SCT survival. This supports the assumptions that there is a GVL effect also in SCT of childhood leukaemia which is related to the occurrence of chronic GVHD.

P533
Discrepancy in response of different metastatic sites after allogeneic peripheral blood stem cell transplantation for renal cell carcinoma
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Background: Graft-versus-tumor effects after allogeneic hematopoietic stem cell transplantation have been shown to be effective in patients with renal cell carcinoma. Patients and methods: In October 2000 a 46-year old man was diagnosed with renal cell carcinoma in the left kidney with metastases in the brain and lung. After tumor-nephrectomy, resection and radiation of the cerebral metastasis the patient was scheduled for an allogeneic hematopoietic stem cell transplantation from his HLA-identical sister. On admission to the transplantation unit in March 2001 he presented with a swollen right palatinal tonsil and a small subcutaneous nodule at the left flank. Pulmonary metastases had increased in size since initial
P534

Use of donor leukocyte infusion (DLI) for the treatment of relapse after related allogeneic myeloablative or nonmyeloablative stem cell transplantations: analysis of toxicity and efficacy

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DLI for relapse after allogeneic stem cell transplantation (allo-SCT) demonstrated its efficacy in terms of eradication of the malignant clone. Non-myeloablative allogeneic stem cell transplantation (allo-NSCT) is a new promising therapeutic approach, although attaining complete chimerism seems more difficult and often requires DLI. We retrospectively analyzed 2 groups of patients: DLI for relapse after conventional allo-SCT (group 1) or DLI in the context of allo-NSCT (group 2). 30 patients were studied in group 1: 19 males, 11 females, median age: 34 years (17-47). There were 14 CML and 16 AL patients. In group 2, 39 patients were analysed: 27 males, 12 females, median age: 42 (18-62), 8 AL, 4 Chronic leukemias, 12 MM and 12 lymphomas. Chimerism were analyzed according to the semi-quantitative VNTR technique. In group 1, we observed 10 cytogenetical responses for CML and 3 CR for AL. Median number of DLI necessary to obtain complete chimerism was 2, and the delay was 3.5 months (0.25-13.2). We observed 5 acute GVHD (1 grade I, 2 grade II and 2 grade IV) and 3 chronic GVHD. SVHD after DLI appeared after median delay of 2.2 months (1.5-6). In group 2, we observed 5 CR (lymphomas and AL) and 3 PR (MM). 10/39 patients could be analysed for GVHD (p=0.7). However, DLI following allo-NSCT converted faster patients to complete chimerism, without significant increase of GVHD incidence (p=0.08).

P535

The importance of patient and donor CMV status in unrelated stem cell transplantation


Patient and donor CMV status prior to unrelated stem cell transplantation are considered to be important factors affecting outcome. It has been reported that a T cell depleted and T replete setting that patient seropositivity is associated with worse overall survival. Reports of the impact of donor CMV status and patient/donor CMV combinations are contradictory and confusing. We have studied the effects of CMV in a cohort of 218 unrelated transplants, where donors were provided by the Anthony Nolan Trust, with well characterized high resolution tissue typing to allele level. Of the donors provided 25% were CMV positive and 27% of the patients in this cohort were CMV positive. In 59% of the transplants both donor and recipient were CMV negative, in 11% both were positive, in 16% the patient was positive and donor negative and in 14% patient was negative and donor positive. We found a significant survival advantage in patients who were CMV negative (p=0.0164), irrespective of the donor CMV status. In multivariate analysis this result remained significant with a hazard ratio of 1.8 (1.1, 2.9; p=0.01). There was no significant difference in the incidence or severity of acute or chronic graft versus host disease (cGVHD) between any of the groups. However patients who were CMV positive had a significantly increased chance of relapse (p=0.0049), once again irrespective of the donor CMV status. In multivariate analysis this result remained significant with an hazard ratio of 2.2 (1.2,4.1; p=0.01). CMV negative patients who had CMV positive donors appeared to be protected from relapse. Donor CMV positivity was associated with a lower incidence of chronic graft versus host disease (p=0.0443). This remained a significant factor in multivariate analysis with a hazard ratio of 0.4 (0.1, 0.9; p=0.02). The presence of chronic graft versus host disease conferred a survival advantage, in this largely T cell depleted cohort of patients, with a hazard ratio of 0.3 (0.17,0.56; p=0.00). Therefore while donor CMV seropositivity protects from relapse, this is balanced by a decrease in cGVHD which would tend to increase relapse; the net effect is no alteration in overall survival. We conclude that in a CMV positive patient, donor CMV status does not alter outcome, however in a CMV negative patient, the consequences of donor status should be considered for the individual patient.

P536

The immunosuppressive impact of idarubicine in the conditioning regimen before partially T-cell depleted allogeneic SCT in patients with chronic myeloid leukemia


Eighty-nine patients (pts) were transplanted for CML-CP1 with T-cell depleted (counterflow centrifugation) stem cell grafts from HLA-identical siblings. Nineteen pts were conditioned with cyclophosphamide (2x 60 mg/kg) and TBI (2x 4.5 Gy) and in 70 pts the conditioning regimen was intensified by the addition of Idarubicine (Ida) 42 mg/m2. The 5-year probability (prob.) of hematological and/or cytogenetic relapse was 70% in the standard conditioned pts. The introduction of Ida in the conditioning regimen resulted in a significantly (p=0.002) lower 5-year prob. of relapse (41%). The addition of Ida correlated with significantly more acute GVHD. (p=0.017). Transplant related mortality was not influenced by Ida. The 5-year prob. of current leukemia-free survival defined as survival in first or second (after therapeutic DLI) remission were identical for both conditioning regimens (64% and 63%, respectively). Ten pts were randomized between the standard or the intensified conditioning. In these 10 pts chimerism was monitored frequently in highly purified subsets of PBMC's and granulocytes using a newly developed real-time quantitative PCR.
of single nucleotide polymorphisms. Pts conditioned with Ida developed significantly earlier (median, 6 versus 12 months) complete donor chimerism in the T-lymphoid (CD3+/CD4+ and CD3+/CD8+) subsets compared to the patients conditioned with the standard regimen (p=0.017). In the intensified conditioned pts this immunosuppressive effect is additionally demonstrated by significantly lower (p=0.028) percentages of autologous T-lymphocytes in the first 6 months after SCT. The addition of Ida in the conditioning had no influence on the time to reach complete donor chimerism in the investigated non-T-lymphoid subsets. We conclude that the addition of Ida to the conditioning regimen in the setting of allogeneic T-cell depleted alloSCT resulted in a rapid clearance of autologous T-lymphocytes that may explain the increased prob. of acute GVHD and the decreased relapse rates. However in pts suffering from CML, the addition of Ida to the conditioning regimen did not influence current leukemia-free survival due to the impact of therapeutic DLI. In pts transplanted for hematological malignancies that do not respond well to DLI for treatment of relapse this observation may have consequences for the conditioning regimens before T-cell depleted SCT.

P537
Incidence and features of large granular lymphocytes (LGL) following allogeneic stem cell transplantation: a long-term analysis
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Large granular lymphocyte (LGL) proliferation follows a chronic course during which major features are cytopenia and immune abnormalities. Persistently elevated numbers of LGL have been shown to be increased in few cases after allogeneic stem cell transplantation (allo-SCT). In the present report, we undertook a retrospective analysis of all LGL cases occurring following allo-SCT in a large cohort of 201 patients. The aim of the study was to determine clinical and biological features associated with LGL expansion following allo-SCT. Six cases could be documented over a long follow-up period of seven years. We demonstrate that LGL expansion occurs more frequently following a reduced preparative regimen (4 cases), as compared to conventional myeloablative regimens (2 cases) (incidence 8.2% vs. 1.3%, P=0.04). Expansion of LGL was seen between 3 and 15 months following allo-SCT. Hematopoiesis, with mild to severe cytopenia, was a privileged target for LGL. Different autoimmune manifestations like polyarthritis and hypergammaglobulinemia were also observed. LGL proliferation was observed in the context of chronic antigenic stimulation associated with recurrent viral infections especially CMV. Moreover, 5 patients out of these 6 high risk patients, achieved a long term complete remission concomitant or following LGL expansion. These data suggest that LGL are a subset with the properties of effector lymphocytes which may represent an important component of the graft versus tumor effect.

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Hematogones in the healthy bone marrow
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Multiparameter flow cytometry may be used to detect minimal residual disease in acute leukemia because leukemic cells often display aberrant phenotypes when compared to normal cells. One limitation of this approach in B-lineage Acute Lymphoblastic Leukemia (B-ALL) is that rare subpopulations of normal marrow B lymphoid cells , expressing immunophenotypes typically found in B-ALL, were sought by multiparameter flow cytometry in the marrow of both children and adults (p=0.017). In adults, after autologous or allogeneic transplantation or conventional chemotherapy for acute leukemia. These cells, in some patients, may account to greater than 50% of bone marrow cells, creating a picture that can be confused with Acute Lymphoblastic Leukemia (ALL) or metastatic tumor. Although originally called hematogones (HG), a variety of other names have been proposed for these unique cells. The clinical significance of expanded HGs has not been resolved, and the biologic features are incompletely described. This study included the immunophenotypic characterization of different precursor and mature B cell populations in the bone marrow samples taken from 15 healthy individuals, who served as bone marrow donors. The samples were analyzed with different antibody combinations using four-colour and CD45 -gating strategies:

CD10FITC/CD34PE/CD45PerCP/CD19APC; D20FITC/CD34PE/CD45PerCP/CD19APC; D22FITC/CD34PE/CD45PerCP/CD19APC; HLA-DR FITC/CD34PE/CD45PerCP/CD19APC; skappa FITC/sLambda PE/CD45PerCP/CD19/APC; cyKappa FITC/cyLambda PE/CD45PerCP/CD19/APC.

In 13/15 samples two immature CD19+ subpopulations with different SSClog/CD45 distribution on the cytogram and co-expressing B cell-associated antigens were identified: CD34+/CD19+/CD22dim+/CD10dim+/HLA-DR+/CD45dim CD19+/CD22dim+/CD10dim+HLA-DR+/CD45dim Representing 0.2% (0.1-0.7) and 0.68% (0.2-1.5) of the total BM nucleated cells, respectively. The patterns of antigen expression were very reproducible in all 13 samples and were not found to be age related. These two populations never merged even with mature CD19+ population, or ALL-B lineage. We conclude that Hematogones aren’t an unusual finding in the healthy bone marrow and their particularly SSClog/CD45 distribution allows the discrimination between normal and malignant precursor B-cells and can therefore be used for MRD studies.

Additional abstracts to this topic

Early immunotherapy to reverse mixed chimerism in children with malignancies after allogeneic stem cell transplantation (alloSCT)
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Mixed chimerism (MC) after alloSCT is associated with high risk of relapse or graft rejection. To prevent these life-threatening complications immunotherapy was initiated in patients with autologous cells detected after transplantation.

Patients: Immunotherapy was started in 12 children with malignant diseases (ALL 7, AML 4, CGL 1) who developed MC after alloSCT. Eight children received hematopoietic cells from HLA id siblings (MSD) and 4 from mismatched relatives (MFD). Busulfan-based conditioning regimens were used. GvHD prophylaxis consisted of CsA +/- MP (MFD), T-cell depleton only (MFD) or CsA+MTX+MP for a patient transplanted from MFD with unmanipulated graft.

Methods: Hematopoietic chimerism was monitored in PB MNCs and BM by semiquantitative fluorescent VNTR-PCR assay. MNCs were isolated by density gradient centrifugation. In 7 patients on CsA prophylaxis, when stable or increasing MC was detected in 2 consecutive assessments with no signs of GvHD, CsA was withdrawn. If no GvHD appeared and no decrease in autologous DNA was detected after CsA had been tapered, patients were scheduled for DLI. Patients with MC after T cell depleted transplants received T-cell addbacks as front-line therapy. In one patient (UPN 223) after alloSCT from MSD on CsA+MP prophylaxis, steroids were tapered and CsA level decreased.

Results: Patient UPN 223 responded to withdrawal of steroids and converted to complete chimera (CC) with no signs of GvHD. Four patients responded to cessation of CsA and converted to CC. Two of them developed grade II and grade III aGvHD, which responded to reintroduction of CsA. One experienced CNS relapse while being CC in both BM and PB. Among 3 patients who
failed to respond to CsA withdrawal, 2 subsequently relapsed. One refused further treatment and died in relapse. One received chemotherapy followed by DLI with transient response and died due to disease progression. One patient received DLI and gradually eliminated autologous cells, remains in CR with no symptoms of GVHD. Three patients developed increasing MC after transplantation of TCD graft. Two did not respond to T-cell add-backs and finally rejected the graft. One rapidly eliminated autologous cells, converted to CC and developed grade III aGVHD with good response to steroids.

Conclusion: Our limited data suggest that chimerism-guided immunotherapy may eradicate residual host cells. Further studies are required to prove clinical efficacy of this therapeutic approach.

Role of chimerism in prevention and/or treatment of post-transplant leukemia relapse in children

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We present the role of hematopoietic chimerism (HC) in prediction of post-transplant outcome and our experience with adoptive immunotherapy (AI) in prevention and treatment of leukemia relapse after HSCT.

Methods: Between 1/1997 and 12/2000 43 patients (ALL 16, AML 15, CML 5, MDS 6, JMML 3, CMML 1) underwent 46 unmanipulated allogeneic HSCT from identical siblings (23) or unrelated donors (23). We have analyzed chimerism in PB samples using PCR of VNTR in 44 evaluable follow-ups. AI was used in 11 pts (ALL 3, AML 5, CML 2, JMML 1/2x). The treatment was initiated on the basis of increased mixed chimerism (inMC,?), in molecular relapse (or) in hematological relapse (4). Withdrawal of immunosuppression was performed in 9 patients, 5 of them received also donor lymphocyte infusion (DLI) and in 3 DLI was applied as a front-line therapy. AI was combined with chemotherapy in 2 children. CD3+ doses varied between 1x10^5 and 2.4x10^8/kg bw according to type of donor and indication.

Results: Complete donor chimerism (CC) after day+28 was documented in 25/44 follow-ups. Mixed chimerism (MC) after D+28 was found in 19/44 follow-ups. Transient MC(trMC) was detected in 7; inMC in 12 follow-ups. Transplant related mortality day+100 was 7/25 in CC and 1/19 in MC group; 36 patients were evaluable for relapse free survival (RFS). At a median follow-up of 19 months (2-51 months) RFS for CC group was 17/18, MC group was 7/18. Hematological relapse was documented in 1/7 children with trMC, in 1 patient molecular relapse was detected and AI was started before the appearance of trMC. InMC was followed by hematological relapse in 9/12 follow-ups. 3/12 patients with inMC responded to AI and re-achieved CC. Complete response to AI was defined as sustained recurrence of CC and CCR was documented in 4/12 children (1 AML, 2 CML, 1 JMML) at a median follow-up of 30 months (15-40 months), 1 patient (ALL) achieved CC but died due to severe GVHD. 3 patients (2 AML, 1 JMML) showed transient decrease or disappearance of MC and developed relapse subsequently 8, 9 and 20 months after intervention. No response was seen in 4/12 follow-ups (2 ALL, 2 AML).

Conclusions: Frequent monitoring of HC allows identification of patients with high risk for hematological more than extramedullary relapse and therefore indicated for AI. In some children with leukemia methods of AI allow to prevent or treat posttransplant relapse. This work was supported by grants IGA-MZ ER NC 6512-3 and IGA-MZ ER NC 5902-3.

Three different methods for detection of minimal residual disease in neuroblastoma

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Aim: Detection of neuroblastoma cells in bone marrow and circulating neuroblasts during treatment may predict clinical outcome and correlate with tumor recurrence. The aim of study was to confirm usability of methods for detection of minimal residual disease (MRD). We evaluated methods per these criteria: sensitivity, speed and cost.

Methods: The methods for metastasis detection in neuroblastoma include reverse transcription-polymerase chain reaction (RT-PCR), fluorescent in situ hybridization (FISH), and flow cytometry (FC). RT-PCR detects tyrosine hydroxylase (TH) mRNA, FISH amplification of N-myc oncogene (amplified in approximately 30% neuroblastoma with poor prognosis). Cytometric detection of antigens CD45-, CD81+ and CD 56+.

Results: Four neuroblastoma cell lines from advanced neuroblastoma with amplification of N-myc oncogene were investigated. We confirmed sensitivity RT-PCR 10(-4-5), FISH 10(-3-4), Flow cytometry 10(-2-3). Least time spend FC (1-2h), FISH 2 days and RT-PCR 2-3 days. Flow cytometry is cheaper, FISH and RT-PCR are more expensive. TH mRNA was detected by RT-PCR in 30 bone marrow from 14 patients; eight samples from 5 patient were positive. Five PBSC from 2 patient were without tumor cells. Thirty-eight bone marrow samples from 25 patients were examined by FC using CD81+/CD56+/CD45-MoAbs; three samples from 3 patients were positive. Seventy percent (35/51) patients with N-myc amplification had positive bone marrow using FISH technique.

Conclusions: Combining multiple molecular markers and independent screening techniques, we may be able to overcome tumor heterogeneity and expedite the detection of microscopic disease in the model of MR of neuroblastoma.

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Recommendations for quantitation of minimal residual disease in multiple myeloma

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Objective: Changes in the level of minimal residual disease (MRD) in patients with multiple myeloma (MM) might be predictive of relapse. However, the level of MRD in MM may span 6 logs, and methods with high sensitivity and reproducibility over a broad dynamic range is thus necessary for quantitation of MRD in MM. We have tested flow cytometry, patient-specific real-time PCR and patient-specific nested RT-PCR combined with limiting dilution statistics for quantitation of MRD in MM patients.

Materials and Methods: Nine patients were included in the study. All patients received an induction program of three cycles of VAD (vincristine, doxorubicin and dexamethasone), followed by high-dose cyclophosphamide (4g/m2) and granulocyte colony-stimulating factor (G-CSF) followed by peripheral blood stem cell (PBSC) harvest and finally high-dose therapy with melphalan (200 mg/m2), followed by PBSC transplantation of a minimum of 2 x 108 CD34+ cells/kg total weight. The flow cytometric method used CD56, CD28 and CD117 as “tumor specific antigens” whereas the PCR based methods used allele-specific oligonucleotides (ASO) corresponding to the complementary determining region 3 (CDR3) of the rearranged immunoglobulin heavy chain gene (IgH).

Results: The sensitivity was 1:104, 1:105, 1:106 for flow cytometry, patient-specific real-time PCR and patient-specific nested RT-PCR, respectively. The tumor burden determined in BM by the three methods was in relatively good agreement with values obtained by microscopic examination and the measured serum M-protein. Consistently low levels of clonal cells were seen in patients considered in complete remission and increased levels were seen at relapse in both BM and PB. BM and PB samples were tested positive at all time-points using PCR based methods whereas 1/3 of blood samples were negative during treatment when measured by flow cytometry.

Conclusions: This study shows that different quantitative methods can monitor MRD in patients considered to be in complete remission and offers an opportunity to enhance our understanding with regard to the clinical relevance of residual malignant cells. PCR based methods have high sensitivity but are time-consuming and applicable to only 70% of patients. Flow cytometry has a lower sensitivity but allows a fast evaluation of all patients.
Establishment of stable continuous molecular remission by immunotherapy in combination with STI-571 in a patient with molecular relapse of BCR/ABL positive ALL after allogeneic stem cell transplantation


We report on a 39 year old male patient diagnosed with bcr/abl-positive ALL. After induction therapy according to the Hölzler-protocol he achieved a complete remission but experienced relapse 3 months later and received an allograft from his HLA-identical sibling without reinduction chemotherapy. Conditioning therapy consisted of cyclophosphamide (120 mg/kg b.w.) and fractionated total body irradiation of 13.2 Gy. After infusion of 7.9 x 10^6 CD34+ cells obtained from the peripheral blood from his HLA identical brother he was treated with standard immunosuppressive therapy (cycosporine A and methotrexate). Posttransplant course was uneventful and one month after allogeneic SCT both STI and DLI may result in improved outcome donor cells is observed between 6 and 32 weeks. Thus the interval between molecular and haematological relapse in patients with STI-571 may result in rapid and specific suppression of the leukemic clone. Treatment with STI-571 may result in rapid and specific suppression of the bcr/abl positive clone. The antileukemic effect of alloreactive donor cells is observed between 6 and 32 weeks. Thus the combination of both STI and DLI may result in improved outcome of patients relapsing with bcr/abl positive ALL.

10. Infections Viral

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Virus surveillance in pediatric HSCT patients: single center experience

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Intensive PCR based viral screening was performed during the course of allogeneic transplants in 53 children with the following characteristics. Donor: MRD 22, MMRD 6, MUD 25. Diagnosis: leukemias 35, inborn errors 16, SAA 2. Age: recipient 5,5 (1 – 19) years, donor 15,7 (1-63) years. CMV-seropos.: recipients 63 %, donors 40 %. EBV seropos.: recipients 74 %, donors 88 %. HHV-6 seropos.: recipients 55 %, donors 44 %. Adenovirus seropos.: recipient 92 %, donors 63 %.

Results: During the early post transplant course 34 % of the patients became positive for CMV, 14 % for EBV, 13 % for HHV-6, and 36 % for adenovirus. There were only 22 % of patients without proof of any virus. 48 % of patients showed at least 1 virus, 13 % 2 viruses, and 15 % 3 and more viruses. In multiple viral infections treatment related mortality went up from 10 % to 22 %, aGVHD II from 18 % to 44 %, aGVHD III-IV from 0 % to 22 %, and severe chronic GVHD from 0 % to 56 %. The most often detected virus was the adenovirus which was highly associated with critical morbidity in 22 % of the positive patients. Comparing such adenovirus positive with negative transplant recipients severe aGVHD III-IV occurred in 21 % compared to 3 %, severe cGVHD in 32 % compared to 6 %, severe intestinal involvement in 37 % compared to 6 %, and severe liver involvement in 32 % compared to 3 %. The virus was most often detected in stool followed by urine and nasal wash/BAL. All serotypes could be demonstrated. Patients receiving ATG were at higher risk than patients without ATG.

Conclusions: Treatment related mortality and morbidity in allogeneic transplants is highly associated with the detection of multiple viruses. Adenovirus infections/ reactivations belong to the most critical events during such transplants. Pre-emptive treatment strategies have to consider the critical virus spectrum and multiplicity, and the ideal therapy to control all of these viruses at the same time without major side effects still have to be demonstrated.

P541

The role of polyomavirus in the development of post-transplant haemorrhagic cystitis

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Polyomavirus (PV) and, particularly, BKV has been associated with the development of haemorrhagic cystitis (HC) in transplanted patients. It is known that PV infection is common and most frequently assymptomatic in humans and that PV can remain in latent status in the renal tissue until they become reactivated. Objectives of this study were to know the frequency of PV found in urine of patients receiving an HCT before and after transplant, the correlation with the development of HC and the possible predictive value of a PCR qualitative technique of detection. Patients and methods: A total of 59 children (ages between 3 months and 16 years, median 7 years) undergoing an HCT (allogeneic: 41, autologous: 18) were included in the study over a 18 month period (April 2000-September 2001). Urine samples were taken pre-transplant and at 15, 30 and 90 days post-transplant. The method for detection of BKV and JCV consisted on a multiplex nested PCR after DNA viral extraction with guanidine thiocyanate.

Results: Prevalence of urinary excretion of PV before transplant was 45% (BKV: 34%, JCV: 11%, both: 2%). After HCT, 80% of patients excreted PV (BKV: 72% and JCV:8%). Eleven patients (18,6%) (10 after allo-HCT and 1 after auto-HCT) developed an HC: grade I:1, grade II: 8 and grade III: 2. The median time to HC from HCT was 31 days (20-66).Fifty percent of the patients with HC excreted BKV before transplant and all of them become excretors after transplant. Duration of HC ranged from 8 to 90 days (median 31 days). All the patients were treated with hyperhydration, urinary alkalinization and diuretics; two patients suffered severe obstruction and required vesical lavages. Antibival agents were not given in any case. Eigth of the eleven patients with HC had a favourable outcome; the other 3 died with HC but this was not considered the main cause of death.

Conclusions: 1/ A reactivation of BKV infection after transplant was seen; the proportion of patients excreting virus increased from 34% to 72%. 2/ All patients with HC excreted BKV. 3/ The qualitative PCR method of detection of BKV was not valid for predicting the development of HC. A quantitative PCR should be used in future studies.

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Active parvovirus B19 infection in hematologic patients

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Objective: To study the incidence of active parvovirus B19 infection in hematologic patients, all patients admitted to a hematologic ward were screened on a regular basis by PCR for a time period of 6 months. Methods: 121 samples were drawn from 56 patients, a median of 2 (1-7) per patient. Patient characteristics

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Establishment of stable continuous molecular remission by immunotherapy in combination with STI-571 in a patient with molecular relapse of BCR/ABL positive ALL after allogeneic stem cell transplantation

were as follows: 56 patients, mean age 44 years (18-69), 22 (39%) women, 34 (61%) men. Diagnosis: 14 NHL, 10 ALL, 9 CML, 8 AML/MDS, 7 multiple myeloma, 4 Hodgkin, 4 other. Patients were heavily pretreated for a mean of 21 (0-148) months and had undergone a median of 5 (0-13) treatment cycles. Of the 56 patients, 19 patients had undergone autologous stem cell transplantation, 26 patients were transplanted allogeneic. Of these allogeneic transplanted patients, two had been transplanted for the third time, one patient was transplanted for the second time. 29 patients (52%) had additional immunosuppression with CSA/FK506, prednisolon or both, 26 patients received immunomodulatory treatment on a regular basis. 

Result: One out of the 56 patients, was tested positive (1,8%). This patient was a 47 year old woman suffering from multiple myeloma for more than 14 months. She was intensively pretreated, including an autologous and an allogeneic blood stem cell transplantation. At the time of the positive test, day +79 post PBSCT, cyclosporine A and prednisolone was administered. The patient developed an acute febrile monoarthritis of the right knee. Joint puncture showed few granulocytes, microbiologic diagnostic was negative. Under antibiotic treatment the symptoms improved rapidly. Anemia was moderate and most likely attributable to post transplant bone marrow insufficiency. 63 days later the PCR-test still was positive after hybridisation so that the clinical symptoms most likely were not caused by parvovirus B19. 

Conclusion: Incidence of active parvovirus B19 infection in heavily pretreatd hematologic patients was low (1,8%) and did not contribute to morbidity and mortality.

Lethal adenovirus reactivation complicating immunoablative conditioning in pediatric haplo-identical peripheral blood stem cell transplantation (haplo-PBSCT).

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Objective: Haplo-PBSCT for children with high risk haematological malignancy is feasible. Transplant across major histocompatibility antigens is achieved by CD34+ stem cell selection, with sufficient reduction in donor T-cells to reduce fatal GVH reactivity. Graft failure or rejecion may be overcome by infusing more than 20x10.6/kg recipient weight. In our patients with graft failure or early rejection after haplo-PBSCT, further transplantation with non-myoeloblative conditioning was performed immediately after the first attempt.

Methods: Out of 14 consecutive unselected haplo-PBSCTs, four were second attempts. 3 children underwent two haplo-PBSCTs, 1 child two haplo PBSCTs with paternal donor and two with a concomitant transplant from alternate parents. The fourth received paternal stem cells after rejecting a matched unrelated donor bone marrow. All four received different immunomodulatory conditioning prior to the rescue attempt. In 3/4 fluodarabine 30 mg/m2/day x 5 was included.

Results: Prior to the start of the second conditioning all four index cases were adenovirus negative by culture of throat, faeces and retrospective study using real time PCR analyses of stored sequential serum samples. Despite successful engraftment documented by 100% donor chimerism of peripheral lymphocytes all patients died due to infection in the immediate second post-transplant period (+38, 64, 84, 119 days). 3 children died from disseminated adenovirus infection (serum PCR- with one/more culture(s) positive), despite initiation of antiviral therapy. 1/4 died from Klebsiella pneumonia with concurrent CMV infection and localised colonisation by adenovirus. Serum was negative for adenovirus by PCR.

Conclusion:
1. Immunoablative conditioning therapy allows for successful engraftment in children in whom previous transplant has failed.
2. However the associated severe immune suppression led to a 0% survival rate in our group, owing to overwhelming infection in the post-engraftment period, not seen in our survivors of haplo-PBSCT following myelo-ablative conditioning therapy.

3. Adenovirus infection is problematic and warrants further investigation as to whether regular surveillance or preemptive antiviral therapy alters outcome in these potentially salvageable children.
(25mg/Kg twice a day) or combined. Intravenous immunoglobulin was added for all cases of measles. Duration of treatment with Ribavirin ranged from 4 to 45 days.

Eight of the cases followed stem cell transplantation, including two autologous. Mortality was higher in the transplant group compared to the chemotherapy patients. Four of the cases were transplants performed for Hurler's disease and resulted in two deaths. Lymphopenia was another identified risk factor for a worse outcome with no deaths seen in non-lymphopenic children.

We conclude that the isolation of sporadic non-herpetic respiratory viral infections, especially RSV, appears to be increasing. Despite a drop in hand/wash practices following a reduction in immunisation uptake we have not yet seen a case of measles since 1995. Stem cell transplantation, lymphopenia, adenovirus and Hurler's disease were associated with a worse outcome following infection. Ribavirin appears to have reduced the expected mortality of RSV pneumonia.

P546
Hepatitis B virus (HBV) vaccination in patients undergoing hematopoietic transplantation (HT)
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Background: Patients undergoing haematopoietic transplantation (HT) are immunosuppressed for a variable period of time, ranging from months to several years after HT. For different reasons, such patients are at risk from HBV infection, occasionally leading to a fulminating hepatitis. The ideal timing for the successful active immunization against HBV is not well known and it probably varies between the different types of patients and HT.

Patients and methods: Since March’00, thirty-nine patients who underwent allogeneic HT (25%) and twelve autologous HT (63%) showed Ab levels higher than 100 mUI/ml. Among the fifteen allogeneic HT who did not get 100 mUI/ml, fourteen had chronic GVHD and two of the autologous and none of the allogeneic HT got those levels after the two additional doses of vaccine. Five allogeneic HT (25%) and twelve autologous HT (63%) showed Ab levels higher than 100 mUI/ml. Among the fifteen allogeneic HT who did not get 100 mUI/ml, fourteen had chronic GVHD and thirteen were on immunosuppressive therapy. Vaccination response was not influenced by any of the other factors analysed. Conclusions: Patients undergoing autologous HT show acceptable response rate to HBV vaccination administered after six months post-transplant. On the other hand, patients undergoing allogeneic HT with chronic GVHD on immunosuppressive therapy show a very poor response to vaccination, in spite of being started after twelve months post-transplant.

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Occurrence of CMV infection and disease - a comparison of recipients undergoing related donor and unrelated bone marrow transplantation
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Cytomegalovirus (CMV) infection is common life-threatening complication in recipients undergoing allogeneic bone marrow transplantation (BMT). We evaluated the occurrence of CMV infection and disease, and its frequency after related and unrelated BMT (UBMT). Patients and Methods: 111 patients after allogeneic BMT (74 related, 37 unrelated), transplanted between XI/1996 – VIII/2001, were tested. Methods: nested-PCR (MIE-gene) and pp65 antigenemia. Active CMV infection was defined: antigenemia > or = 5 positive cells/100 000 cells or > two consecutive positive PCR. Proven active CMV infection was treated pre-emptively with ganciclovir or with foscarnet. Definition of CMV syndrome and disease: Ljungman P., Plotkin S. 1995. Results: 40 patients were examined by both methods, 71 only PCR. At least one positive test occurred in 70 (63.1%) recipients. The first positive test appeared on median day +46 (6-321 days after BMT), before day +100 in 63 patients (90%), after day +100 in 7 (10%). Overall active CMV infection was proven in 45%; syndrome in 28.8%; CMV interstitial pneumonia (CMV-IP) in 7 (6.3%) cases, IP was diagnosed on median day +86 (57-281 days after BMT), 4 people died (= 57% mortality of CMV-IP). After related BMT median day of 1st positive test was +52, CMV infection was proven in 35.1%, syndrome in 21.6%, CMV in 2 (2.7%) recipients, one patient died. After UBMT median day of 1st positive test was +40; CMV infection had 64.9%, CMV syndrome 43.2%, CMV-IP 5 (13.5%) patients (one case of two episodes of CMV-IP), three of them died. Conclusions: Risk of CMV infection and disease is relatively high in both of the two groups of recipients, in UBMT group was found significantly higher occurrence of CMV infection (p< 0.05), syndrome (p< 0.05), and pneumonia (p< 0.05). Appearance of 1st positive test was found nearly 2 weeks earlier in UBMT group. We concluded CMV infection should be periodically monitored, because of its high incidence it is necessary to use at least 2 methods for CMV detection.

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Presence of CMV DNA in blood is a predictor of shorter survival following both allogeneic bone marrow and peripheral stem cell transplantation

One of the major infectious complications observed following stem cell transplantation is CMV. CMV viremia can be determined with viral culture, CMV serology or CMV DNA assays. CMV viremia may not always cause infection. Factors that may delay immune reconstitution may also increase the incidence of CMV viremia. To investigate this association we analysed our transplant data retrospectively, CMV DNA was detected by hybrid capture assay (Digene Hybrid Capture System,USA), sensitivity of the assay was 2 pg/ml. Values above 15 pg/ml were accepted as positive. Since March 2000, 58 patients with sequential CMV DNA follow-up were included in this analysis. Median follow up was 16.5 mos after PBSCT and 52% following BMT (p<0.05). In the case of CMV DNA > 15 pg/ml patients received preemptive or therapeutic ganciclovir. Patients without CMV viremia had better overall survival at 2 years ( 96% vs 78%, p=0.03). Presence of CMV DNA was an independent factor on survival. CMV DNA was detected
more frequently during day 0 – 100. Following both BMT and PBSCT, CMV was more frequent during AGVHD as shown below. In conclusion stem cell source did not determine the incidence of CMV follow up duration, CMV was observed more frequently during AGVHD and thus in early post transplant period. The follow up is too short to comment on the role of CGVHD. However at least one event of CMV antigenemia was associated with shorter overall survival (19.4 vs 30.9 mo).

In conclusion stem cell source did not determine the incidence of CMV infection or disease was 56% compared to 41% in the total material. 72% of the patients with CMV disease died, whereas the mortality in the group with CMV infection was 48%.

Conclusion: In this retrospective single centre study, CMV infection or disease was found in only 66 patients (22%); of whom 71% were diagnosed as having CMV infection whereas in 29% CMV disease was identified. 56% of the patients with CMV infection or disease died (versus 41% in the total material).

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From 1985 to 010401 285 patients were treated with allogeneic stem cell transplantation for malignant disease/severe aplastic anemia. We have examined the incidence of CMV infection and disease. Criterion for the diagnosis CMV infection was identification of the matrix protein CMV pp65, that subsequently led to anti-viral treatment(252 evaluable patients after 1992; from 1985-91 33 patients were examined by CMV culturing and analysis of IgG/IgM in serum; none of these patients were however positive). For the diagnosis CMV disease the criteria according to the "Fourth International Cytomegalovirus Workshop, Paris, 1993" were used. 86% of the patients were conditioned with Bu4Cy2. Graft versus host disease (GVHD) prophylaxis was cyclosporine A with conventional short course of i.v. methotrexate. 96 patients (34%) were transplanted with an unrelated donor, 92(32%) developed GVHD grade II and needed immunosuppressive treatment with steroids and/or T cell antibodies.

Results: CMV infection or disease was detected in a total of 62 patients (22% of the total material). CMV infection was diagnosed in 44 patients (15%), whereas CMV disease was identified in 18 patients (6%). Of the patients transplanted with a family donor, CMV infection or disease was detected in 17%, compared to 31% in those receiving stem cells from an unrelated donor. 50% of the patients with CMV infection or disease had GVHD grade II compared to 52% in the total material.

5 patients were diagnosed as having primary CMV infection or disease; 3 of these were transplanted with a CMV positive donor, whereas 2 were transplanted with CMV negative donors. Only in 2 patients CMV pp65 was detected in the neutropenic phase (granulocytes <0.5x10^9/l). In 4 patients (6%) CMV infection or disease was detected between day 1-29; in 43 patients (69%) between day 30-59; in 13 patients (21%) between day 60-99 and in one patient CMV was detected after day 100. One patient was diagnosed post mortem. Mortality among patients with CMV infection or disease was 56% compared to 41% in the total material. 72% of the patients with CMV disease died, whereas the mortality in the group with CMV infection was 48%.

Conclusion: In this retrospective single centre study, CMV infection or disease was found in only 66 patients (22%); of whom 71% were diagnosed as having CMV infection whereas in 29% CMV disease was identified. 56% of the patients with CMV infection or disease died (versus 41% in the total material).

P550
Predictors of CMV reactivation following allogeneic hematopoietic stem cell transplant (AHSCIT) for hematological malignancies
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Amongst 537 patients treated with AHSCIT over a period of 1/90 to 5/01 CMV status on both donorrecipient pairs was available on 383 patients (M: 231, F: 152; median age 30 yr, range:1-56 yr.). Transplants were done for ac. leukemia (n=230), chr. leukemia (n=71) or other diseases (n=82) and 165 patients had good risk disease while 218 had bad risk disease. Conditioning therapy was with (n=310) or without TBI (n=73) and cyclophosphamide alone (n=45) or with short course MTX (n=338) was used as GVHD prophylaxis. Donor was sibling (n=104), or non related family member (n=10). Source of stem cells was marrow (283) or PBSC (100). Amongst recipients 181(47.3%) and amongst donors 185 (48.3%) were CMV serum IgG positive. Patients were regularly monitored for CMV reactivation by performing weekly test for early antigen or buffy coats. CMV reactivation was defined as detection of early antigen or isolation of virus from body fluids. 96 patients had evidence of CMV reactivation (25.1%) at a median of 50d (range: 8-415d). The 3yr. probability of developing CMV reactivation post-transplant was 35%. CMV reactivation was more common in patients >30 yr. (33% vs. 17%, p=0.001), CMV positive recipients (43% vs. 9%, p=0.001) or donor (33% vs. 16%), CyA+Mtx prophylaxis (28% vs. 7%, p=0.002) and chronic GVHD (41% vs. 19%, p<0.0001). Reactivation was least common in donorpatient recipients where both were CMV negative (2.3% vs. 37% when at least 1 was positive, p<0.0001). In multi-variate analysis CMV seropositive recipient (RR: 5.9 95% CI: 3.5-10, p<0.0001), age above 30 yr. (RR:1.6 95% CI:1.02-2.4, p=0.042) and CyA+Mtx prophylaxis (RR: 4.1 95% CI:1.3-13.1, p=0.017) were independently associated with increased risk of CMV reactivation. Since May 1992 all patients with evidence of CMV reactivation were pre-emptively treated with 3 week course of Ganciclovir or Foscarnet. Of the 96 patients who had CMV reactivation 4 (4.2%) developed fatal CMV infection. In conclusion, even though the incidence of CMV reactivation is high risk of CMV infection seems to be very low, probably because of early pre-emptive therapy. It will be worth evaluating if universal CMV prophylaxis and pre-emptive therapy after CMV reactivation are equally efficacious in this group of patients.

P551
Anti-cmv prophylaxis after transplant significantly reduces the risk of disease in thalassemic patients
Cytomegalovirus (CMV) infection is a relevant cause of morbidity and mortality in patients undergoing allogeneic BMT. Many strategies have been tried for the treatment of CMV infection/disease and to understand the beneficial effect of a prophylactic Ganciclovir treatment. As the viral load seems to correlate with the risk of CMV disease, we used the Antigenemia test (pp65 protein detection on leukocytes) for early diagnosis of CMV infection and treatment. We thus studied 61 thalassemic patients (mean age 9.7 years, range 1-27) submitted to bone marrow transplantation from December 1998 to July 2001 (57
patients received HLA identical family donor, 2 haploidentical donor and 2 unrelated high risk patients included class 3 patients, adult patients, unrelated and mis-matched donor patients, patients with acute and chronic GVHD, patients undergoing a second transplant. After transplant every patient performed CMV Ag screening test twice a week from day + 14 until the out patient discharge. Patients in the high risk group received prophylactic Ganciclovir at the dose of 5mg/Kg/3days a week from day + 30 to day + 90. Fourteen (14) high risk patients received CMV prophylaxis, 47 standard risk patients did not. Thirty (30) patients (49%) never presented CMV positive Antigenemia; 31 (51%) did. CMV infection occurred in 4/14 (28%) pts receiving prophylaxis and in 27/47 (57%) of patients without prophylaxis (P = 0.05). CMV disease was documented in 4 patients (1 pancytopenia, 2 Interstitial Pneumonia, 1 GI tract + IP). All 4 patients were in the “no-prophylaxis” group and presented recurrent CMV infections. Only 1 patient died by CMV disease.

Marrow toxicity (neutropenia) occurred in 50% of pts receiving prophylactic Ganciclovir but resolved with dose modification and GCSF use. In comparison 20% of patients without prophylaxis required GCSF for neutropenia. We conclude that Ganciclovir prophylaxis was effective in preventing CMV infection in a group of high risk patients with acceptable marrow toxicity and should be considered as a mandatory prophylaxis for thalassemic patients submitted to allogeneic BMT.

P552
Dose-adjusted pre-emptive therapy for cytomegalovirus disease based on real-time polymerase chain reaction after allogeneic hematopoietic stem cell transplantation
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Cytomegalovirus (CMV) disease still remains as one of the serious infectious complications following allogeneic hematopoietic stem cell transplantation. Preemptive treatment for CMV infection guided by either CMV antigenemia assay or polymerase chain reaction (PCR) has shown to be effective to prevent CMV disease by several investigators. However, the appropriate method to monitor CMV infection and the dosage of anti-CMV agents have not been fully elucidated. We have prospectively evaluated the efficacy of real-time PCR-guided preemptive therapy for CMV diseases in allogeneic hematopoietic stem cell transplant recipients with grades II-IV acute GVHD. The dose of ganciclovir was adjusted according to the viral load determined by real-time PCR. On detecting CMV reactivation in the plasma, ganciclovir was initiated at a dose of 5mg/kg body weight once daily, and the dose was increased to twice daily if viral load continued to increase after initiating ganciclovir. In 39 evaluable patients, CMV reactivation assessed by real-time PCR became positive in 30 (77%). One developed CMV gastroenteritis before positive PCR result. Thus the remaining 29 patients were treated preemptively with ganciclovir. The dose of ganciclovir was increased in 12 patients (41%) of preemptively treated patients for increasing viral load. CMV diseases were diagnosed in two patients (one gastroenteritis and one retinitis), and late CMV disease was diagnosed in one patient (gastritis). The treatment was generally well-tolerated, but three patients (10%) developed neutropenia (neutrophil count less than 1000/mcl). In conclusion, real-time PCR-guided preemptive therapy with decreased dose of ganciclovir is feasible, which does not increase the frequency of CMV diseases if the dose is adjusted according to the viral load.

P553
Delayed Cytomegalovirus Reactivation following Allogeneic Transplantation when using Polymerase Chain Reaction Surveillance and Pre-emptive Antiviral Therapy

We determined the frequency of delayed cytomegalovirus (CMV) reactivation when using polymerase chain reaction (PCR) surveillance and pre-emptive antiviral therapy in allogeneic transplant recipients in our unit. We studied 127 patients who had survived more than 100 days after stem cell transplantation. All patients at risk of CMV reactivation received high dose aciclovir prophylaxis. CMV reactivation was defined as two consecutive CMV DNA positive results by PCR from peripheral blood. A total of 50% experienced at least 1 CMV reactivation episode. Among high risk patients, defined as a CMV seropositive donor and/or recipient (93 patients), the incidence was 54%. The median time to the first reactivation episode was 40 days, with a range of 3 to 206 days. Within the high risk group, 17 patients (18%) experienced a CMV reactivation episode after day 100 post allogeneic transplantation. Twelve (70%) of these patients had experienced at least one previous CMV reactivation prior to 100 days.

Delayed CMV reactivation can occur following allogeneic transplantation when using a strategy of CMV PCR surveillance and pre-emptive antiviral therapy, and it is therefore our practice to continue monitoring beyond 100 days.

P554
Optimization of antigen presentation by CMV antigen-pulsed dendritic cells
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Our in vitro co-culture system uses CMV antigen-pulsed DC generated from CMV-seropositive donor blood to present antigen to autologous PBL, which results in proliferation of CMV-specific CTL and T helper cells over a period of 2-3 weeks. The T lymphocytes generated can then be given to stem cell recipients in an attempt to prevent post-transplant CMV disease and thus avoid antiviral chemotherapy.

To optimise antigen presentation, immature monocyte-derived DC were exposed to different maturation cytokines or cytokine combinations previously published, to explore their effect on CMV antigen presentation in this system. The DC were pulsed with CMV antigen or control antigen one day before addition of the maturation signal. The ability of the DC to initiate antigen-specific T cell proliferation was assessed using 3H-Thymidine incorporation assays. DC were immunostained for maturation markers.

DC matured with either 300ng/ml CD40L, 100ng/ml TNFa, or 30ng/ml LIGHT initiated similar levels of T cell proliferation. These levels were significantly higher (~30%) than those obtained with immature DC. Maximum DC maturation was achieved after a 48-72h exposure to these maturation agents. Combining any two of these agents did not result in any significant further increase in T cell proliferation. DC treated with 200U/ml TNFa and 5microMol PGE2 for 24h were slightly less able to initiate CMV-specific T cell proliferation than immature DC. These DC appeared morphologically more mature in culture (more non-adherent cells) and on cytoadherence (cells had more dendrites). More of the TNFa/PGE2-treated DC, as well as TNFa (20ng/ml)- and CD40L-treated DC expressed the maturation markers CD83 (31-69%), CD86 (73-96%) and CD40 (84-95%), than did immature DC (CD83: 4-15%, CD86:33-79%, CD40: 69-84%). HLAII expression/cell was down-regulated in TNFa-treated DC compared to immature DC, whereas it was up-regulated in CD40L-treated DC.

These results suggest that maturation marker expression does not always predict DC function. CD40L, TNFa, and LIGHT are promising candidates for the optimisation of CMV antigen
Development of hematopoietic chimerism and dynamics of virus-specific DNA occurrence in HSCT patients
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Objectives: It is of interest to estimate the correlation of engraftment, characteristics and results of viral DNA detection in HSCT patients. The aim of study was to follow the levels of donor hematopoietic chimerism (HC) and reactivation of viral infections after hematopoietic stem cell transplantation (HSCT). Recent methods of HC detection, especially under nonmyeloablative conditioning regimens, are focused on highly sensitive DNA PCR approaches. Similarly, DNA PCR is commonly used among diagnostic tools for active viral infections.

Patients and methods: HC dynamics was studied in 23 patients undergoing allogeneic HSCT. AmpliType technique (Applied Biosystems) based on multiplex PCR/dot-blot hybridization using a panel of ubiquitous gene probes was adapted at our department. In parallel, cytomegalovirus (CMV), Herpes Symplex (HSV) and Epstein-Barr virus (EBV) was assayed by detection of viral DNA sequences in blood leukocytes by means of one-step PCR. Results: The AmpliType technique proved to be effective for screening of hematopoietic reconstitution, allowing to detect >5% of recipient cells in leukocyte populations. Full donor chimerism occurred, approximately, by d+25 after HSCT, being in accordance with renewal of peripheral leukocytes and platelets. Positivity for CMV and HSV DNA before transplantation was noted for, resp., 2.3% and 4.6% of leukocyte samples. Meanwhile, increased occurrence of viral DNA (CMV, HSV) in blood leukocytes was noted as late as from d+40 to d+60 post-HSCT, with a peak of >20% positives, thus corresponding to the latent periods of 2 to 4 weeks known for these infections.

Conclusions: (1) AmpliType system is quite informative for semi-quantitative detection of mixed haemopoietic chimerism post-HSCT. (2) Time-dependence of donor chimerism-based hematopoietic engraftment correlates with increased occurrence of common viral infections, thus confirming the opinion about donor leukocytes harbouring viral genomes. (3) DNA diagnostics of CMV, EBV and HSV proved to be an informative for viral reactivation/reinfection, but their predictive power for subsequent clinical infections in transplanted patients is doubtful.

Contribution of liver biopsy in allografted patients - A retrospective study
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Background: liver dysfunction is a common problem in patients treated by allogeneic stem cell transplantation (SCT) due to diversity of the causes and different authors recommend to perform liver biopsy. We reviewed retrospectively 54 allografted patients during a period of 3 years. The objective of this study was to further evaluate the impact of liver biopsy on the medical treatment of the patients and to confirm the results of previous studies.

Population: We reviewed the liver function before and after SCT in 54 consecutive patients. Patients routinely underwent clinical examination, scintigraphy and biological liver function tests and viral serology. All patients received hepatotoxic drugs. A liver biopsy was performed to determine accurately the cause of liver injury and to adapt the appropriate medication. Pre-SCT isotopic exam (aminopyrine *C14 breath test) was normal in all the patients.

Results: 16/54 (30%) patients underwent a transjugular (18%) or transparietal (82%) liver biopsy during their course: 2 patients before day 30, 4 patients between d30 and d100, 9 patients between day 100-day 360 and one patient after the first year. All patients had biological liver dysfunction: cytology before day 100 and cholestasis after day 100. In these 16 patients, 9/16 (56%) had a prior liver disease: viral hepatitis B positive serology (n=6), steatosis (n=2) and drug-induced hepatitis (n=1). All the liver samples were analysed morphologically, phenotypically, microbiologically and genetically for malignant or infectious disease. All the time of biopsy, graft-versus-host-disease (GVHD) was suspected in 3 patients and veno-occlusive disease (VOD) features were present in 8 patients. None of the 16 patients experienced side effects due to the procedure. In 12/16 (76%) patients, analysis was contributive: hemosiderosis (n=3), toxic hepatitis (n=3), HHV6 viral hepatitis (n=2), hepatitis B (n=1), chronic GVHD (n=2) and steatosis (n=1). In 8/13 (61%) patients, a modification of the therapy resulted from the results of the biopsy. Conclusion: Liver biopsy is a useful diagnostic procedure in allogeneic SCT patients mainly where several etiologies are possible. In these situations, the pathology report can answer the question. In 60% of the cases, this leads to a treatment modification.

Severe gastroenteritis with graft failure due to HHV-6 infection after peripheral blood stem cell transplantation
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Reactivation of HHV-6 following BMT was first reported in 1991 with clinical manifestations such as pneumonitis, encephalitis, GVHD, sinustitis and CMV co-infection. Myelo suppression was described in 67% of BMT recipients with HHV-6 infection compared to 10% of patients without HHV-6. Since 1995 HHV-6 infection has also been known to be associated with bone marrow failure. Two children, one with Hodgkin’s disease and one with high risk ALL were transplanted using autologous or matched unrelated peripheral blood stem cells (PBSC), respectively. One child who received autologous PBSC had delayed granulopoietic engraftment and remained erythrocyte and platelet transfusion dependent. The second child showed complete engraftment but developed secondary bone marrow failure on day 82. In both patients bone marrow was morphologically aplastic with evidence of hemophagocytosis. Both children were tested continuously once a week for presence of HHV-6 and cytomegalovirus-DNA in the peripheral blood using qualitative and quantitative PCR-based methods. However 6 months and 2 months, respectively, after transplantation both children developed severe mucositis, diarrhea and emesis. Intestinal endoscopy showed only few lesions, but biopsies revealed 5.3 x 103 and 11 x 103 HHV-6 DNA copies/ml, respectively, measured by quantitative PCR. Both children were treated with a combination of gancyclovir and foscarnet on alternating days and cidofovir at 14 day intervals. One patient is now transfusion independent with stable WBC two months after discontinuation of antiviral therapy. The other patient is still erythrocyte and platelet transfusion dependent but also shows a significant increase of WBC. A remarkable temporal association between the initiation of antiviral therapy, improvement of gastroenteritis and bone marrow recovery was observed in both children. Although both patients primarily presented with bone marrow failure followed by gastroenteritis, evidence of virus replication could only be detected by PCR in gastrointestinal biopsies. We conclude that HHV-6 should be included in the differential diagnosis of graft failure or delayed engraftment after stem cell transplantation even if samples of peripheral blood are tested negative. It is therefore necessary to perform a more aggressive search for viral infection in all tissues of concern. The most appropriate antiviral therapy and the optimal duration of treatment remain to be investigated.
Association of BK viruria with hemorrhagic cystitis and tubulo-interstitial nephritis in recipients of stem cell transplants


Virus associated hemorrhagic cystitis (HC) is a common complication of allogeneic stem cell transplantation. Late onset and the excretion with the urine of BK human polyomavirus (BKV) characterize HC. Between June 2000 and May 2001, 725 pts, who underwent an allogeneic transplantation, developed HC with macrohematuria, dysuria and excretion of BKV in the urine, confirmed by nested-PCR analysis. Five pts received a T-depleted transplant from a related HLA-haploidentical donors, 1 pt from a related HLA-identical donor and 1 pt an unrelated cord blood unit. The last 2 pts presented aGVHD, treated with ATG+PDN. Clinical signs of HC appeared after a median of 31 days (range 20-70) from the transplant with a median duration of hematuria of 10 days (range 7–15). All had persistent BK viruria (2 or more consecutive positive samples). Three pts developed a tubulo-interstitial nephritis like syndrome and showed proteinuria and drug-refractory hypertension. Treatment with adequate hydration was started and one of them spontaneously improved renal function, two pts developed an acute renal failure that required dialysis, which was irreversible in one case. Hematuria was associated to proteinuria in 3 pts and to proteinuria and hypertension in 1. In 2/7 pts the HC spontaneously resolved; in 3 cases estriol therapy was given at dose of 20 mg/day e.v. once a week to control hematuria, but the efficacy was moderate. One pt received specific antiviral therapy with Cidofovir at dose of 3mg/kg (two fortnightly doses) and hematuria and BK viruria rapidly disappeared. In conclusion, BKV reactivation may account for a late-onset, long-lasting HC and in few cases for tubulo-interstitial nephritis like syndrome in highly immunosuppressed recipients of stem cell transplant. Monitoring of BKV viruria is necessary in all transplant recipients to prevent the clinical effects of viral reactivation.

The effect of acyclovir and valaciclovir on CMV infection and disease in allogeneic bone marrow transplant recipients; a single institution’s experience over twenty years

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A retrospective analysis was made for CMV infection and disease of all patients receiving allogeneic bone marrow transplantation between 1980 and the end of 1999 at a single institution. There were 184 allografts performed. The analysis was divided into the 3 periods 1980-1986, no CMV prophylaxis, 1987-1994, reflecting the introduction of high dose valaciclovir prophylaxis and 1995-end 1999, reflecting the introduction of high dose acyclovir prophylaxis. In first period of 2 of 10 (20%) patients developed CMV infection both developing interstitial pneumonitis, 1 dying of CMV disease. In the second period 4 of 60 (6.6%) patients developed CMV infection all developing CMV disease, all deceased of CMV disease. In the third period 28 of 114 patients (24.5%) developed CMV infection, 7 progressing to CMV disease, all dying. The increased incidence of CMV infection in the third period reflects the introduction of PCR technology, including quantitative PCR to monitor CMV infection, but a lower proportion progressed to CMV disease, in part attributable to preemptive ganciclovir therapy. Acyclovir and valaciclovir were effective in reducing the incidence of CMV end organ disease, but when end organ disease developed the mortality rate has remained high (12 of 13 patients).

Fatal herpesvirus 6 encephalitis following an allogeneic nonmyeloablative peripheral blood stem cell transplant

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A 67 years old female with diagnosis of acute myelogenous leukemia standard risk by cytogentic, in 1st complete remission after two chemotherapy induction treatment underwent a non-myeloablative allogeneic peripheral blood stem cell transplant from an HLA identical sibling. The patient was conditioned with fludarabine 30 mg/m2 on day –4, -3 and –2, and low-dose TBI (200 cGy) and postgrafting immunosuppression with mycophenolate moftil (MMF) and cyclosporine (CSA). MMF was discontinued by day 27. Day +28 assessment for donor chimerism showed: donor T cells 90%; granulocytes 100%; bone marrow 95%.Bone marrow evaluation showed complete remission by morphology and flow cytometry. By day +31 the patient experienced grade II aGVHD (skin and upper gastrointestinal tract) and methprednisolone was added to cyclosporin. Suddenly at day +35 the patient appeared lethargic but without neurological focal signs. The day after, cyclosporine was discontinued in the suspicion of cyclosporine toxicity, a CT scan was performed which showed no focal lesions, neurological assessment was again negative for focal signs and a lumbar puncture was performed. Extensive virological and microbiological assay for cerebrospinal fluid allowed the identification of HHV6 DNA. The patient became progressively more lethargic and died two days later. This case illustrates the importance of considering HHV6 as a possible pathogen also in the setting of non-myeloablative allogeneic peripheral blood stem cell transplant.

Danger of infection transmission by the transplanted graft - system of prevention


Objectives: Cumulative evaluation of grafts shows the clear danger of infection in some percentage of grafts. It exists not enough experience with the possibility of decreasing the risk of contagiosity (e.g. toxoplasmosis, viruses) by cryoconservation. The safety system of infection prevention is necessary in tissue bank, too. We describe our experience.

Methods: We demonstrated usage of different temperatures below -80°C for storage of haematopoietic progenitor cells for clinical transplantation. It is possible during one year in mechanical freezers or liquid nitrogen vapour phase. The longer preservation is possible only in the liquid phase of nitrogen. However it is considered hazardous due to described cases of contamination among damaged bags with the stored material.

Results: To evaluate the risk of infection the groups of 45 patients were tested according to common standards of EBM and ISHAGE Europe. The panel of tests included the proof of following infections: retroviruses (HIV, HTLV), hepatitis (A,B,C), herpes viruses (CMV,EBV,VZV, HSV), syphils and toxoplasmosis. Active infection that required clinical treatment of donors/patients was confirmed in 2 stem cell concentrates (1 varicella, 1 toxoplasmosis). Laboratory signs of active infection were detected in the following number of patients and/or donors: CMV IgM: 6, VZV: 2, HSV2, toxoplasmosis: 1 case). Previous infection by hepatitis A was detected in 2, by hepatitis B in 3 cases. The risk of infections seems to be clear and not very rare. As the prophylactic measure we established the system of quarantining of all concentrates in the vapour phase of liquid nitrogen until the results of all tests mentioned above were completed. Than the bags can be stored in the liquid phase of nitrogen. That can save a spare container for the conservation in liquid nitrogen.

Conclusion: Using the conservation of stem cell concentrates in the vapour phase of liquid nitrogen till the solution of all problems with the infectiosity of concentrates is a suitable, safe and cheap method for prevention of infection transmission by the graft in tissue bank.
CMV infection/reactivation in patients treated with allo-SCT after myeloablative and nonmyeloablative conditioning

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(Wroclaw, PL)

Non-myeloablative conditioning regimen involves aggressive immunosuppression. This causes high risk of infection complications. The present analysis focused on the incidence of CMV infection/reactivation in two groups of patients receiving myeloablative and non-myeloablative regimen.

Group 1 (transplanted after myeloablative regimen): 45 patients
(male:female = 28:17) with a median age 23 years (16 – 47) suffering from CML – 15, ALL - 14, AML – 13, NHL – 3. Type of allo-SCT: MUD - 6, Sib - 35. Conditioning of Sib transplants: BuCy and in four cases extra VP-16 and in other four Thiotepa was added. Conditioning of MUD transplants: Bu or Mel FluATG. Patients were followed for the presence of CMV early and immediate early antigen in blood mononuclear cells. In addition all symptoms suggesting viral infection were noticed. Both groups of patients receive Acyclovir viral prophylaxis, which was replaced by Gancyclovir during ATG administration. Gancyclovir as a pre-emptive treatment was introduced when CMV test was positive and/or clinical symptoms involved: marrow suppression, haemorrhage cystitis, interstitial pneumonia and viral hepatitis appeared.

The positive tests for CMV EA and IEA were seen in similar proportion - about 50% in two groups of patients irrespective of conditioning. However positivity of CMV test was significantly more frequently associated with clinical symptoms in patients receiving non - myeloablative conditioning - 8 patients in group 2 vs. 3 patient in group 1 (p=0.01). Also the profile of clinical symptoms was different in this groups: interstitial pneumonia was seen in one patient, but patients on non-myeloablative regimen presented frequently liver symptoms (8pts) suggestive viral hepatitis. They lacked either hepatitis B or C markers. Therefore we can assume that hepatitis could be associated with CMV infection/reactivation.

It appeared that viral hepatitis was frequently present in patients receiving ATG and Fludarabine as a part of conditioning regimen and these symptoms were associated with CMV infection/reactivation.

11. Hemoglobinopathy / Inborn Errors

P555

Successful allogeneic T-cell-depleted peripheral blood stem cell transplantation (PBSC) in a child with alpha-mannosidosis

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Introduction: a-mannosidosis is a storage disease characterised by accumulation of oligosaccharides in various tissues leading to symptoms such as macrocephaly, coarse face, hepatosplenomegaly, dysostosis multiplex, infections, hearing disabilities and mental retardation. Without treatment this autosomal recessive disease leads to progressive neurodegeneration.

Case report: We report a child who came to clinical attention due to a shortened Achilles tendon and mental retardation at the age of 20 months. He exhibited characteristic features such as frontal bossing, skeletal abnormalities, hearing disorder, vacuolated lymphocytes and recurrent otitis. A-mannosidosis was diagnosed by decreased enzyme activity levels of a-mannosidase in leukocytes and typical pattern of urinary oligosaccharide secretion. At the age of 24 months he underwent PBSC with T-cell-depletion by CD34-positive-selection (21*10^6 CD34+cells/kg, 27*10^3 CD3+cells/kg) from his HLA phenotypically identical mother. Conditioning was carried out according to the EBMT protocol for inborn errors with busulfan (20 mg/kg) and cyclophosphamide (200 mg/kg). OKT3 and methylprednisolone were used as GvHD-prophylaxis. The post-transplantation period was uneventful; no GvHD occurred and the patient 1 year after transplantation. On day +46 he developed mixed chimerism with 30% cells of recipient origin in the mononuclear cells which has been stable since. Enzyme activity levels have been shown to be about half of the normal level. This was expected since the donor is a carrier of the disease. One year after PBSC the patient is alive and well and has made significant developmental progress, urinary oligosaccharide secretion has returned to normal and changes in brain MRI have disappeared.

Discussion: Stem cell transplantation was first shown to be an effective treatment for a-mannosidosis in a feline animal model. Decreasing glycoprotein substrate accumulation in skeleton and brain after transplantation was noted and thought to be mediated by donor peripheral blood mononuclear cells and microglial cells. In the report patients reporting presence of skeletal abnormalities and improvement of neuropsychological capabilities have been observed. We report a patient who has been successfully transplanted by CD34+-PBSC from his mother.

Conclusion: CD34+-PBSC is a feasible option for patients with a-mannosidosis and if chosen as therapy should be carried out early in life to obtain optimal results.
Successful unrelated cord blood transplantation (UCBT) in two children with severe combined immunodeficiency syndrome (SCID)


Haemopoietic stem cell transplantation is the treatment of choice for SCID. We describe successful immunological reconstitution following myeloablative cord blood transplantation in two patients affected by a Zap-70 deficiency and an Omenn-like syndrome.

Patients: the patients were hospitalised after recurrent infections at the age of 13 and 2 months, respectively. In both no related donor was found and the search for a suitable cord blood unit began. The time to transplantation was 55 and 58 days from initiation of search: one unit was mismatched for loci A and DPB1 and the other for locus A after high resolution typing. The conditioning regimen consisted of cyclophosphamide, busulfan and anti-thymocyte globulin. The GVHD prophylaxis was cyclosporine A and steroids. The total number of cell infused was 15.1 and 17 x 10^6/Kg. CD34+ were 8 and 14 x 10^5/Kg and CFU-GM were 2.9 and 1.7 x 10^4/Kg.

Results: neutrophil engraftment was achieved on day +15 and +23, platelet count >50000 was achieved on day +21 and +52. One patient presented acute GVHD grade I (skin) and the second grade III (skin, liver). Chimerism was mixed and full donor, respectively. Immunological reconstitution is summarised in the figure. Normal lymphoproliferative response to mitogens and alloantigens was detectable from 6 months after UCBT in both patients. The patients are alive at 48 and 13 months from transplantation with complete haematological and immunological reconstitution.

Conclusion: haemopoietic stem cell transplantation needs to be considered as soon as possible after diagnosis of SCID because these disorders usually run an unpredictable course and may be rapidly fatal. Umbilical cord blood is a valid alternative source of haemopoietic stem cells because of the rapid availability of a matched or mismatched unit and the successful reconstitution observed.
median follow-up time of 39 months, 51/54 patients are alive, 50 with donor-derived normal haematopoiesis. The actuarial survival and disease-free survival at 5 years are 94.7% and 92.9% respectively. Seven/8 patients who underwent a 2nd transplant engrafted properly with minimal toxicity and have restored normal haematopoiesis. One patient transplanted initially with CB failed to engraft with BM from the same donor. In this population of thalassaemic children results are encouraging with low morbidity, and no mortality in class I and II patients. A second transplant in children who reject the graft, has been proven to be a safe and effective option.

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Increased cell dose and outcome of transplantation in thalassemia major patients
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Introduction: For allogeneic stem cell transplantation, it is common practice to infused at least 2 -4×10^8/kg and 5-7×10^8/kg cells in Bone marrow (BM) and peripheral blood (PB) stem cell transplantation respectively, to ensure engraftment. We studied the effects of increasing the threshold leads to a better outcome in transplantation.

Methods and Materials: We enrolled 140 patients with thalassemia major in this study (class I: 38 patients, class II: 64 patients and class III: 38 patients; Female: 65, Male: 75; Mean age: 6.5years, Range: 2.5-17years). 96 patients received bone marrow transplantation (BMT) and 44 patients received peripheral blood stem cell transplantation (PBSC). And median follow up of patients were 737 days post transplantation.

Results: We assessed relation between cell dose and engraftment with regression test. For each unit cell dose increased, engraftment (PMN >500 and platelet >20’000) occurred 1.2 day in BMT earlier and 0.25 day in PBSC, and actually not considering graft type, the engraftment occurred 0.5 day earlier. Also we have detected date of engraftment in patients who have received PBSC was 9 days earlier than those who have received BMT. Incidence of GVHD in BMT was less than PBSC (odds ratio=0.11, P value=0.03). Relative risk of GVHD in children with age <6 years in comparison with age >6 years was 1.27 and for each year increase in age incidence of GVHD reduced 0.82%.

Conclusion: Cell dose transfused for transplantation is effective for date of engraftment without any effect on incidence of GVHD. But graft type and age are effective factors in occurrence of GVHD.

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Treatment outcome and chimerism status after cord blood or bone marrow transplant in transfusion-dependent thalassemia patients
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Objective: We review the treatment outcome and chimerism status in patients with transfusion dependent thalassemia who underwent stem cell transplantation in our hospital using either sibling bone marrow (BM) or cord blood (CB) from 1991 onwards.

Methods: Seventeen patients underwent BM and 6 CB transplantation. Conditioning with busulphan and cyclophosphamide (Bu/Cy) was used in the initial 7 patients. Due to the occurrence of graft rejection, anti-thymocyte globulin (ATG) was added in subsequent cases. Fludarabine-containing regimen was used in 4 recent Class III patients. Methotrexate and cyclosporine were used for GVHD prophylaxis. Chimerism status was analysed using either FISH or conventional cytogenetics in sex-mismatched cases and DNA microsatellite analysis in the recent 2 years.

Results: In the BM group, graft failure occurred in 3 of 7 patients conditioned with Bu/Cy, and 2 of the 10 with ATG-containing regimens. GVHD was more severe in BM group, and contributed to death in 2 cases. 10/17 (58.8%) patients became transfusion independent (follow up:0.8 – 9.5 yr, median: 3 yr.). In the CB group, all 6 patients (100%) became transfusion independent (follow up:0.9-7 yr, median:3.3 yr.) With ATG-containing regimens, incidence of GVHD was decreased (9.3%) in patients became transfusion independent. Assessment of chimerism showed that in the CB group, 5 of the 6 patients achieved 100% donor chimerism early after the transplantation. One patient had 60% donor cells at 1 month post-transplantation, which gradually increased to 91% after 9 months without intervention. For the BM group, 5 patients were not evaluable (graft failure or study not performed). Mixed chimerism was documented in 3 out of the 12 evaluable patients. One patient had donor cells of 95% and remained so at 2 years’ follow-up. Two patients had mixed chimerism early after transplantation with donor cell proportion less than 50%. Both were treated with repeated doses of donor lymphocyte and peripheral blood stem cell infusion. One reverted to full donor chimerism and became transfusion independent and one finally rejected the graft.

Conclusion: CB achieved better results than BM transplantation in our patients. ATG-containing regimen possibly gives superior outcome than the one without. The outcome of patients with mixed chimerism was variable. We succeeded in improving engraftment for a patient with low donor cell proportion post-transplantation by donor lymphocyte and stem cell infusions.

P562
Immunosuppression and engraftment after allogenic BMT for Thalassemia major
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Graft failure is one of the major problems in transplantation for severe beta-thalassemia. Therefore, current conditioning regimens include intensive myelotoxic and immunosuppressive medications.

We describe the case of a four year-old girl with class 2 thalassemia major. She received a bone marrow transplant from HLA-identical grandmother following busulfan (14 mg/kg) plus cyclophosphamide (200 mg/kg). Engraftment was early on day +15 (chimerism: 92% donor in peripheral blood; 87% in bone marrow of day +30). The girl was successfully treated for CMV reactivation (hepatitis) and grade II skin GVHD.

On day +87 she developed secondary graft failure with drop of hemoglobin and a drop of donor chimerism in total nucleated blood cells of 42% (total nucleated bone marrow cells: 22%). The analysis of subsets of nucleated cells showed, that T-lymphocytes were 96% of donor origin in peripheral blood (92% in bone marrow). As a consequence of these events the immunosuppressive medications were reduced and stopped on day +91 (corticosteroids) and day +98 (cyclosporin A), respectively. The proportion of donor cells in total nucleated cells increased to 63% on day +144 and is now stable at higher than 90%. The course, however, was complicated by GvHD of the skin (grade III) and severe fungal pneumonia. On day +250 the child is alive and well with a full donor chimerism.

We conclude, that regulation of immunosuppression is important for the long term engraftment after BMT for thalassemia. Therefore, the chimerism of whole blood cells and when indicated of the T-cells should be assessed repeatedly.

P563
Comparison of the effects of cyclosporin A/methotrexate versus cyclosporine A on GVHD and hematopoetic recovery in thalassemia major after blood and marrow transplantation
A. Ghavamzadeh, M. Iravani, M. Jahani, B. Bahar, K. Alimoghaddam, M. Yunesian, S. Gholibeikian, S. Samiee (Tehran, IR)

In this trial we compared effects of Cyclosporin A (CSA) alone as a GVHD prophylactic agent versus Cyclosporin A in combination with Methotrexate (MTX) in thalassemic patients.
Liver disease during the first post-transplant year in 113 beta-thalassemic patients at a bone marrow transplantation center: the Shariati experience

A. Ghavamzadeh, S. Keyhanian, M. Iravani, B. Bahar, M. Jahani, M. Arshi, M. Hashemieh, A. Mousavi, S. Shafiee (Tehran, IR)

Liver dysfunction (LD) is a common problem in BMT recipients, thus it is important to determine the etiology and incidence in order to appropriate therapy.

A total of 113 beta-Thalassemic patients who had been transplanted at the Shariati BMT Center between March 1991-July 2000 are included in this study. 60 pts were male and 53 were female. We transplanted 27 pts of class I, 56 pts of class II and 30 pts of class III. The mean age in class I was 5.67±0.7, in class II 6.32±0.7 and in class III 8.75±1.4. Before transplantation all pts underwent liver biopsy to identify the fibrosis and HBsAg, HBsAb, HCVAb and CMVAb were assessed. Also ALT, AlkPh and total bilirubin were measured. Busulfan (3.5mg/kg) and CY 50mg/kg were used as conditioning regimen for class I and II pts, but for class III busulfan (4mg/kg) and CY (40mg/kg) were used. GVHD prophylaxis in classes I and II was only with CYA, but CYA and MTX were used in class III. Before transplantation 36 out of 113 pts were HbsAb+, one patient was AntiHCV+ and 43 pts were CMVAb+. HBsAg was negative in all pts. LD occurred in 86 out of 113 (76.7%) recipients during the first post transplant year. The causes of LD were GVHD (53.09%), CYA toxicity (15.92%), VOD (1.76%) and conditioning regimen toxicity (5.3%). Hepatic GVHD developed in 11 out of 27 pts in class I, 28 out of 56 pts in class II and 21 out of 30 pts in class III. CYA toxicity developed in 2 out of 27 pts in class I, 10 out of 56 pts in class II and 6 out of 30 pts in class III. VOD developed in 1 out of 56 pts in class II and 1 out of 30 pts in class III. No pts in class I developed. Total mortality of our transplanted pts was 17 (15.01%). The causes of death were bacterial infection (4 pts), CMV infection (3 pts), VOD (1 pt), acute hepatic GVHD (2 pts), chronic hepatic GVHD (2 pts), lung fibrosis (1 pt), renal failure (1 pt) and unknown (2 pts). This study shows that the incidence of LD following BMT in thalassemic pts is 76.07%. Although many etiologies are responsible, hepatic GVHD was the main cause of LD (53.09%) in these pts. The first post transplant year was divided into 3 periods: 1-30 days, 31-100 days and 101-365 days after BMT. CYA toxicity, drug hepatotoxicity and VOD were the main causes of LD during the first 30 days.

The most common causes of LD in first post-transplant year in Thalassemic pts were hepatic GVHD and drug hepatotoxicity (75%). Also hepatic GVHD was the cause of death in 23.5% of our pts.

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P565

Myelodysplastic features in Griscelli syndrome

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Griscelli syndrome (GS) is a rare, autosomal recessive disease, caused by mutations in the MYOSA gene, and the RAB27A gene. Located on the same chromosome (15q21). The clinical findings are silvery hair associated with diffuse cutaneous hypopigmentation, recurrent infections and accelerated phases with lymphohistiocytic infiltration of multiple organs leading to hepatosplenomegaly, lymphadenopathy and fever. Hematologic findings include pancytopenia and coagulopathy during phases associated with hemophagocytosis. Especially RAB27A gene mutations are associated with prominent hemophagocytosis. The only possible cure is allogeneic bone marrow transplantation. We report on two patients with GS associated with a RAB27A gene mutation and myelodysplastic features. A 17-month old girl of consanguineous Turkish parents presented a history of severe varicella infection, Staph Aureus skin lesions and disseminated infection after BCG vaccination. She underwent peripheral SCT but died of multiple organ failure due to adenoviral infection. The other patient was successfully treated with steroids for a virus-associated haemophagocytic syndrome and will be transplanted in the future. In the 2 patients myelodysplasia was noted in all three cell lineages. This may be the result of an impaired production of precursor cells during viral illness. Alternatively, the RAB27A and MYOSA mutations, known to interfere with intracellular transport might be directly involved in hematopoiesis resulting in dysplastic morphology. In addition to myelodysplasia, patient 1 had a slightly increased number of myeloid blasts (8%). In both cases, evidence of clonality of hematopoietic cells is lacking. Therefore, we are reluctant to entertain the diagnosis of MDS. A transient increase in myeloblasts in a slightly dysplastic marrow can occasionally be noted during infectious illness in otherwise healthy infants. Together, we believe that there is not enough evidence to make a firm diagnosis of MDS in Griscelli syndrome.

Additional abstracts to this topic

Cord blood Transplantation in three thalassemic patients

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UBC (Umbilical Cord Blood) as a source of HSCS can be used in patients suffering from hematological disorders, like thalassemia, which is common in Iran. Three patients transplanted with sibling HLA-matched UBC-HSCS.

The patients, a 3-years-old girl and tow 9-years-old boys were 13.5kg, 37.5kg and 24kg respectively.
Conditioning regimen was 3mg/kg Busulfan for 4 days and 50mg/kg Cyclophosphamide for 4 days. Nucleated cell counts were $4.82 \times 10^8$, $0.24 \times 10^7$ and $2.1 \times 10^7$ respectively.

GVHD prophylaxis began in the –2 with 3mg/kg for the first case, added by 12.5mg/kg (oral) in the day +5.

For the 2nd and 3rd cases GVHD prophylaxis began with cyclosporin 1mg/kg in the day +5, which was changed to 4mg/kg in the day +23.

No GVHD was observed in the 1st case. In the 2nd case a stage III Skin GVHD, a stage I GI GVHD and a stage I Liver GVHD was observed.

In the 3rd case a stage I Skin GVHD, a stage I GI GVHD was observed (there was no sign of liver GVHD in this case).

For the 2nd and the 3rd case neutrophil count recovery (neut > 500 for 3 successive days) was established on the day +19 and +29 respectively and platelet recovery (plt count > 50,000 for 3 successive days) occurred on the day +31 and +78 respectively.

All three patients are alive now (4 years). The transplant was rejected in the 1st case and she is transfusion dependent. But the other two cases are transfusion independent and the transplant is engrafted.

12. Stem Cell Biology

Role of different medium and growth factors on placental blood stem cell expansion. An in vitro and in vivo study

M. Berger, F. Fagioli, W. Piacibello, F. Sanavio, K. Mareschi, E. Biasin, F. Nesi, S. Lijoi, M.E. Saroglia, E. Madon, M. Aglietta (Turin, I)

Introduction: Successful expansion of haemopoietic stem cells from placental blood has been obtained with a combination of Kit-ligand (KL), FLT3-ligand (FL), Thrombopoietin (TPO) with or without Interleukin-6 (IL6) in serum replete medium. For clinical use, cell expansion in the absence of serum is a clear advantage. Therefore stem cell expansion in serum free (SF) medium with a combination of three (KL, FL, TPO) or four (KL, FL, TPO, IL6) growth factors was compared with the results obtained using foetal calf serum (FCS) or human serum (HS).

Methods: Human CD34+ placental blood cells were cultured in the presence of FL, TPO, KL +, -IL6 with foetal calf serum (FCS), human serum (HS) and SF medium for up to 8 weeks. To assess progenitor cell expansion, CD34+, CFC, LTC-IC content was measured at various intervals. Moreover, to determine in vivo repopulating capacity of expanded cells, CD34+ expanded cells were transplanted in sublethally irradiated NOD/SCID mice.

Results: With the three growth factor combination CD34+ cell number increased steadily up to the 8 weeks of culture. CD34+ cells were expanded 67.5 fold with SF; 11.7 with HS and 49.2 with FCS. However when CFCs and LTC-ICs were considered a continuous expansion was observed only with FCS and HS, whereas in SF medium after 6 weeks culture their number started to decline (Fig.1). The addition of IL-6 did not change the expansion significantly (Fig.2).

To assess in vivo repopulating activity, cells grown ex vivo for 14 days were transplanted into NOD/SCID mice. The engraftment of human cells in mice was higher for serum replete expanded cell, nevertheless, SF cultured cells were also able to engraft both marrow and spleen in all animals (Tab.1). In addition, engrafted human cells still maintained clonogenic ability.

Conclusion: With KL, FL, TPO +, -IL6 it is possible to expand haemopoietic progenitor cells in SF medium. Although compared with serum repleted cultures, the absolute number of clonogenic cells and in vivo repopulating cells is lower. The degree of expansion is significant and warrants a clinical trial to test its efficacy to reduce post-transplant aplasia.

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Cytometry analysis has been used to identify these stem cells, but also lineage committed progenitors. Multicolor flow cytometry in a homogeneous population – they contain not only pluripotent hematopoietic stem cells defined as CD34+ cells do not consist of administration of G-CSF after a chemotherapy regimen. In peripheral blood, maximum wbc count was reached on day +15 (21.9x10^9 cells/L), relative CD34+ count reached maximum day +14 (1.25 % of wbc), and absolute CD34+ count reached maximum day +15 (0.3x10^9 cells/L). Maximum yield in collected PBSC grafts was reached day +14 (2.1 % of CD34+ cells, 6.6x10^9 CD34+ cells/L). Average expressions of antigens on CD34+ cells were as follows: CD38 92.2%, CD2 6.0%, CD5 10.6%, CD14 13.7%, CD3 13.7%, CD10 1.8%, CD33 13.2%, CD15 14%, CD41 42.7%, CD61 34.6%, CD7 12.5%, CD135 6.4%, Glycophorin A 40.8%, CD86 5.6%, HLA-DR 52.3%, CDw90 1.6%, CD19 21.9%, CD20 8.6%, CD45 99.8%, CD145 14.5%, CD13 22.6%, CD11b 25.2%, CD117 5.9%, CD64 23.7%, CD71 77.2%, CD1a 8.8%. There were no significant time dependent changes in expression of these antigens on CD34+ cells. A conclusion can be drawn from the observed data that a major proportion of CD34+ cells in peripheral blood stimulated by G-CSF is likely to contain committed progenitor populations which cannot contribute to a long-term engraftment. In average, approximately 12% of CD34+ cells were committed to T-cell lineage, 22% to B-cell lineage, and over 40% to myeloid, megakaryocytic or erythroid lineages in our samples. Only minority of CD34+ cells showed phenotype of more primitive hematopoietic stem cells (3.2% of CD34+38- cells, and 1.9% of CD34+w90+ cells). These results underline the importance of possible new markers for evaluation of long-term engrafting stem cell populations within the PBSC harvests.

**P567**

The growth of marrow from elderly individuals in hemopoietic long-term culture (LTC) is the same as for normal stem cell donors

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Background: Traditionally bone marrow (BM) for research has been obtained from normal donors at the time of bone marrow harvest. Following the recent trend towards of allogeneic mobilised peripheral blood stem cells, the number of BM samples available for research has decreased. During total hip replacement (THR) surgery bone marrow is removed from the femoral shaft to make space for prosthesis. We have compared orthopaedic patient marrow (OBM) with normal stem cell donor marrow (NBM) for endogenous haemopoiesis and stromal support of third party haemopoiesis in LTC.

Methods and Results: OBM was obtained from the femoral shafts of 12 orthopaedic patients undergoing THR, median age 67.8 (61-87), 2 were male. Patients with malignant disease and/or those receiving immunosuppressive or cytotoxic treatment were excluded. All patients had normal pre-operative blood counts. 13 NBM donors were studied, median age 31 (19-53), 6 were male. T25 flasks were seeded at 3x10^6 buffy coat cells/ml and CFU-NBM donors were studied, median age 31 (19 -53), 6 were male. All patients had normal pre-operative blood counts. 13 NBM donors were studied, median age 31 (19-53), 6 were male. T25 flasks were seeded at 3x10^6 buffy coat cells/ml and CFU-GM and BFU-E production over 10 weeks was compared. There was no difference in the ability of the OBM and NBM stroma to support haemopoiesis.

Conclusion: Marrow obtained from patients undergoing THR is a satisfactory source of bone marrow for Stem Cell research. There is no significant age effect on the activity of endogenous haemopoiesis or on stromal support of third party haemopoiesis in LTC.

**P568**

Analysis of subpopulations of CD34+ cells in peripheral blood mobilized by G-CSF

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Hematopoietic stem cells are routinely mobilized to peripheral blood of patients undergoing autologous PBSC transplantation by administration of G-CSF after a chemotherapy regimen. Hematopoietic stem cells defined as CD34+ cells do not consist of a homogeneous population – they contain not only pluripotent stem cells, but also lineage committed progenitors. Multicolor flow cytometry analysis has been used to identify these subpopulations. Samples of peripheral blood were taken from 10 patients with the diagnosis of lymphoma undergoing stimulation chemotherapy with cyclophosphamide, etoposide, and G-CSF. CD34+ cells were analyzed daily from the beginning of G-CSF administration, and phenotype of CD34+ cells was investigated daily from day 10 of chemotherapy to the end of stimulation in peripheral blood, and in all PBSC harvests. A wide panel of monoclonal antibodies was used to analyze samples by multicolor flow cytometry. In peripheral blood, maximum wbc count was reached on day +15 (21.9x10^9 cells/L), relative CD34+ count reached maximum day +14 (1.25 % of wbc), and absolute CD34+ count reached maximum day +15 (0.3x10^9 cells/L). Maximum yield in collected PBSC grafts was reached day +14 (2.1 % of CD34+ cells, 6.6x10^9 CD34+ cells/L). Average expressions of antigens on CD34+ cells were as follows: CD38 92.2%, CD2 6.0%, CD5 10.6%, CD14 13.7%, CD3 13.7%, CD10 1.8%, CD33 13.2%, CD15 14%, CD41 42.7%, CD61 34.6%, CD7 12.5%, CD135 6.4%, Glycophorin A 40.8%, CD86 5.6%, HLA-DR 52.3%, CDw90 1.6%, CD19 21.9%, CD20 8.6%, CD45 99.8%, CD145 14.5%, CD13 22.6%, CD11b 25.2%, CD117 5.9%, CD64 23.7%, CD71 77.2%, CD1a 8.8%. There were no significant time dependent changes in expression of these antigens on CD34+ cells. A conclusion can be drawn from the observed data that a major proportion of CD34+ cells in peripheral blood stimulated by G-CSF is likely to contain committed progenitor populations which cannot contribute to a long-term engraftment. In average, approximately 12% of CD34+ cells were committed to T-cell lineage, 22% to B-cell lineage, and over 40% to myeloid, megakaryocytic or erythroid lineages in our samples. Only minority of CD34+ cells showed phenotype of more primitive hematopoietic stem cells (3.2% of CD34+38- cells, and 1.9% of CD34+w90+ cells). These results underline the importance of possible new markers for evaluation of long-term engrafting stem cell populations within the PBSC harvests.

**P569**

Mobilization of peripheral stem cells after 131I-nuclide therapy with G-CSF plus SCF for support of subsequent nuclide application


Autologous stem cell support is a promising approach to overcome dose limiting haematotoxicity in patients treated with systemic irradiation with radioactive nuclides such as 131I. Autologous stem cell support after radiotherapy could allow dose intensification or shortening of treatment intervals. Few data about stem cell support after systemic radiotherapy have been published so far. Five cycles of steady-state stem cell mobilisation with G-CSF and SCF were carried out in four patients suffering from metastasised thyroid cancer (n=3) and metastasised paraganglioma (n=1). Measurement of CD34+-cells was performed following ISHAGE guidelines. Patient #1 reached a maximum of 3.9 CD34+-cells/µl and failed criteria for successful harvest. Patient #2 mobilised twice. In the 1st cycle a peak of 21.5 CD34+-cells/µl was measured. A total of 1.75*10^6 CD34+-cells/kg bodyweight was harvested by three apheresis procedures. The second cycle did not led to a successful stem cell mobilisation with a peak of 8.7 CD34+-cells/µl. Patient #3 and #4 mobilised well with peaks of 32.3 and 12.1 CD34+-cells/µl. From patient #3 a total of 2.12*10^6 CD34+ cells/kg was harvested. Patient #4 could not be subjected to apheresis due to concomitant cardiac problems probably not related to growth factor application. Stem cell mobilisation in patients after systemic radiotherapy with 131I was successful in 3 of 5 cycles, however, with moderate overall results. Stem cell mobilisation after therapy with radioactive nuclides is feasible. Factors predicting a poor mobilisation needs to be identified in future.
Stem cell processing with an automated system
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The SEPAX system from Biosafe provides a polyvalent and flexible platform for the separation and volume reduction of various stem cell sources (cord blood, leukapheresis material, peripheral blood, and bone marrow). The system consists of a closed disposable set associated with a compact instrumentation that manages the cell separation process automatically.

Cord blood and leukapheresis material was processed by conventional separation procedures (centrifugation with cell separator (Optipress) or plasma extractor (Baxter)) and compared to the processing with the SEPAX system. The fast (20 minutes) and efficient volume reduction (>64%) of the SEPAX system was accompanied with excellent recoveries of WBC (>88% with cord blood, and >98% with leukapheresis material) and CD34+ progenitor cells (>97% with cord blood, and >87% with leukapheresis material) compared to the conventional separation (Table1, Figure 1a and 1b). The fully automated protocols of the SEPAX system are user independent, lead to high quality and reproducibility of the final product.

Cryopreservation of peripheral blood progenitor cells (PBPC) with 10% dimethylsulfoxide (DMSO) has been the standard procedure in most hospitals for several years. However, the infusion of PBPC with DMSO is associated with toxicity for the patients. The incidence and severity of infusion related toxicity, ranging from mild nausea and vomiting to renal failure and cardiac arrest, is proportional to the amount of DMSO reinfused to the patients. The present study was undertaken to find out whether the viability of CD34+ cells would be altered if cells were frozen with only 5% DMSO. We have earlier demonstrated that there was no difference in colony formation whether PBPC were frozen with 5% or 10% DMSO(1).

Methods: Duplicate samples of PBPC from 18 patients were frozen in parallel with 5% and 10% DMSO with controlled rate to minus 160 degree Celsius for 3-22 months. The two samples were diluted 1:20 and handled in parallel after thawing. Two different flow cytometric methods were used: 1) The lyse, no wash one-platform analysis which includes triple staining with anti-CD34PE, anti-CD34 FITC and the vital dye 7AAD. This method gives information on the number and percentage of viable CD34+ cells. 2) The annexin V method, which includes triple staining with anti-CD34PE, annexin V FITC and 7AAD and gives information on the fraction of viable, apoptotic and necrotic CD34+ cells.

Results: The number and percentage of viable CD34+ cells were higher in all the PBPC samples that were frozen with 5% rather than 10% DMSO. Median calculated number of viable CD34+ cells was 4.0 versus 3.6 x 10^6/kg in the PBPC concentrates frozen with 5% and 10% DMSO, respectively, p= 0.001. Median percentage of viable CD34+ cells was 1.15% versus 0.69% in the PBPC concentrates frozen with 5% versus 10% DMSO, p= 0.001. The difference in CD34 cell viability was mainly due to less necrosis in the 5% DMSO samples, as compared to the 10% DMSO samples (11% versus 25%, p= 0.001). The fraction of apoptotic CD34+ cells were slightly lower in the 5% as compared to the 10% DMSO samples (10% versus 12%, p= 0.04).

Conclusion: This flow cytometric investigation suggests that freezing PBPC with 5% rather than 10% DMSO results in better cell viability post thaw. Infusing PBPC concentrates with 5% DMSO may give less toxic side effects to the patients and better PBPC quality.

(1) Abrahamsen, Bakken, Bruserud, Blood 96,11,p379A,2000

Long-term cryopreservation of peripheral blood and bone marrow hematopoietic progenitors
(Pamplona, E)

Objectives: Evaluate the clonogenic capability and viability of the hematopoietic progenitors cryopreserved for 5 years or longer in our department.

Materials/Methods: A total of 47 peripheral blood and 11 bone marrow samples, collected between 1993 and 1996, were retrospectively evaluated. The diagnosis of the patients were: 9 Hodgkin’s lymphoma, 4 High grade Lymphoma, 2 Multiple Myeloma, 1 Chronic Myeloid Leukemia, 2 Chronic Myeloid Leukemia, 1 Plasmacytoma, 4 Multiple Mieloma and 7 Breast cancer. Bone marrow was obtained under general anesthesia by multiple aspirates in posterior iliac crests. Sample concentration and processing, as well as peripheral blood progenitors apheresis were done with the help of a COBE Spectra cell sorter. No sample was purged “ex vivo”. Freezing was done in a NPII Hemofreeze bag from Gambro. We used 10 % dimethyl sulfoxide (DMSO) and 40% albumin as cryopreservative solution, with a volume equivalent to cell concentration. Cryopreservation was done with an automatic program (Cryosson BV-25) at a controlled-rate of −1°C/min, and cells were afterwards kept in a liquid nitrogen tank at −196°C. CFU-GM cultures were done in semi-solid agar media supplemented with fetal bovine serum and McCoy, after elimination of the buffy-coat using Ficoll gradient centrifugation. Reading was performed at 14 days of incubation. Cells viability estimation was carried out with Tripan Blue vital tinction.

Results: The Wilcoxon matched-pairs test was used for the statistical analysis. No significant difference (p = 0.487) was found in CFU-GM recovery after five or more years of preservation. No significant different was neither found in the cell viability (median=80%, p = 0.194). The results were similar in all diagnostic groups. No differences were either found when the
Cord blood (CB) as transplantation material optimization of method of preparing blood for long-term storage

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Cord blood is a rich source of primitive hematopoietic stem cells. In clinical hematology it has been transplanted instead of bone marrow or peripheral blood stem cells. To optimize storage space of cord blood after HLA testing it is necessary to reduce its volume. The aim of our study was to find methods of isolating leukocytes from cord blood within a closed system. Six methods of isolation have been tested: 6% HES, 3% HES, 1% HES, "buffy coat", 3% gelatin, and leukoreduction filters. We have used the centrifuge and sedimentation methods. The final volume of cord blood was about 25 ml for each unit. Results are presented in table under text.

The best results were obtained for sedimentation with 3% gelatin. With 6% HES sedimentation 100% of WBC, 70,7% of MNC; and 90,3% CD34+ were recovered; with 1% HES the results were: 87,1%; 86,4%; and 91,4% respectively and with 3% gelatin sedimentation the results were: 75,1%; 78,6%; and 81,3% respectively. The similar results have been obtained for two methods: "buffy-coat" and leukoreduction filters. The best waste of RBC were obtained for leukoreduction filters and sedimentation with 3% gelatin. No change in viability of progenitor cells was observed.

HES, gelatin and leukoreduction filters are approved for clinical use therefore methods of isolating leukocytes based on them are safe for recipients. The use of close system, recommended by Eurocord Transplant, prevents bacterial contamination. No bacterial (aerobic and anaerobic) contamination has been observed after processing.

P574

A new method for the determination of CD34+ cells viability by flow cytometry

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Objectives: One of the most important applications of flow cytometry is in studies of the mechanisms of cell death and the identification and quantification of apoptotic and necrotic cells. This study was designed to detect and quantitate the number of CD34+ hematopoietic precursor cells and to determine the cellular viability. For that purpose, a new method for identification of apoptotic events by staining with annexin V has been proposed. A total of 50 samples leucapheresis products from 16 patients were analyzed.

Materials and Methods: the specimens were incubated, at the end of harvesting before they were cryopreserved, with a mixture of CD45 PE (BD), CD34 APC (8G12, BD) Mabs, protein annexin V FITC and 7AAD. Erythrocytes were lysed using the lyse-and-non-wash technique by addition of fixative-free buffer QUICKLYSIS (Cytogons, Spain). Data acquisition was performed using FACScalibur flow cytometer (BD). A total of 100 CD34+ cells were acquired on seven parameters using Cell Quest software.

Determinations of viable CD34+ cells were analyzed with a single-platform version of the ISHAGE protocol. During data acquisition, Boolean gating to resolve the CD34+ HPCs from irrelevant cell populations was applied. Those progenitors cells were analyzed on new histogram annexin / 7AAD to include living cells, apoptotic cells and necrotic cells. After erythrocyte lysis without washing, an identical volume of fluorescent microbeads at a known concentration was added. Those progenitors cells were analyzed on new histogram annexin / 7AAD to include living cells, apoptotic cells and necrotic cells. The Thy 1 antigen has been shown to be expressed on various murine and human hematopoietic stem and progenitor cells other than cells committed to T lymphoid lineage. Hence, it should play a role as minor histocompatibility antigen and immunization of recipient against this antigen should result in a failure of hematopoietic graft. In order to investigate this issue we have taken advantage of availability of inbred strains of mice that differ in alleles of this antigen. Thus, AKR/J-Thy 1.1 mice were hyperimmunized against Thy 1.2 antigen using thymus cells from CBA/J-Thy 1.2 mice. One hour after lethal irradiation they were transplanted with marrow cells from AKR subline that possess Thy 1.2 instead of Thy 1.1 namely AKR/Cum mice. Despite using recipient with very high titer (at least 1:256) of anti-Thy 1.2 antibodies the effects observed were subtle. CFU-S self-renewal in both spleen and marrow was reduced two-fold, but no effects on the kinetics of engraftment have been observed in the marrow, spleen or thymus. All surviving (17/20) hyperimmunized AKR/J recipients were permanently (for at least 6 months - end of observation) repopulated with AKR/Cum Thy 1.2 cells. The frequency of survival was not different from recipients of syngeneic AKR/J cells. These data suggest, that either Thy 1...
antigen is not present on stem cells reconstituting hematopoiesis or these cells possess some not yet understood capacity to escape from the cytotoxic action of anti-Thy 1 antibodies. Since Thy 1 antigen was used to isolate murine stem cells the first possibility is unlikely. To our knowledge, it is the first description of successful engraftment of hematopoietic stem cells in a host hyperimmunized to any of their antigens.

P576
Adult bone marrow (BM) is a rich source of human mesenchymal stem cells but umbilical cord (CB) and adult blood are not
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Background: In post-natal life mesenchymal stem cells (MSC) can self-replicate, proliferate and differentiate into bone, fat, tendon, muscle and bone marrow stroma. Possible clinical applications for MSC in stem cell transplantation have been proposed.

Methods and results: BM mononuclear cells (BM MNC) were cultured at 37 degrees centigrade in DMEM and 10% Foetal calf serum at a concentration of 1x10^6 cells/ml in T25 flasks. During culture BM MSC proliferated to confluence in 10-14 days, maintaining a stable phenotype, CD29+, CD44+, CD90+, CD106+, CD45-, CD34- and CD14-. The frequency of MSC in BM MNC was 1.1x10^4. After three passages there was no morphological evidence of reduced proliferative potential or lineage differentiation. In lineage specific culture conditions both adipogenic and osteogenic differentiation of BM MSC was obtained. In paired experiments cultured BM MSC and mature BM stroma were seeded with 1x10^4/ml CD34+ CB cells and incubated in long-term haemopoietic cell culture conditions. CFU-GM from the culture supernate were measured at weekly intervals for 10 weeks. Similar numbers of CFU-GM were produced by MSC and mature stroma cultures. In contrast, CB MNC cultured in MSC conditions for two passages produced an adherent, non-confluent fibroblast-like cells with a haemopoietic phenotype, CD45+, CD34+, CD14+, CD44+, CD90- and CD106-. Cytokine stimulated adult blood MNC cultured in the same way produced a scanty CD45+, CD34+ adherent cell population which did not survive after two passages.

Conclusion: Adult bone marrow is a reliable source of functional cultured MSC, but cord and adult blood are not.

P577
Allogeneic mesenchymal stem cell engraftment in the infarcted rat heart: Timing and delivery route
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Previous data from this lab has demonstrated the potential therapeutic utility of autologous mesenchymal stem cells (MSC). However, the period required for MSC isolation and expansion may limit their clinical application in acute injury settings. Therefore the purpose of the present study was to examine the ability of allogeneic (allo) MSCs to engraft and differentiate in infarcted myocardium. Fisher rats (n=31) were infarcted by occluding the LADS for 45 minutes followed by reperfusion. Allo MSCs were isolated from ACI rats, expanded in culture, and labeled (Dil or DAPI) prior to implantation. Approx. 2 million MSCs in suspension (50ml saline) were injected into the infarcted region. Fisher rats received ACI MSCs at the time of reperfusion (n=9), or 2 weeks post-reperfusion (n=9). Rats were sacrificed at various time points, and the hearts frozen for histological examination. Immunohistochemical staining and confocal microscopy were utilized for identification of engrafted MSCs, and the expression of muscle-specific proteins. Examination of implanted hearts demonstrated significant engraftment and retention of ACI MSCs in Fisher hearts at all timepoints examined (1-12 weeks). Myogenic differentiation of MSCs could be identified in all animals implanted 2 weeks post-reperfusion (9/9 animals). Furthermore, cells implanted at reperfusion also exhibited robust engraftment and differentiation (9/9 animals). The implantation of allogeneic MSCs was not associated with ectopic tissue formation or inflammation. Studies have also been conducted to examine the fate of MSCs delivered systemically at reperfusion (n=13). In these animals, MSCs delivered IV via tail vein at reperfusion “home” to the site of infarction. The degree of engraftment in the infarct was similar in animals receiving MSCs IV and by direct injection. When the IV delivery of MSCs was delayed until 2 weeks post-reperfusion, the degree of engraftment in the infarcted heart was markedly reduced, with most cells returning to the bone marrow. These data indicate that “homing” of MSCs to the site of injury is a time dependant phenomenon. In conclusion these data suggest that allogeneic MSC cardiomyoplasty is feasible, safe, and a potential therapy for the treatment of acute myocardial infarction. These data also suggest that such a therapy could be delivered systemically and at various times post-reperfusion.

Additional abstracts to this topic

Does granulocyte-colony stimulating factor (G-CSF) inhibit megakaryocyte proliferation/differentiation process after peripheral blood stem cells (PBSC) transplantation - A clinical observation

In these past years the use of granulocyte growth factors reduced the time of ANC and all the complications of a prolonged aplasia. The problem concerning platelet recovery, on the other hand, still remains a major concern. Thus, every experience regarding platelets could be important to overcome this problem. We observed that, after high-dose chemotherapy supported by PBSC transplantation, even if rapid and efficient engraftment of all hematological cell lines had been achieved, when G-CSF was not terminated for some reason, the platelet count decreased. Based on this experience, we performed a case-control study to confirm or disconfirm our observation.

Patients and Methods: Twenty-six patients entered the study. All received high dose chemotherapy as follows: Thiotepa 300 mg/m² plus Melphalan 140 mg/m², or Thiotepa 300 mg/m² plus Cyclophosphamide 2 g/m², or Busulfan 480 mg/m² plus Melphalan 140 mg/m². The patients were divided into two balanced groups. Group 1 received G-CSF from 24 hours after PBSC infusion to 48 hours after ANC >500/ml, while group 2 received G-CSF up to the day of ANC 2000/ml.

Results: In group 1 median time to ANC >500/ml was 8 days (range 6-14) and to platelets 30.000 was 12 days (range 9-15). In group 2, median time to ANC >500/ml was 12 days (range 8-16) to ANC 2000/ml and 12 days (range 10-15) to platelets 30.000/ml. It was interesting to observe that in all group 2 patients there was a decrease in the number of platelets within 48 hours after reaching 30.000/ml as well as a prolonged time to platelets 50.000/ml (p < 0.01).

Conclusion: We didn’t examine the bone marrow, but our impression is that in some way G-CSF influences the proliferation/differentiation process of megakaryocytes in vivo. We are planning to perform a study including bone marrow examination to ameliorate the use of G-CSF and to avoid any possible mistakes that could lead to a prolonged thrombocytopenia.

Analysis of transplanted material obtained from cytopheresis according to the contents of progenitor stroma cells of bone marrow (CFU-F).
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In standard culture conditions in a liquid medium containing foetal calf serum (FBS), progenitor cells of the bone marrow stroma reveal a CFU-F activity, i.e. form cell colonies corresponding to fibroblasts. The aim of this study was to identify CFU-F in cytopheresis material from healthy donors of progenitor cells and
patients with haematological disorders in complete remission. Methods: 33 products of cytapheresis were examined: 6 from healthy donors, 10 from patients with AML, 7 with Hodgkin Disease, 6 with non-Hodgkin Lymphoma and 4 with multiple myeloma. Mononuclear cells (MNC) were incubated for 14 intervals in a liquid medium (RPMI 1640, 20% FBS, 2% l-Glutamine, in a quantity of 100 000 MNC/ml of the medium/1,8cm2 of the surface of the of the culture bottle. The number of adherent cells before fixation and after MGG, alkaline phosphatase, alpha-naphyl and chloroacetate esterase examinations were assessed. Results: The number of adherent cells showed a positive correlation with the number of monocytes entered into the culture. Among adherent cells, those corresponding to macrophages and monocytes dominate. Cytochemical straining showed activity of non-specific esterase in adherent cells (>98%). Activity of specific esterase and alkaline phosphatase was not revealed. Conclusions: Among adherent cells present in the products of cytapheresis from both donors and patients in haematological remission, macrophages and monocytoidal cells dominate. Progenitor cells of bone marrow stroma (CFU-F) do not undergo peripherisation under mobilisation by means of growth factors (G-CSF) nor chemotherapy combined with the administration with G-CSF.

13. Minitransplants

P578

Reduced-intensity conditioning followed by allogeneic transplantation is an effective salvage treatment for patients with hematologic malignancies failing a previous autograft


Conventional allogeneic hematopoietic stem cell transplants have a high incidence of acute GVHD (60-70%) and treatment-related mortality (TRM) (50-80%) when used to rescue patients relapsing after an autologous transplantation. With the aim of decreasing nonrelapse mortality, and to enhance its postulated graft-vs-tumor effect, we employed reduced-intensity conditioning in 30 patients failing a previous ASCT. Initial diagnoses were lymphomas (high-grade= 10 pts, low-grade= 7 pts, HD= 4 pts), multiple myeloma (n= 2 pts), acute leukemia (n=1 pt) and RAEB (n= 2 pts). The median age was 51 years (range, 20-69) with a median time from diagnosis to allograft of 40 months (range, 7-120). Before transplant, 19 patients had refractory and 11 chemosensitive disease, respectively. After conditioning with thiopeta (5-10 mg/kg), fludarabine (60 mg/m2) and cyclophosphamide (60 mg/kg) patients received bone marrow (n= 5) or peripheral blood hematopoietic cells (n= 25) from a HLA identical siblings. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine A, and short course methotrexate (anti-T lymphocyte globulin 7.5 mg/kg was used in the conditioning of myeloma pts only). Donor lymphocytes infusions (DLI) were given to 7 patients relapsing after the allograft. All patients engrafted, and chimerism studies showed that all of them were full donor by day +60. CMV reactivation occurred in 60% of seropositive recipients. Acute GVHD occurred in 32 patients (3 after DLI). It was scored grade II in 18 (58%) pts and grade III in 4 of 29 (13%) evaluable pts. Chronic GVHD developed in 9 pts (n=5 limited, n=4 extensive). Five of 30 pts died of transplant-related complications (infections=2 pts, GVHD grade III= 1, respiratory failure during endoscopy= 1, PTI=1). All the latter five patients had GVHD. Thirteen patients relapsed at a median of 90 days (range, 13-663) after allograft and 7 of them achieved again remission after CSA withdrawal (n= 3) and/or chemotherapy+DLI (n=4). At a median follow-up of 357 days, 19 patients (63%) are alive: 12 in complete remission (with 3 molecular remission by PCR analysis), 5 in partial remissions, and 2 have progressive disease. Six patients died of progressive disease, so far. In conclusion, despite the poor prognosis of pts failing a previous autograft, we have observed a high overall response rate (57%), possibly reflecting a graft-vs-tumor effect, and a relatively low TRM (17%).

P579

Transplant-related mortality (TRM) in patients receiving reduced vs conventional intensity thiotepa-cyclophosphamide (THIO-CY) conditioning regimen for allogeneic hematopoietic stem cell transplantation (HSCT)


Objective: The aim of this retrospective study was to investigate whether a dose-reduction of the THIO-CY based preparative regimen might have a beneficial effect on the outcome following HSCT. Patients: Between January 1994 and March 2001, 184 patients with hematological malignancies underwent an HLA-identical sibling donor transplant after receiving a reduced intensity conditioning including THIO 10 mg/Kg and CY 100 mg/Kg (THIO10-CY100, n=52) or a conventional regimen which included THIO 15 mg/Kg and CY 120-150 mg/Kg (THIO15-CY150, n=132). Bone marrow was the preferred source of stem cells in 29 (56%) cases of the THIO10-CY100 group and 50 (38%) cases of the THIO15-CY150 group, whereas peripheral blood stem cells (PBSC) were used in 23 (44%) and 82 (62%) cases of the two groups respectively. 48% (25/52) of the patients in the reduced intensity group had advanced disease as compared to 67% (88/132) in the conventional group (p=0.03). The median age was 52 (range 34-65) and 45 years (range 18-59) in the two groups respectively (p=0.0001). Patients received unmanipulated grafts with cyclosporine (CyA) and MTX (n=179), CyA alone (n=3) or CyA+other (n=2) as GVHD prophylaxis. Results: neutrophil recovery > 0.5 x 10^9/l was achieved by all patients receiving THIO10-CY100 and by 95% of the patients who received THIO15-CY150 at a median of 18 days (11-40) and 15 days (10-29) respectively (p=0.0001). Acute GVHD grade II-IV was observed in 44 % of the THIO10-CY100 recipients and 42% of the THIO15-CY150 recipients (p=0.9); 19 out of 47 (40%) THIO10-CY100 recipients vs 35 out of 112 (31%) THIO15-CY150 recipients developed extensive chronic GVHD (p=0.3). Seven (13%) patients who received a reduced intensity conditioning and 29 (22%) patients who received a conventional regimen died from transplant-related causes. The cumulative incidence of TRM at 180 days was 5% vs 14% in THIO10-CY100 and THIO15-CY150 group respectively (p=0.003). The corresponding figures for the TRM at 180 days were 7% vs 19% (p=0.02) respectively. With a median follow-up for surviving patients of 31(2-69) months in the THIO10-CY100 group and 32 (3-77) months in the THIO15-CY150 group, the OS rate was 58% and 46% respectively (p=0.12).Conclusion: The study demonstrates proofs of principle that reduced intensity conditioning regimens may lead to a lower risk of TRM and allow the use of HSCT for older patients and those with comorbidities which preclude high dose chemotherapy.

P580

Low incidence of acute GvHD in nonmyeloablative allografting with early introduction of FK506

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Introduction: Non-myeloablative allografting (NMA) has been introduced in the hope of minimizing TRM, maximizing the graft-versus-malignancy effect, and yielding better survival rates. We present herein a variant regimen with early introduction of FK506 characterized by low TRM, low incidence of acute GVHD, and prompt engraftment. Regimen: CY 300 mg/m2 and FLU 30 mg/m2 were given from D-7 to D-3, prior to infusion of at least 5 x 10E6
CD34+/kg from a matched sibling donor. FK506 was initiated on D-7 and tapered by D=100. MMF was started on D+2 and discontinued on D+50. DLIs were prospectively scheduled for patients not achieving complete donor chimerism (CDC), complete remission, or with progressive disease. Results: Between July 2000 and November 2001, 31 patients have been enrolled, and results are available for 22. Indications for NMA-allografting included older age (6) or higher risk of toxicity (16). Diagnoses included: MM (9); MDS/AML (5); NHL (5); ALL (1); HD (1); and CML (1). Patients experienced no mucositis, short cytopenias (median duration of neutropenia and thrombocytopenia were 7 and 0 day(s), respectively), and very low transfusion requirements. We only documented two episodes of neutropenic fever, but no septicemia. CDC was achieved in 19/20 evaluable cases (median time 42 days). Two patients with progressive/relapsing disease failed to achieve CDC, while one patient with CML-AP on STI571 had primary graft failure. Two patients required DLIs for persistent mixed chimerism. We report only one case of cutaneous (SLL00) acute GVHD in one patient with suboptimal tacrolimus levels. With a median follow-up time of 165 days, we report a 45% incidence of chronic GVHD. We observed only one TRM due to systemic aspergillosis in a patient with refractory cGVHD. Four patients died of relapsing/progressive disease. A graft-versus-myoeloma effect is already observed in the MM subgroup, often correlated with onset of chronic GVHD.

Discussion: Our regimen is associated with low morbidity and mortality, and a very low incidence of acute GVHD. Our regimen could be particularly attractive for non-malignant conditions requiring allogeneic transplant. We hypothesize our results to be linked to early introduction of FK506 which has been shown to induce polarization of dendritic cells toward a DC2 phenotype.

P581

Allogeneic stem cell transplantation with reduced-intensity conditioning regimen: incidence and risk factors for conditioning related toxicities

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In an effort to decrease the transplant related morbi-mortality associated with allogeneic stem cell transplantation (allo-SCT), reduced-intensity conditioning regimens (RIC) have been developed. The use of these RIC regimens would allow the performance of the allo-SCT in an outpatient setting, even in elderly patients. We analysed the incidence of neutropenic fever and => grade 2 gastrointestinal (GI) toxicity as hallmarks for hospital admission and risk factors associated to both of them in 41 patients allografted in early phase and 19 (46%) beyond the early phase.

We report our experience of 42 consecutive patients allografted from an HLA identical sibling donor with myeloid malignancies and fludarabine (150 mg/m2 iv) plus melphalan (140 mg/m2 iv) for chronic myeloid leukemia (n=4). The RIC regimen consisted of cyclosporine A plus short-course methotrexate and 6-thioguanine (200 mg/m2 po) in a 2:1 ratio for myeloid malignancies or cyclophosphamide (150 mg/m2 po) daily for lymphoid malignancies and fludarabine (150 mg/m2 po) plus busulphan (10 mg/kg po) with dosage adjustment to plasma levels for myeloid malignancies. Graft versus host disease prophylaxis consisted of cyclosporine A plus short-course methotrexate and fludarabine prophylaxis of norfloxacin 400 mg bid po, acyclovir 800 mg bid po and fluconazole 100 mg q24h. All patients had been previously treated before allo-SCT; median number of previous treatments was 2 (0-6) including an autologous stem cell transplantation (ASCT) in 12 patients. 22 (54%) patients were allografted in early phase and 19 (46%) beyond the early phase. Twenty-nine patients (70%) developed neutropenic fever and 13 (32%) => grade 2 GI toxicity at a median number of 17 (6-28) days after allo-SCT. 11 (27%) patients would have never been hospitalised in the immediate post-allo-SCT period. Time of hospitalisation was 27 (17 – 50) days [median (range)]. If allo-SCT had been performed in the Outpatient Department (OPD), inpatient days would have decreased to 9 (0 – 33) days, p < 0.001. There were no significant differences in the incidence of neutropenic fever or grade => 2 GI toxicity between groups of age, sex, underlying disease, number of previous treatments, previous ASCT, ECOG, disease status at allo-SCT, time from diagnosis, serum creatinine, serum albumin and leukocyte count before allo-SCT. Allo-SCT protocol is a well tolerated procedure, which can be performed entirely in the OPD in a significant proportion of patients.

P582

NMSCT and DLI can cure advanced acute leukemias, but relapse rate is still high

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Introduction: Nonmyeloablative stem cell transplantation (NMSCT) has been performed increasingly in the last years in patients with contraindications against standard SCT. Preemptive donor lymphocyte infusions (DLI) could be used as a therapeutic adjunct after transplantation in high-risk patients. The value of NMSCT and DLI in the treatment of acute leukemias has not been defined so far.

Patients: 25 pts., 2 of them with a 2nd NMSCT because of relapse or rejection (M: 14, F: 9; median age 43 years, range 19-61 y.) received NMSCT from related (n=16) or unrelated (n=7) donors. Diagnoses: ALL (n=7), AML (n=14), high-grade NHL (n=2), Stage I-III myelodysplastic syndrome (n=1), advanced disease (n=18). Indications for NMSCT: age > 50 y. (n=5), bad general condition (n=2), infection (n=10), relapse after standard SCT (n=8). Conditioning therapy: fludarabine, busulfan and ATG (Slavin, Blood 1998). DLI were given as available after NMSCT, if no GVHD had developed.

Patients were followed by repeated chimerism analysis of leukocyte subpopulations by multiplex PCR.

Results: 21/25 pts. engrafted, 2/25 reconstituted with leukemia, 2 had a graft failure. 14 pts. received DLI in escalating doses, 10/14 pts. developed GvHD after DLI. Mixed chimerism (MC) was observed in 10/12 evaluable pts. and DLI were given to 9 of them. MC was converted to full donor chimerism (CC) in 7/9 pts. Enduring MC resulted in relapse in all patients. After a median follow up of 7 months (1-28 mo.) 10 pts. are alive, 9/10 in CR; 6/10 have acute and/or chronic GvHD. 13 are dead, 12/13 from primary disease and 1/13 from GVHD and infection. 4/10 survivors had advanced disease before NMSCT.

Conclusions: Acute leukemias can be cured with NMSCT and DLI even at advanced stage, but relapse rate is still high. The risk for severe GvHD after DLI is high. MC bears a high risk of relapse in these patients. Chimerism analysis could guide preemptive DLI.

P583

Nonmyeloablative stem cell transplants: the Belgian Hematological Society Dose-finding Study


Background: Recent efforts to reduce transplant toxicity involve non-myeloablative preparative regimens combined with pre- and post-transplant immunosuppression (IS). A non-myeloablative conditioning regimen using elemental doses of cyclophosphamide (CPA) for lymphoid malignancies or cytarabine (ARA-C) for myeloid malignancies, combined with Fludara/ATG/cyclosporine as IS was prospectively studied. The primary objectives of this phase I-II trial are: engraftment rate and chimerism results.

Population: 36 pts received fludarabine 30 mg/m2/d x 4, ATG 10 mg/kg/d x 4, cyclosporine A (3 - 5 mg/kg/d IV) and CPA (1 g/m2/d x 3) or ARAC (2 g/m2/d x 4). CPA and ARA-C are reduced by 25% after 10 evaluable pts/arm. G-CSF mobilised HLA id (donor peripheral blood stem cells (5 x 106/kg CD34+ cell/kg pt weight) were used. Chimerism was assessed at day 30-45-60-90 for donor engraftment.
Results: Diagnoses included 6 AML, 7 MDS, 1 CML, 5 NHL, 9 MM, 5 CLL, 2 HK, 2 ALL. Median age was 51 (19-68) y.o. 42% pts were in complete remission (CR) before transplant. Median follow up is 10 months. No pt developed VOD or chemotherapy-related toxicity. All pts but one (MDS with MF) engrafted after transplant. Before d90, none of the pts in the CPA arm (0%) and 3/10 (30%) in the ARA-C arm developed acute GVHD. Chronic GVHD was observed in 2 pts. Infectious complications occurred in the CPA arm (27%). Overall survival in the good prognostic group is 92% and 50% in the poor prognostic group.

21 pts are evaluable for chimerism at day 60 and 12 (57%) pts have full T cell chimerism (> 90%), 8 pts (38%) have mixed chimerism and 1 pt (5%) has < 10% donor T cells. DLI were administered in 11 pts to push mixed chimerism to full chimerism. 10 Pts with CR before transplant are still in continued CR. 1 Pt with active or residual disease is in CR after transplant. 1 Pt relapsed after transplant. 1 Pt progressed after transplant. 6 Pts (19%) died of treatment-related complications.

Conclusions: These preliminary results suggest that this regimen allows haematological engraftment and can be administered safely to this older population. However, the low level of full T cell chimerism remains a concern. We are currently reducing the IS to improve donor engraftment.

P584

Minimal GVHD following nonmyeloablative in vitro T-cell depleted allogeneic stem cell transplantation which allows the infusion of graded increments of donor lymphocytes in patients with hematological malignancies and solid tumors.


High incidence of acute and chronic GVHD is observed after unmanipulated nonmyeloablative allogeneic stem cell transplantation. An in-vitro T-cell depleted nonmyeloablative stem cell transplantation protocol was investigated in eleven patients with high risk malignancies who failed conventional chemotherapy for leukaemia (4), lymphoma (3), breast cancer (2) and renal cell cancer (2). Median age was 47 years. Recipient conditioning consisted of fludarabine (30mg/m2, 6 days), ATG (10mg/kg, 4 days) and busulphan i.v. (3.2 mg/kg, 2 days). Oncology patients also received Cyclophosphamide (750 mg/m2, 2 days). High numbers of G-CSF mobilized peripheral blood stem cells from HLA-identical siblings were collected by leukapheresis (median 12 x 10.6 CD34+/kg). The graft was T-cell depleted by incubation with 20 mg Campath-1H for 30 minutes at room temperature and washed. The donors were HLA-identical siblings. All pts were grafted with unmanipulated total body irradiation /200 cGy; 7cGy/min/. The donors were HLA-identical siblings. All pts were grafted with unmanipulated PBSC and DLI and patients 19-21 CD34-selected PBSC and DLI and patients 19-21 CD34-selected PBSC followed by CD8-depleted DLI. Post-transplant immunosuppression was carried out with CyA and MMF. No patient developed significant regimen-related toxicities. Initial engraftment was seen in all patients, but 2 CML patients (patient 1 and patient 7) (13%) later rejected their graft. Total white blood cell, CD3-positive cell, CD13-positive cell and BM chimerisms were 90% (48-100), 94% (37-100), 95% (71-100) and 90% (7-99), respectively on day 100. The lowest figures were obtained in patient 1 who rejected her graft after day 100. The actuarial 180-day incidence of grade II-IV acute GVHD was 80% for patients 1-5 versus 11% for patients 6-21 (p=0.0002) and the 1-year incidence of extensive chronic GVHD was 100% for patients 1-5 versus 57 % for patients 6-19 (NS). Seven of 17 evaluable patients were in CR 180 days after the transplant and 3 others had partial responses. The 1-year survival rate was 54% and nonrelapse and relapse mortality rates were 24 and 22%, respectively. We conclude that CD8-depleted or CD34-selected NMSCT followed by CD8-depleted DLI is feasible and considerably decreases the incidence of acute GVHD while preserving engraftment and apparently also the GVL effect. Further studies are needed to confirm this encouraging preliminary report.

P586

Nonmyeloablative conditioning and peripheral hematopoietic stem cell grafting in poor-prognosis patients /pts/ with hematological malignancies

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We report our experience on 49 high-risk pts /11-chronic myeloid leukemia, 10-acute myeloid leukemia, 10-chronic lymphatic leukemia, 9-multiple myeloma, 5-non-Hodgkin’slymphomas, 3-myelofibrosis, l-chronic eosinophilic leukemia/. Twenty eight pts were male and 21 female /median age 51, range34-67/. The following coexistent diseases were present: arterial hypertension /4/, postinfarction angina /3/, mitral regurgitation with stenosis /2/, constrictive pericarditis /1/, kidney agenesis /1/, ulcerative colitis /1/, gastic ulcer /1/. For conditioning 45 pts received Fludarabine /30 mg/m2 for 6 days/, Busulfan /4 mg/kg for two days/, Antithymocyte globulin /10 mg/kg for 4 days/, and 4 pts low-dose total body irradiation /200 cGy; 7cGy/min/. The donors were HLA-matched identical siblings. All pts were grafted with unmanipulated peripheral blood stem cells (5.0 /1-13/ x106/kg CD34+/ cells). After grafting all pts received Cyclosporine A, and pts conditioned with TBI in addition mycophenolate mofetil. Seven pts did not reveal myelosuppression, with granulocytes >0.5 G/L and platelets > 20 G/L. Among the remaining, granulocytes > 0.5 G/L was reached at median of 14 /10-21/ days, and the recovery of platelets > 20 G/L was observed at median of 20 /13-30/ days.
Eight /16%/ pts died during the first 100 days. The following transplant-related complications were observed: acute GVHD /27/, CMV-infection /22/, bacterial infection /15/, chronic GVHD /14/, fungal infection /8/, renal failure /3/, hemolytic uremic syndrome /2/, aplastic anemia /1/, graft rejection /1/. At day +30, complete chimerism was obtained in 42% of pts and mixed chimerism in 46%. At day +60, complete chimerism was achieved in 29% of pts and mixed chimerism in 61%, and at day +90, complete chimerism demonstrated 38% of pts and mixed chimerism 48%. Nine pts received donor lymphocyte infusion and 5 achieved full chimerism. Twenty /40%/ pts died due to relapse, acute GVHD, fungal infection, CMV-pneumonitis, hemolytic uremic syndrome, acute renal failure, graft rejection and cardiac arrest. With a median follow-up of 12 /range: 1-33/ months, the overall and disease-free survival is 57% and 49%, respectively. In summary, our results are encouraging, however, a longer follow-up and larger group of pts is needed in order to evaluate the role of reduced conditioning and allografting in pts with hematological malignancies in advanced age and/or poor medical condition.

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Infectious morbidity after non-myeloablative allogeneic hematopoietic stem cell transplantation (AH SCT)

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Objective: Non myeloablative conditioning regimen (NMCR) for AH SCT have been developed to reduce procedure-related toxicity. The aim of this approach is to extend indications of AH SCT to patients (pts) who are not eligible previously for high dose chemotherapy or total body irradiation. We describe infectious morbidity related to this new procedure in our unit in a retrospective way.

Results: 45 AH SCT with NMCR were performed in our unit between 1997 to 2001, 32 males, 13 females, with a median age of pts and of donors was respectively 53 years (range, 39 -65). Hematologic diagnosis were 8 AL, 12 MM, 2 CML, 5 NHL, 3 MDS, 6 Hodgkin diseases, 3 CLL, 6 solid tumors. Engraftment was observed in all pts with a median of 21 days (range 12-49) for PNN > 0.5 G/l, and 18 days (range 0-201) for platelets > 50 G/l. Ten pts received donor lymphocyte infusions (DLI). Nineteen pts (48.7%) presented acute GVHD: 7 without any DLI, 4 before DLI and 8 after DLI (2 grade 1, 7 grade 2, 4 grade 3 and 2 grade 4).

Nineteen pts (42%) presented 39 infections: 1 episode, n = 7; 2 episodes, n = 7; 3 episodes, n = 3; 4 episodes, n = 1; 5 episodes, n = 1. Twenty two infections (56.5%) occurred in 13 pts during 3 months post transplant: 18 sepsisemia (12 Gram-negative bacilli, 5 Gram-positive cocci, one yeast), 1 septic shock without documentation, 2 cytomegalovirus (CMV) infections, 1 zoster. Only 4 of these infections occurred after engraftment (3 bacterial septicemia and 1 CMV disease). The pt with fungal septicemia died before engraftment. We observed 17 late infections (43.5%) in 11 pts: 3 septicemia, 1 septic shock without documentation, 2 CMV diseases, 6 probable invasive aspergillosis, 1 zoster, 1 Campylobacter colitis, 1 Staphylococcus aureus pneumonia, 1 EBV lymphoma and 1 enterovirus encephalitis. Overall, bacterial infections were observed in 56% of cases, viral in 20% and fungal in 18%. No infection occurred in 26 pts (58%).

Five pts with hematologic malignancy relapse presented a probable invasive aspergillosis.

Twenty eight pts died (62%), 16 in relapse, 9 (32%) with infection: 1 fungal septicemia, 1 viral encephalitis, 1 hepatitis, 6 invasive aspergillosis.

Conclusion: Most of infections observed after AH SCT with NMCR were bacterial in our experience.

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Low Mycophenolate mofetil (MMF) draft serum levels correlate with the occurrence of acute GVHD grades II-IV after allogeneic hematopoietic stem cell transplantation (HSCT) following low dose TBI with/without Fludarabine and immunosuppression with CyA/MMF

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Background: A combination of 200cGy TBI and postgrafting immunosuppression with CyA and MMF allowed stable allogeneic engraftment in a canine model. This regime has been recently applied with low transplant related mortality (TRM) in clinical protocols using related and unrelated donors in patients. Acute GVHD incidence, however, was high ranging between 50 and 60 %. In order to identify risk factors for the occurrence of GVHD, MMF draft levels were analysed and correlated with the presence of acute GVHD.

Patients and Method: Seventy-four patients with haematological disease transplanted between 1998 and December 2000 were included in this study. Median age was 55 years. Thirty-nine patients (52.7%) received grafts from related donors, the remaining from unrelated donors. MMF was given p.o. 15 mg/kg q 12 hours starting on day 1 and continued until the observed period (28 days after HSCT). Because of gastrointestinal side effects, MMF was given i.v. 15 mg/kg q 12 hours to 11 patients on day 7 and 6 patients on day 21 instead of p.o.. Serum levels of the active metabolite mycophenolic acid (MPA) were measured before drug delivery on day 7 and 21 after allogeneic HSCT by the EMIT-MPA assay (Behring).

Results: Median draft MPA serum levels were 1,01µg/mL and 1,18 µg/mL on day 7 and on day 21 respectively. After oral administration values were lower (median 0,96µg/mL; range: 0,2-10,2µg/mL on day 7 and 0,97µg/mL; 0,19-1,69µg/mL on day 21) than the one observed after i.v. infusion (median 1,64µg/mL; range:0,61-15,0µg/mL on day 7 and 1,64µg/mL; 0,57-1,9µg/mL on day 21). Five patients rejected their graft. No differences in serum draft MPA levels were observed between patients with and without rejection. However, patients with acute GVHD grades II-IV had lower MPA levels on day 21 after p.o. administration compared to patients without GVHD ( median 0,69µg/mL vs. 1,24µg/mL, p<0,005 Mann-Whitney U-test).

Conclusion: Slightly higher draft MPA levels were observed after i.v. compared to p.o. administration. A statistical significant difference in draft serum MPA levels on day 21 after HSCT was observed in patients with acute GVHD grades II-IV showing lower values than patients without GVHD. This finding confirms the importance of evaluating MPA levels after HSCT with minimal preparative regimen. Protocols with dose-adjusted MMF therapy are currently investigating the possibility to reduce acute GVHD incidence by optimising MMF dosing.

P589

Allogeneic peripheral blood progenitor cells (PBPCs) transplantation after nonmyelo ablative regimen (NMR) in patients with hematological malignancies


NMR followed by grafting of allogeneic PBPCs is an attractive strategy for reducing the toxicity of allograft procedures and may permit the exploitation of any potential GVT effect. Between Feb.2000 and Nov.2001,11 patients (pts.) underwent PBPCs transplantation from HLA identical siblings after NMR. The underlying disease were MM in 4pts,(2 PR and 2 refractory) NHL in 2pts,(1 in CR-bcl-1 positive and 1 refractory), AML in 2pts(CR), HDG in 1pt.,1 solid tumor and 1 acute myelofiobrosis. The median age of pts. and of donors was respectively 53 years (range,39-65) and 50 years (range,42-64). There were 5 sex mismatched transplantations. As conditioning regimen pts received Mel
and longterm observations are necessary to prove this. 

Patients with DC/HH and presumably in other diseases with DNA-molluscum contagiosum disappeared completely. One year after including TBI is a well tolerated and safe conditioning procedure in rejection-and GVHD-prophylaxis. The transplant was completely cessation of CSA. Combined immunodeficiency, hematological autosomal recessive DC. These symptoms disappeared after transplant and complete chimerism (CC) was obtained with stable engraftment. CC was obtained in 62% of patients after a median time of 42 days (15-56). DLI was used in 1 patient affected by MM for persisting disease:after 2 weeks skin and gut GVHD developed. Acute GVHD was seen in 6 patients (54%): I°in 4pts (36%) and >=II°in 2 (18%). 1pts. died in +65 for gut acute GVHD. Two pts. developed extensive chronic GVHD (18%) and 4pts. (36%) limited cGVHD. After a median follow-up of 318 days (range,28-652) overall survival (OS) was 73%; 7pts. achieved complete remission (64%) with full donor chimism,2pts. relapsed respectively 14 and 13 months after transplantation without loosing donor chimera. TRM at 11 months was 18%. These preliminary results show that NMR is well tolerated, have a low risk of TRM and is able to ensure a sustained engraftment.

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Successful stem cell transplantation after non-myeloablative conditioning (Flu/2 Gy TBI) in autosomal recessive Dyskeratosis congenita (DC)/Hoyeraal-Hreidarsson (HH) syndrome

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A 9-year old girl of Swiss consangineous origin with combined immunodeficiency presented with recurrent bacterial, pneumocystis carinii, and disseminated molluscum contagiosum skin infections. She showed neither symptoms of DC nor neurological impairment and was transplanted with G-CSF stimulated PBSC (10 x 106/kg CD34; 96 x 106/kg CD3) from her 11 year-old HLA-identical healthy brother. Eight years ago her older brother had succumbed to Hoyeraal-Hreidarsson Syndrome. Chromosomal breakage studies had been normal in all family members. We chose a non-myeloablative conditioning regimen consisting of Fludarabine 30 mg/m2 (days -5, -4, -3) and 2 Gy TBI (0.07 Gy/min), since we assumed autosomal recessive dyskeratosis congenita and worried about the well-known high transplant-related mortality. MMF/CSA was administered for rejection-and GVHD-prophylaxis. The transplant was completely uneventful and successful with a neutrophil take at day +16 and discharge at day +57. After PBSC, she developed lingual leukoplakia and nail dystrophies, confirming the diagnosis of autosomal recessive DC. These symptoms disappeared after cessation of CSA. Combined immunodeficiency, hematological abnormalities and therapy-refractory skin infections with molluscum contagiosum disappeared completely. One year after PBSC donor chimerism was 100%, lymphocyte mitogen and antigen proliferation as well as pulmonary function tests were normal. Conclusion: PBSC with a minimal conditioning regimen including TBI is a well tolerated and safe conditioning procedure in patients with DC/HH and presumably in other diseases with DNA-repair or cell cycle disorders, like Fanconi’s anemia. Larger studies and longterm observations are necessary to prove this hypothesis.
Nonmyeloablative stem cell transplantation in refractory or relapsed Hodgkin’s disease


Conventional allogeneic stem cell transplantation (allo-SCT) is associated to a high transplant related mortality (TRM) in Hodgkin’s disease (HD). In an effort to decrease TRM, reduced-intensity conditioning regimens have been developed. We have analyzed the outcome of a group of 28 relapsed or refractory HD pts conditioned after a reduced intensity regimen using an HDLK identical sibling (n = 24) or an unrelated donor (n = 4). There were 11 males and 17 females with a median (range) age of 35 (19 - 58) years at transplantation. All pts had been heavily pre-treated before allo-SCT: 24 (86%) had been previously autografted. Fourteen pts were autografted in resistant relapse (RR), and 14 in sensitive relapse (SR). Seven pts (25%) had an ECOG = or > 2 at allo-SCT. Conditioning regimens consisted of the combination of fludarabine (150 mg/m2 iv, total dose) plus melphalan (80 – 140 mg/m2 iv, total dose) in 18 pts, plus busulfan (8 mg/kg po, total dose) in 5 pts or plus cyclophosphamide (500 mg/m2 iv x 5 days) in 2 pts and fludarabine (90 mg/m2 iv, total dose) + TBI 200 cGy in the remaining 3 pts. Acute GVHD prophylaxis was with cyclosporine A (CyA) + methotrexate in 21 pts. CyA + mycophenolate mofetil in 4 pts, CyA + prednisone in 1 and CyA alone in 2 pts. Hematological toxicity was low with a number of days with neutrophils < 0.5 x 10⁹/l and platelets < 20 x 10⁹/l of 10 (range 0 to 16) and 2 (range 0 to 27), respectively. Grade = or > 2 aGVHD developed in 9 out of 26 evaluable pts (34%); 6 out of 17 pts evaluable for cGVHD developed an extensive form. Fourteen pts were alive with a median follow up of 8.5 (range, 2 – 27) months after transplantation and 14 have died of transplant-related complications (n = 9) or disease progression (n = 5). Actuarial TRM at 6 mo is of 25%+/−9% (10%+/−8% in SR and 39%+/−13% in RR). One year progression free and overall survival of the whole series are 33%+/−11% and 54%+/−11%, respectively. Allogeneic transplantation after a reduced-intensity conditioning protocol is feasible in heavily pretreated HD pts and represents a therapeutic option for pts otherwise not candidates for other strategies. Acute and cGVHD remain a significant problem and TRM is still high in patients allografted in RR. Longer follow up, higher numbers of pts and probably, more restrictive inclusion criteria are needed to evaluate the potential role of allo-SCT after a reduced-intensity conditioning regimen in HD.

Effective immunotherapy of resistant indolent lymphoma/CLL by reduced-intensity allogeneic stem cell transplantation (SCT)

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In indolent lymphoma the fludarabine / cyclophosphamide combination (FC) is an effective salvage therapy. As this regimen is also a potent immunosuppressant, we studied the use of FC for conditioning for allo-SCT of patients who had failed conventional treatment or auto-SCT for these diseases. 21 patients (CLL/immunocytoma=13, follicular lymphoma=4, mantle cell lymphoma=2, myeloma=1, AILD=1) were eligible. Median age was 51 (35-63) years. Previous therapy consisted of 4 (1-7) regimens. Autologous SCT had failed in 9 patients. 18 patients were in partial remission and 3 patients were refractory at time of transplant. Conditioning regimen consisted of daily F 30mg/m² and C 500 mg/m² over 5 days. GVHD prophylaxis was performed with CSA/short course MTX. PBSC were obtained from HLA-identical donors. Median dose of CD34 positive cells was 4.6x10E06 (1.2-10.9) x10⁶/kg. Results: In 14 patients with a median time to neutrophil recovery of 14 (5-26) days and a median number of 2 (0-63) transfused platelet units. Chimerism kinetics were delayed (median time to >95% chimerism 108 days) and required cessation of CSA (n=6) or DLI (n=3) in the majority of cases. The probability of being a complete chimera at 9 months post SCT was 93%. 2/21 patients succumbed to early treatment related death. Otherwise, non-hematological toxicity was very low. 11 of 15 evaluable patients (73%) experienced acute and/or chronic GVHD. Ongoing responses developed over time in 13 of 14 patients evaluable (time to best response 3 (0-6) mos) and were preceded by the onset of chronic GVHD in 5 cases. With 12 (1-32) months of follow-up, 2-year overall survival is 89% (95%CI 84-100%) and progression free survival is 83% (95%CI 66-100%). Conclusions: FC allo-grafting is well tolerated, allows establishment of complete hematopoietic chimerism, and mediates potent immunotherapeutic activities by graft-versus-lymphoma effects. It promises to be a potentially curative salvage regimen for resistant indolent lymphoma or CLL.
Allogeneic stem cell transplantation (SCT) with dose-reduced conditioning for chronic lymphocytic leukemia (CLL): role of consolidation with rituximab

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Allogeneic SCT can induce durable responses in 40-60% of pts with CLL. However, it may take up to 24 months for maximum responses to occur. In addition, 50% of allografted pts ultimately die from persistent or recurrent CLL. We hypothesized that consolidation therapy with the anti-CD20 monoclonal antibody rituximab (R) would improve residual disease control and, hence, the prerequisites for late GvL effects.

Patients and Methods: 10 pts (median 50 years) with advanced B-CLL were enrolled. Median time from diagnosis to SCT was 32 months (range 1-172). Pts had received a median of 4.5 (1-9) treatment cycles. At SCT, 77% had progressed, 23% had stable disease. Median white cell (WBC) count was 7.0 (1-29) x 10^9/L. 6 pts had symptomatic disease. All pts were HLA-matched, 7 with good quality HLA-A, B, DR typing.

Conditioning included bendamustine (Bend), cyclophosphamide (Cy), fludarabine (Flud) with/without rituximab (Rtx), tapering from d+100 to d+128. While on stable WBC counts, A with “short course” MTX was given for GvHD prophylaxis and cyclosporin A until day 100 and short course Methotrexat.

Cytometric quantitation of marrow CD5+/CD20+(CD19+) cells as well as qualitative and quantitative clonotypic PCR using patient-specific CDRIII primers.

Results: At the time of writing, 2 pts were too early for evaluation and 1 pt died early from PD after secondary graft failure. The other 7 pts transplanted from matched related (6) or unrelated (1) donors had complete (6) or partial (1) donor chimerism at engraftment. Five of those received R consolidation. One pt was withdrawn because of a history of hepatitis B. R was tolerated without clinically overt adverse events. Two pts subsequently experienced grade 2/3 bacterial infections, while grade 4 infections, reactivation or infection of CMV did not occur. Acute GvHD < III° developed in 4, chronic in 4 pts (extensive 2, limited 2). At d+45, 4/5 pts receiving R were in PR, and one had SD. Two pts were PCR-positive, one with detectable levels of CD5+/CD20+(CD19+) lymphocytes in the marrow. In both pts with immunophenotypic or molecular markers, a significant reduction of marrow CLL cells could be demonstrated. After a median follow up of 331 days (107-560), all 5 pts are alive and well: 3 in immunophenotypic and molecular remission, 1 in improving PR, and 1 with relapse on d+516 scheduled to receive DLT.

Conclusions: Consolidation therapy with R after allogeneic SCT for CLL is well tolerated and adds to the control of residual disease, thus optimizing the conditions for late GvL effects.

Fludarabine reduced conditioning followed by allogeneic stem cell transplantation in patients with myelofibrosis with myeloid metaplasia


Myelofibrosis with myeloid metaplasia (MMM) is a chronic myeloproliferative disorder with a median survival of less than 3 years. Allogeneic stem cell transplantation might cure this disease, probably due to graft versus myelofibrosis effect. We report on 3 patients with MMM who received stem cell transplantation from HLA matched related (n=2) or HLA-matched unrelated donor (n=1) after a dose reduced conditioning regimen consisting of busulfan (8mg/kg), fludarabine (180 mg/m²) and ATG (4x10^8 cells). Host disease (GvHD) prophylaxis was carried out with cyclosporin A until day 100 and short course Methotrexat.

All 3 pts engrafted with a leukocyte count >1/nl after a median of 18 days (range 16-20). At day 30 complete chimerism was seen in all three pts. One patient developed grade II acute skin GvHD. Chronic GVHD was observed in 2 of the pts, one with limited and one with extensive chronic GvHD. Liver toxicity was observed according to the Bearman score level I (n=1) and level II (n=2). All 3 pts developed mucositis according to the Bearman score level II.

Stem cell source was bone marrow (n=1) or peripheral blood stem cells (n=2). Host disease (GvHD) prophylaxis was carried out with cyclosporin A until day 100 and short course Methotrexat.

Allogeneic transplants use conditioning based on experiences from the 1970's. Now minitransplantation protocols have shown the possibility for allografting with window immunosuppression, providing opportunities for graft-versus-malignancy (GvM). However, 2nd generation protocols have limitations from early tumor progression, mixed chimerism requiring DLI to achieve full chimerism, high risk for GvH during conversion, and slow development of GvM. We have developed a 3G protocol aiming at optimizing tumor control with retained low non-hematopoietic toxicity, and early full donor chimerism. We sought for oral treatment and easy handling for patients and staff, using experiences in part from non-transplant studies. Our current protocol includes Fludarabine 50 mg and oral Cyclophosphamide 300 mg/day, d –5 through –1 (Ara-C 1 g/sm qm iv instead of Cy for acute leukemia), Melphalan inj d –1, 40-80 mg/sm depending upon tumor status, Campath sq inj 30 mg days –3 through –1, oral cyclosporin and MMF. Unmanipulated mobilized blood stem cells were preferred, and aliquots were frozen for future use as DLI. Frequent monitoring of tumor markers and chimerism were mandatory, and dose-adjusted DLI given if required. We have treated 45 patients with our adjusted protocols, aged 54 years (9-66), with ALL 3, AML 8, CML 10, CLL 3, myeloma 19, NHL 1, other 1. 11 had low risk score (AML CR1, CML COP1), 11 had intermediate risk (ALL CR, AML CR2, others in remission), whereas 23 had high risk (CML BC, myeloma progression post AutoSCT, or refractory).

Stem cell source was blood from siblings in 30, unrelated blood cells in 10 and unrelated marrow in 5. Conditioning induced rapid lymphopenia, but delayed other cytopenias. A median of 5.8 units red cells and 4.5 units platelets were given, and median days with fever>38°C was 6.2. CMV-reactivation was frequent, but GvH moderate. With a median observation of 1 year, the transplant-related mortality was 8/23 (35%) in high risk and 0/22 in low and intermediate risk patients, (n=4) and 0 meditation mortality 2/23 (8.7%) in high risk and 0/22 in low risk. Overall 2-year survival is 75%, (100% in 22 low- and intermediate risk patients). These results compare beneficially with reported data, while being a higher and risk profile. We believe that our protocol is cost-effective, and improves short-term toxicity, transplant-related mortality, but also tumor control as compared to conventional regimens.

Third generation (3G) conditioning for allogeneic transplantation in a single center setting: Low transplant-related mortality and relapse mortality

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Most allogeneic transplants use conditioning based on experiences from the 1970's. Now minitransplantation protocols have shown the possibility for allografting with window immunosuppression, providing opportunities for graft-versus-malignancy (GvM). However, 2nd generation protocols have limitations from early tumor progression, mixed chimerism requiring DLI to achieve full chimerism, high risk for GvH during conversion, and slow development of GvM. We have developed a 3G protocol aiming at optimizing tumor control with retained low non-hematopoietic toxicity, and early full donor chimerism. We sought for oral treatment and easy handling for patients and staff, using experiences in part from non-transplant studies. Our current protocol includes Fludarabine 50 mg and oral Cyclophosphamide 300 mg/day, d –5 through –1 (Ara-C 1 g/sm qm iv instead of Cy for acute leukemia), Melphalan inj d –1, 40-80 mg/sm depending upon tumor status, Campath sq inj 30 mg days –3 through –1, oral cyclosporin and MMF. Unmanipulated mobilized blood stem cells were preferred, and aliquots were frozen for future use as DLI. Frequent monitoring of tumor markers and chimerism were mandatory, and dose-adjusted DLI given if required. We have treated 45 patients with our adjusted protocols, aged 54 years (9-66), with ALL 3, AML 8, CML 10, CLL 3, myeloma 19, NHL 1, other 1. 11 had low risk score (AML CR1, CML COP1), 11 had intermediate risk (ALL CR, AML CR2, others in remission), whereas 23 had high risk (CML BC, myeloma progression post AutoSCT, or refractory).

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Allogeneic blood stem cell transplantation after treosulfan and fludarabine conditioning


Treosulfan and fludarabine, used as conditioning agents for allogeneic blood stem cell transplantation, have been used in a phase II study (29 patients otherwise noneligible for allogeneic transplantation with a median age of 49 years have been transplanted with CML, AML, MDS, multiple myeloma, NHL, cbcc, t-NHL and CLL). 11 matched related donors and 15 matched unrelated donors (MUD) donated bone marrow or peripheral blood stem cells. Conditioning consisted of treosulfan 10 g/m² i.v. d-6 to d-4 and fludarabine 30 mg/m² i.v. d-6 to d-2. Patients with unrelated donors received ATG (rabbit) 10 mg/kg d-4 to d-2. Cyclosporin A was given as GvHD prophylaxis only. Leukocyte recovery > 0.5 Gpt/l occurred by median day +9. Platelet recovery (>50 Gpt/l) occurred by median day +15. Extramedullary toxicity was mild not exceeding CTC °II except for ALAT/ASAT elevation in 7, bilirubine in 3 pat. and creatinine in 1 pat.. In the pat. with CTC °IV toxicity was most likely related to severe septic shock. Three of these 7 patients had started the conditioning therapy with a liver enzyme elevation. Additionally in one patient a peripheral polyneuropathy occurred (CTC °III) – this patient had been heavily been pretreated and the cause of this polyneuropathy remains still unclear. Complete chimerism was reached in 21 of 26 pts. by day +28 (1 not evaluated due to septic shock). One pat. had a primary graft failure and recovered with his own hematopoiesis. One pat. had a therapy refractory CLL before transplantation and the other had an increasing chimerism after day +28. Once reached complete donor chimerism has been maintained by all pts. except for 4 pts.. Three pts. with CML had an cytogenetic relapse and one pat. with a multiple myeloma – only in PR after 2 directly preceding autologous transplantations – had only a short lasting disappearance of the aneuploid cell clone. After reduction of the immune suppression (2) or DLI (3) all came into a complete remission. 1 pat. with a high grade NHL relapsed d+113. The treatment related mortality after a median observation time of 345 days is 19% (6/26). One additional patient with multiple myeloma died due to complications of his accompanying cardiac amyloidosis. With an overall survival of 73% (19/26) further evaluation of the treosulfan and fludarabine combination may lead to a promising toxicity reduced conditioning regimen with sufficient stem cell toxicity, immunosuppression and anti-leukemic activity.

P600

Tacrolimus (FK506) and mycophenolate-mofetil (MMF) promote engraftment and prevent severe acute GvHD after allogeneic blood stem cell transplantation with dose-reduced conditioning

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Low intensity conditioning transplants have become a novel tool in the treatment of hematological neoplasias. Minimal organ toxicity and absence of aplasia are main arguments. Still, few data about kinetics of haematopoietic reconstitution are known. We analysed 26 patients with hematologial malignancies (14 males, 12 females; median age 51.5 years, range 25 - 63) receiving low intensity conditioning transplants, based on Fludarabine (3x30mg/m²), TBI (2 Gy), Cyclosporine and Mycophenolate. Diagnoses were: AML, 4 CML, 5 CLL, 3 MPS, 4 MDS, 3 MM, 3 NHL. 23 patients were in advanced stage of their disease at HSCT. For 21 patients donors were HLA identical siblings. Four patients received a matched unrelated, 1 patient a one antigen mismatched graft. A median dose of 7.09 x 106/kg unmanipulated CD34+ cells was infused. Three patterns of recovery were observed: 16 patients did engraft (group A), 7 patients failed to engraft (group B) and 2 patients did engraft but they were in aplasia at the time of conditioning (group C). Table 1 shows the neutrophil (PNC) and platelet (PLT) recovery profile.

P601

Hematopoietic recovery after low intensity conditioning transplants


Background: Randomized studies after conventional blood stem cell transplantation have suggested that FK506 has at least non-hematologic toxicity >/=II. In both patients who were switched from CsA to FK506 because of severe nausea and vomiting symptoms disappeared shortly after cessation of CsA. All evaluable patients (7) engrafted and converted to complete donor chimerism (>97% donor cells, PB) after a median of 35 days (range 16-49). One of 7 patients developed acute GvHD (I°) while on targeted FK506 levels. Following FK506 taper between day +50 and +100, 2 of 4 patients developed acute GvHD (1x I°-II°, 1x II-IIII°) which again improved with intensified FK506 therapy. At present 7 patients are alive with a median follow up of 91 days (range 15-238). One patient (NHL) died of progressive disease.

Conclusion: Our data suggest, that the combination of FK506 and MMF is well tolerated after dose-reduced allogeneic blood stem cell transplantation. In this setting trough serum FK506 levels between 8 and 15ng/ml effectively promote engraftment and prevent severe acute GvHD even in matched unrelated donor transplantation.

S152
Are donor leukocyte infusions (DLI) a systematic part of nonmyeloablative hematopoietic stem cell transplantation procedure? Study of their impact

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Although increasing numbers of non-myeloablative allogeneic stem cell transplantation (NSTCT) are performed worldwide, most of them do not include DLI. We analyzed the impact of DLI on chimerism (semi-quantitative VNTR), disease response and graft-versus-host disease (GVHD). 44 patients [29 males/15 females; median age: 48 years (18.5-63)] underwent NSTCT. Diagnosis pre-transplant was: 2 ALL, 5 AML, 2 CML, 2 CLL, 3 Myelodysplasias, 11 MM, 12 Lymphomas (6 Hodgkin, 6 non Hodgkin) and 6 Solid Tumors. 23 patients were responders prior conditioning; 7 in complete remission (CR1; 4, CR1-3; 1) and 16 in partial remission, 21 were non-respondents: 15 in progressive disease and 6 in relapse. For conditioning regimen, 26 patients received the association of Fludarabine/Busulfan/ATG, 8 Cytoxan/ATG, 3 Idrarubicine/Fiudarabine/AraC, 5 Cytoxan/TBI (6g/s)/ATG, all patients received CsA + Mtx as GVHD prophylaxis. Three patients received TBI (2g/s)/Fiudarabine/CsA/MMF. Twenty-six received BMT from HLA identical sibling donors. The other patients (75%) received a median number of 1.5 (1-4) DLI (escalating dose regimen) after transplants at a median interval of 3 months (1-15.15). At the last follow-up 59% of patients receiving DLI achieved a complete donor chimerism and only 40% among patients without DLI, but the difference didn’t reach a significant level (p=0.38). Among pre-transplant responders, 66% of patients with DLI and 66.5% without DLI remained responders (p=1) and among pre-transplant non-responders, 37.5% achieved a disease response after DLI versus 40% for patients without DLI (p=1). Among the 33 patients, who received DLI, 8 (24%) developed aGVHD: 1 grade I, 3 grade II, 3 grade III and 1 grade IV. At the last follow-up, 13 (40%) patients who received DLI and 4 (40%) who did not, remained alive. So far, in our experience, DLI seems to enhance complete chimerism after NSTCT without induction of high incidence of severe GVHD (related to DLI escalating doses), but doesn’t show any effect on disease status yet.

P603
The myeloablative and immunosuppressive properties of treosulfan in mice

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Treosulfan (Tr) has been shown to have myelo and immunosuppressive properties. In the present investigation we compared the myelosuppressive effect of Tr to that of liposomal busulfan (LBu) and cyclophosphamide (CP) and the immunosuppressive effect of Tr to that of CP in Balb/c mice. Mice were treated with either Tr 1.5 g/kg/day for 3 days, LBu 10 mg/kg/day for 4 days, or CP 0.1 g/kg/day for 2 days. The animals were sacrificed at days 1, 3, 6, 9 and 12 post treatment. Bone marrow and spleenocytes were collected. The myelosuppressive effect was assessed using CFU-GM clonogenic assay. The immunosuppressive effect was evaluated by following the lymphoid depletion in spleen, flowcytometric analysis of spleenocytes CD3+/CD19+, CD4+/CD8+ ratios and the intracellular levels of IL-2, TNFa and IFNg after mitogenic stimulation. Tr and CP showed a rapid depletion of CFU-GM in bone marrow reaching nadir on day 1 while a nadir was reached on day 6 after treatment with LBu. Both Tr and LBu showed stable myelosuppression without recovery until day 12. Both Tr and CP showed 6-fold depletion in spleen lymphoid population by day 1, with lymphoid reconstitution by day 9 in CP treated animals. In contrast, lymphoid depletion was stable through day 12 after Tr treatment. Both drugs were more cytotoxic against CD19+ cells compared to CD3+ cells. Moreover, CD19+/CD3+ ratio showed a trend of returning to pre-treatment values in CP but not in Tr treated mice. No change in CD4+/CD8+ ratios was observed after CP or Tr treatment. IFNg response was inhibited in stimulated spleenocytes at day 3; however, IFNg response was normalized to pre-treatment values at day 9. In contrast, the TNFa and IL-2 response to mitogenic stimulation did not change after treatment with both drugs. In conclusion, treosulfan shows stable myelosupression comparable to that observed after LBu with no recovery in CFU-GM until day 12. Tr showed similar immunosuppressive effect expressed as lymphoid depletion and CD3+/CD19+ and CD4+/CD8+ depletion compared to CP. In conclusion, Tr on the lymphoid compartment seems to be stable. Treatment with Tr resulted in decrease in IFN-g. These may decrease the cytokine storm during conditioning prior to SCT.

P604
Kinetics of donor engraftment following nonmyeloablative unrelated donor transplantation using lineage-specific PCR

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Campath-1H, a humanised monoclonal antibody directed against human lymphocytes, has previously been demonstrated to facilitate durable engraftment whilst reducing the incidence of GVHD when given in addition as part of a non-myeloablative transplant conditioning regimen. We investigated the engraftment kinetics associated with this regimen during the first 3 months post transplant in 5, matched unrelated donor, patients. All patients received Campath-1H, 20mg (day - 8 to day - 4), fludarabine, 30mg/m2 (day - 7 to day - 3) and melphalan, 140mg/m2 (day - 2). Cyclosporin A (3mg/kg) was started on day -1 as additional GVHD prophylaxis. Four patients received granulocyte-colony stimulating factor (G-CSF) mobilised peripheral blood stem cells (PBSC) and one received unmanipulated marrow. Using microsatellite polymerase chain reaction techniques, the engraftment kinetics were studied via lineage-specific chimerism analysis of donor and recipient T, B and myeloid cells. Samples for analysis were taken every 3 days from day +1 up to and including day +30, then again on days +60 and +90. Mixed chimerism in myeloid and lymphoid lineages was seen in all patients on days +1 and +3. On day +6 three of the five patients were found to be full donor lymphoid chimeras and by day +12 all patients had achieved full donor chimerism, which continued until day +90. Currently, two patients exhibited full donor chimerism in all lineages at day +60. The other patient had mixed T-cell chimerism whilst retaining full donor chimerism in both B-cells and the myeloid cells at day +90 had mixed chimerism in both T and B-cells, the myeloid compartment however, remaining full donor. The only patient to develop GVHD (grade II cutaneous and gastrointestinal) received PBSC and has retained full donor chimerism in both compartments since day +6. Preliminary results therefore show that non-myeloablative conditioning regimens containing Campath-1H given in vivo can be seen to induce rapid full donor chimerism. There is therefore potential for a GVL effect preventing disease progression, without severe GVHD.

P605
Monitoring of donor cell chimerism (DCC) in marrow CD34+ cells after allogeneic SCT: prediction of relapse in CD34+ acute myeloid leukemia (AML)

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From 05/99 to 10/01 15 patients with high-risk AML underwent unmanipulated allogeneic PBSC (14) or BM (1) transplantation. GVHD prophylaxis included related (9), mismatched related (5), or mismatched related (1) donors after dose-reduced TBI-based conditioning (8Gy/Fludarabine/ATG). Donor derived hematopoiesis was assessed using multiplex PCR amplification of polymorphic short-
tandem repeats (STR-PCR) with fluorescence detection. DCC kinetics were prospectively monitored in serial samples of total nuclear cells (MNC) and of flow sorted cellular subsets from BM (CD3+, CD19+, CD34+). DCC dynamics were compared to data obtained from marrow cytology, cytogenetics, and flow cytometric immunophenotyping. Results: The median number of informative alleles was 6 (range: 2-9). 14 of 15 patients achieved molecular engraftment with complete overall DCC in BM. One patient with incomplete DCC developed graft failure. Relapse was detected by BM cytology in 5 patients and by cytogenetics in one patient (recurrence of informativ chromosomal aberrations). Five of these patients showed increased expression of the CD34 antigen on the leukemic clone and had a > 20% decrease in BM CD34+ DCC to a median of 10% (range: 1-76) by the time relapse was diagnosed. In contrast, DCC in total BM MNC as well as in lymphocyte subsets was not informative (median/range - BM MNC: 65%/18-83%; CD3+: 96%/87-96%; CD19+: 95%/87-99%). Beyond day +30 after transplantation, a CD34+ DCC < 80% in BM was highly indicative for imminent or overt relapse (6/6 patients), while 7/8 patients with sustained CD34+ DCC > 80% remained in remission after a median follow-up of 237 days (range: 58-641) (p < .05). In 2 patients, decreasing CD34+ DCC preceded overt relapse detectable by BM cytology or cytogenetics by 40 and 60 days, respectively. In both patients, complete remission and complete DCC were reestablished after donor lymphocyte transfusions +/- chemotherapy. Conclusions: Monitoring of CD34+ DCC in BM is a valuable method for the detection of imminent relapse in AML with CD34 expression by the leukemic clone. It provides an additional criterion for timely treatment interventions.

P606

Kinetic of CD3, CD33, CD34 positive donor cells and LTC-IC after allogeneic non-myeloablative stem cell transplantation: significant prognostic impact of donor T-cell chimerism on disease free survival and suppression of LTC-IC after non-myeloablative conditioning

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Non myeloablative allogeneic stem cell transplantation (SCT) allows establishment of donor hematopoiesis without eradication of recipient stem cells by chemoradiotherapy. This procedure has a decreased conditioning related toxicity but graft rejection and GVHD remain clinically challenging. In the case of mixed chimerism monitoring of chimerism of T cells and hematopoietic precursor cells is of high importance. It allows to define origin of hematopoietic recovery and to manipulating favorably the graft by increasing donor leukocytes or inducing T-cell rejection. LTC-IC in BM is a method for the detection of immnosuppressive therapy. We performed dose reduced SCT with conditioning the recipient with fludarabine 90 mg/m2 followed by TBI of 2 Gy and infusion of unmanipulated PBSC. Cyclosporine and mycophenolate mofetil were given to prevent GVHD and rejection. Chimerism was evaluated in various cell subsets including myelo (CD3+, CD34+) and lymphatic lineage (CD3+, CD19+ CD4+ CD8+). There was an excellent correlation of donor chimerism between CD33 and CD34 positive cells. In case of sex mismatch between recipient and donor monitoring was performed by FISH, otherwise by quantitative variable number of tandem repeats (VNTR)-PCR. The correlation between both methods was 98.7. Additionally the kinetic of early and late stem cells before and after reduced conditioning therapy was investigated. Although the conditioning therapy was nonmyeloablative we saw a significant decrease of repopulating stem cells defined as long term culture initiating cells (LTC-IC) to 22% but a recovery of LTC-IC to 150% was seen for weeks after transplantation. So far, 31 patients 7 with AML, 4 with MDS, 10 with NHL, 1 with CML, 5 with MM and 4 with renal cell carcinoma were analysed. Median age was 55 with a range from 18 to 70 years. All patients who had less than 40% of donor T cells four weeks after SCT (7 of 31) (Group A) relapsed one experienced graft rejection whereas all other patients (24 of 31 Group B) showed stable mixed or complete myeloid and lymphatic donor chimerism between one and 16 months after SCT. Disease free survival analysed was significantly better in the patients in group B calculated with the product limit method, according to Kaplan and Meier (p<0.00005) if compared with group A. These results emphasize the importance of sequential analysis of lineage specific chimerism to predict outcome after allogeneic nonmyeloablative SCT with dose reduced conditioning.

P607

Lymphocyte reconstitution after transplantation with matched unrelated or related donor PBPC with reduced conditioning (Fludarabin/BCNU/Melphalan)

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The introduction of allogeneic peripheral blood progenitor cell (PBPC) transplantation has allowed for a reduced intensity conditioning (RIC) therapy and has extended its application to patients beyond the age of 55 years. While short term effects have been well documented, longterm immune reconstitution, especially after unrelated donor transplantation, has not extensively been studied so far. Here we present the quantitative lymphocyte reconstitution of 63 patients (median age 55.5 years, range 24-71 years) with hematologic (24 AL, 5 MPS, 9 MDS, 8 MM, 10 NHL) or solid organ disease (4 RCC, 3 melanoma, 3 colorectal cancer) transplanted with PBPC from matched related (n=43, group A) or unrelated (n=20, group B) donors. All patients were treated with a uniform conditioning regimen containing fludarabine, BCNU and melphalan (FBM; Wäsch et al., BMT 26:243, 2000). GvHD prophylaxis consisted of CSA combined with MTX or MMF and additional ATG-S (Fresenius) in unrelated donor transplantation. Groups A and B were separatedly analyzed. T-lymphocytes reached normal levels by 6 months, mainly due to rapid regeneration of CD8+ T-cells (6 months geometric mean of 443/µl and 567/µl in group A and B, respectively). While CD4+ T cell number normalized within the first 12 months posttransplant (620/µl) in group A, CD4+ T cells failed to reach normal levels during the first 1.5 years in group B (279/µl at 12 mo). B cells in both groups reached normal levels by 12 months posttransplant. To address the issue of age at transplantation, the lymphocyte reconstitution of patients below and above the median age in group A, was analyzed. Interestingly, both age groups showed a comparable reconstitution pattern for B- and T-lymphocytes. We conclude that lymphocyte reconstitution after RIC conditioning and allogeneic PBPC transplantation compares favorable to classical conditioning regimens and is comparable in elder patients.

P608

Sequential limiting dilution analysis of interleukin-2 producing helper T lymphocyte precursors after non-myeloablative human leukocyte antigen-identical sibling peripheral blood stem cell transplantation

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Objectives: To quantify alloreactivity in the graft-versus-host direction after allogeneic stem cell transplantation by the use of limiting dilution analysis (LDA) of IL-2 producing helper T lymphocyte precursors (HTLP).

Methods: Nine patients were transplanted with peripheral blood stem cells from their HLA-identical sibling donor. The conditioning regimen consisted of fludarabine and 2 Gy of TBI. Graft-versus-host disease prophylaxis consisted of cyclosporine and mycophenolate mofetil.

Methods: Before the transplant the patients underwent leukapheresis with the aim to acquire 1 x 10E9 mononuclear cells (MNC). After the transplant bloodsamples were obtained from patients at different time-points, and the MNC were isolated. LDA of HTLP was performed using the MNC collected pre-transplant as stimulator cells and the MNC collected post-transplant as
responder cells. A total of 88 LDA experiments were performed with a median follow up of 6 months (range 1-18 months).

Results: The majority of LDA experiments performed post-
transplant showed non-single hit kinetics. Non linear regression
analysis also led to the hypothesis that at least two cell
populations were present in the responder cells: one cell
population produced IL-2 when stimulated with patient MNC and
the other population inhibited the IL-2 production of the first
population.

Conclusions: We had hypothesised that the HTLp frequencies
would rise dramatically post-transplant when the responder cells
had been primed in vivo. A deviation of the patients' NK cell count
Data analysis shows that the response is tightly regulated and that
an inhibitory cell population is present in the MNC collected post-
transplant.

P609
Kinetics of immune reconstitution and incidence of infections
following allogeneic peripheral blood stem cell
transplantation (PBSCT) in patients conditioned with a
reduced-intensity regimen
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We have investigated the immune recovery after reduced-intensity
conditioning and allogeneic transplantation with G-CSF mobilized
peripheral blood stem cells (PBSCT). Lymphocyte subset counts
have been analyzed in 25 patients (n = 13 hematological
malignancies; n=12 metastatic solid tumors) receiving a HLA-
matched transplant. The analysis has been performed by flow
cytometry at day 30, 60, 90, 180, 270, 360 after allograft (median
follow up of 312 days, range 0-736 days). CD19+ cell counts were
reached a plateau early and remained normal throughout the first
year (median NK cell value were 148/µL and 115/µL at 30 and
12 months, respectively). B-cell counts were significantly low
respectively. T-cell reconstitution was not associated with T-cell
activation (median HLA-DR/CD8+ cells were 83/µL and 76/µL at 6
months, range 3.6-21.4 x 108), 5.31 x 106 (range, 1.1 - 9 x 106), 1.95 x
108 (range, 0.57 - 6.24), respectively. All patients experienced a
delayed recovery of T-, B-cells and monocytes but NK cells
reached a plateau early and remained normal throughout the first
year (median NK cell value were 148/µL and 115/µL at 30 and
365 days, respectively). Mean CD4/CD8 ratio was 1.38 on day 30,
then declined to 0.81 at 1 year, with median CD4 value of 188/µL
(range, 6-062) and 353/µL (range, 109-690) at 6 and 12 months,
respectively. T-cell reconstitution was not associated with T-cell
activation (median HLA-DR/CD8+ cells were 83/µL and 76/µL at 6
and 12 months, respectively). B-cell counts were significantly low
through the first year post-transplant. CD19+ cell counts were
110/µL (range, 3-423) and 146/µL (range, 2-611) at 6 months and
at 12 months, respectively. Bacterial infections or pneumonia
occurred in 24% of pts, and invasive fungal infections in 4%, CMV
antigen reactivation occurred in 52% of seropositive recipients
(24/25), but none developed CMV disease. Acute grade I-II GVHD
occurred in 16/25 (64%) pts and grade III in 2/25 (8%). Chronic
GVHD developed in 9 pts (n=1 limited, n=8 extensive). Life-
threatening infections developed in 2 pts with chronic GVHD. In
correlation of the we have observed a slow immune recovery,
comparable to that observed after conventional-dose allografting.
This might be ascribed to GVHD that required long and intensive
immunosuppression.

P610
Immune reconstitution following allogeneic nonmyeloablative
stem cell transplantation (NST)
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Background: immune recovery post-NST has not been studied in
detail. Lymphocyte subset reconstitution after the first 12 months
post-transplant was analyzed in 14 adult patients (pts) receiving
NST and compared to that of 30 pts grafted after a conventional
hemopoietic stem cell transplantation (HSCT).

Patients/Methods: NST recipients (median age 58 years, range
20-66) were conditioned with fludarabine 30 mg/m2 for 3-5 days and
200 cGy TBI (n=11), or other fludarabine-based dose-reduced
conditioning regimens (n=3); PBSC was the source of stem cells
in 13 cases, 1 pt received unmanipulated BM. HSCT pts (median
age 39 years, range 24-50) received a TBI-containing regimen in
11 (36%) cases; PBSC was the source of stem cells in all cases.
85% of the NST pts (12/14) received CSA and MMF as GVHD
prophylaxis, whereas all pts in the HSCT group received CSA and
MTX. The median number of CD34+ (x 100/Kg) and CD3+ (x
108/Kg) cells infused was 8.1 (range 2.2-11.4) and 2.3 (range 0.6-
6.8) respectively in NST pts, and 9.44 (range 4.4-21.5) and 2.5
(1.3-7.6) respectively for HSCT pts.

Results: All pts receiving NST engrafted; by day +56, 6/14 (43%)
NST recipients showed complete donor chimerism in BM, while
dominate donor T-cell chimerism was reached by 2 out of 9 (22%)
evaluable pts. By day +30 after transplant, 45% of the pts in the
NST group and 70% of the pts in the HSCT group reached an
absolute lymphocyte count >500/mcl (p=NS). Absolute numbers of
helper (CD4+) T cells, naive (CD4+ CD45RA+) and memory
helper (CD4+ CD45RA) T cells as well as suppressor (CD8+) T cells,
CD19+ B cells and NK cells were comparable in the two groups at
any time point after transplantation. Median CD4+ T cell counts,
remained below normal up to one year after transplantation in
both groups; only low numbers of CD4+ cells coexpressed CD45
RA antigens. Normal levels of CD6+ T cells were reached in both
groups within 2-3 months. In contrast to T cells, B lymphocytes
showed low counts throughout the entire study period in both
groups. Bacteremia and CMV antigenemia occurred respectively
in 14% and 46% of the pts in the NST group and in 16% and 50%
of the pts in the HSCT group (p = NS).

Conclusion: Our preliminary data indicate that pts receiving a NST
have a lymphocyte reconstitution similar to that observed in pts
receiving a conventional HSCT. Nevertheless, due to the
small sample size, these results should be considered suggestive
rather than definitive.

P611
Comparison of immunological recovery after
nonmyeloablative and myeloablative stem cell
transplantation
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We measured T-cell responses to three different mitogens before,
at 3 months, 6 months, 9 months and 12 months after allogeneic
stem cell transplantation(SCT). Samples from 21 patients
receiving myeloablative conditioning(HSCT) and 14 patients
receiving nonmyeloablative(NSCT) were analysed. Mitogens were
phytohemagglutinin(PHA, 5 microg/ml, final concentration),
concanavalin(Con-A, 5 microg/ml) and protein A from
Staphylococcus aureus(SpA, 10 microg/ml). DNA synthesis was
measured in triplicate samples containing 1.5 x 10E5
lymphocytes/ml. Cells were cultured in 0.2 ml in microtiter plates
and harvested on day 4(PHA) or day 6(Con-A, SpA). One microCi
of 3H-thymidine was added to each culture 24 hours before
harvesting. Radioactivity was measured by an scintillation counter.
In the HSCT group, median age was 39 years, median donor age
31 years,12 patients had a matched unrelated donor and 9 an
HLA-identical sibling donor. In the NSCT group, median age was 55 years, median donor age 46 years, 5 patients had a matched unrelated donor and 10 an HLA-identical sibling donor. The incidence of GVHD was similar in the two groups. Diagnosis in the HSCT group was mainly hematological malignancies and in the NSCT group mainly hematological malignancies and solid tumors. Results: Differences in response were analysed to show the long-term trend in each group. In all groups the response to the mitogens increased over time. Similar response to Con-A and SPA was seen in both groups. The response to PHA increased more rapidly in the HSCT group (p=0.007). The response to PHA, however, was not very low in the NSCT group.

Analyses for viral mitogens will also be performed.

Conclusion: There may be differences in immune reconstitution after myeloablative and non-myoeloblative stem cell transplantation.

P612

Chimerism and minimal residual disease monitoring after reduced intensity conditioning (RIC) allogeneic transplantation

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Since graft versus leukemia is the main weapon for disease eradication after RIC allogeneic SCT, the availability of sensitive techniques to monitor changes in tumor load after transplant would allow an early intervention in the event of low tumor burden, for which immunotherapy is highly effective.

We have analyzed the impact of MRD and chimerism monitoring on the outcome of 34 patients undergoing RIC transplants compared with 9 patients undergoing conventional transplantation. At day +100 75% reached CR, there were 15% PR and three patients progressed. Incidence of grade 2-4 aGVHD and extensive cGVHD were 35% and 58%, respectively. Sixteen percent of patients developing aGVHD relapsed as compared to 47% of those without aGVHD (p=0.03) and also 10% of patients developing cGVHD relapsed as compared to 50% relapses of those without cGVHD (p<0.03). Four patients (12%) died due to early (n=1) and late (n=3) TRM. Projected OS and DFS at 3 years are 68% and 63%, respectively.

Early chimerism analysis showed 67% of patients with complete chimerism (CC) in bone marrow, 86% in peripheral blood, 89% in granulocytes and 68% in T-lymphocytes. On day +180, these figures were 83%, 100%, 100% and 100%, respectively. We observed a trend to a higher incidence of relapse in patients with more chimerism as compared to patients with CC. MRD monitoring by flow cytometry and/or RT-PCR analysis was performed in 23 patients. MRD assessment on days -21+56 after transplant allowed to identify patients at risk of relapse: seven out of 12 patients (58.3%) who had positive MRD on days -21+56 relapsed as compared to 0 out of 11 patients who had negative MRD (p=0.002). Of the seven patients with criteria to monitor MRD who relapsed after transplant, all but one remained MRD positive until relapse. By contrast ten patients remained MRD negative and all of them are in continuous CR. In 9 additional patients persistence of MRD or mixed chimerism was observed after transplant and withdrawal of ciclosporin with or without DLI was performed. Only two out of these nine patients relapsed. MRD clearance was preceded by CC and GVHD. In conclusion, our study shows that RIC transplants can be used in patients considered poor candidates for conventional transplantation with low TRM. Simultaneous studies of chimerism and MRD are a useful tool in order to predict the risk of relapse in patients undergoing RIC transplants and so can be helpful for individualizing treatment strategies after transplant.

P613

Allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning: chimerism studies by short tandem repeat loci amplification


Background: Short tandem repeat (STR) loci amplification is the best method to analyse for degrees of donor-recipient chimerism after allogeneic transplantation. We present the chimerism results in 35 patients treated with an HLA-identical allogeneic peripheral blood stem cell transplantation after reduced-intensity conditioning (RIC) regimen.

Patients and Methods: There were 18 males and 17 females, with a median age of 48 years. Conditioning regimen consisted in fludarabine (150 mg/m2) plus melphalan (140 mg/m2) for lymphoid malignancies (n=23) or plus busulphan (10 mg/kg) for myeloid malignancies (n=12). After transplant, serial samples of peripheral blood and bone marrow were analysed. Chimerism studies were performed with an automated kit (AmpFISTR Profiler Plus Loci) that amplifies 9 short tandem repeat loci plus the gender marker Amelogenin. Products were identified by a semi-automatic system (electrophoresis gel and a motorized scanner). When a pattern of mixed chimerism (MCh) was found, or a disease relapse was suspected, studies were performed in isolated T-lymphocytes and neutrophils.

Results and Conclusions: On day +21, 19 patients (83%) with lymphoid malignancies showed a pattern of complete donor chimerism. Four patients had a MCh in bone marrow samples, 2 with evidence of disease at that time. Three patients showed a transient MCh around day +150, without evidence of disease recurrence. One year after transplant all the patients had 100% donor cells. In the group of patients transplanted for myeloid malignancies, 10 patients (83%) showed a pattern of MCh after transplant, in some cases until day +180, without any evidence of disease. One patient with an acute myeloid leukemia had a MCh in bone marrow on day, with morphologic evidence of relapse at that time. With the RIC used, donor myeloid engraftment preceded T-cell engraftment in the patients studied. The kinetics of lineage-specific chimerism is of interest in the management of patients after RIC transplants.

P614

Lymphocyte reconstitution following reduced-intensity allogeneic stem cell transplantation


We evaluated the phenotypic immunological reconstitution of different peripheral blood subsets of T, B and NK cells for 6 months following HLA-identical allogeneic peripheral blood stem cell (PBSC) transplant with reduced-intensity conditioning regimen in 14 patients affected with hematological malignancies. The median age was 52 years (range 24-54); 9 were males, 6 females. Three patients were affected with myelodysplastic syndrome, 8 were with acute myeloid leukemia (1 chemo-radiotherapy related), with acute lymphoblastic leukemia and 2 with non-Hodgkin lymphoma. Conditioning regimen consisted of Thiotepa 5 mg/kg/day for 2 days and Fludarabine 25 mg/m2/day for 5 days; 3 patients received a single dose of Idarubicine 15 mg/m2 at day – 12. Graft-versus-Host Disease (GVHD) prophylaxis was performed with cyclosporine A 1.5 mg/kg/day i.v. continuous infusion and short course methyltrexate (10 mg/m2 i.v. at day +1, 8 mg/m2 at days +3, +6, +11). Immunophenotypic analysis was performed before transplant, at the engraftment, at day +30, +60, +90, +180 posttransplant; peripheral blood lymphocytes subsets were studied by direct immunofluorescence and flow cytometry using a lyse-no-wash sample processing of total peripheral blood. A chimerism evaluation with microsatellite polymerase chain
reaction was performed at the same intervals. The immunophenotypic analysis showed a rapid recovery of CD4+ and CD8+ T cells; CD4/CD8 ratio was persistently inverted, due to the faster reconstitution of CD8+ T cells with a spike at 60 days posttransplant; the recovery of CD16+CD56+CD3- NK cells was prompt and showed a consistent overshoot 60 days after transplant. B cell reconstitution was delayed and resulted incomplete 6 months after transplant. The preliminary results of our study seem to demonstrate that reduced-intensity allogeneic stem cell transplant may result in an early T cell reconstitution; the prompt recovery of NK cells could have a clinical relevance sustaining an anti-tumor reaction.

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Day +14 donor/recipient peripheral blood T-cell chimerism analysis as predictive indicator of graft loss after low-dose, TBI-based, non-myeloablative conditioning regimen in advanced lymphoproliferative disorders (LD)


Unstable mixed chimerism (MC) may be associated with non-myeloablative allogeneic stem cell transplantation (NM-ASCT) with minimal conditioning or T-cell depletion. It is known that low donor T-cell chimerism levels in peripheral blood (PB) on day +28 on transplanted patients with higher risk of rejection despite the “prophylactic” donor lymphocyte infusion (DLI) usually given after the removal of immunosuppression. In an attempt to predict graft rejection earlier after NM-ASCT, donor/recipient PB T-cell chimerism was evaluated on day +14. Thirteen patients (pts) with advanced LD (7 chronic lymphocytic leukemias, 2 follicular lymphomas, 3 mycosis fungoides, 1 Hodgkin’s disease) were transplanted using G-CSF mobilized PB stem cells from HLA-identical siblings. Conditioning regimen consisted of two cycles of Fludarabine 30mg/m2/day for 3 days and CTX 300mg/m2/day for 3 days (F-C) repeated every 28 days followed by 200Gy TBI 14 days after the second F-C cycle. GVHD prophylaxis included oral CSA 3mg/kg/day b.i.d from day 0 to +100 (target serum level: 400-600 ng/ml) and MMF 15mg/kg/day b.i.d from day 0 to +27, tapered within 2 weeks. All of the patients received >4x10^6/kg CD34+ donor cells as per protocol. Donor engraftment was evaluated by means of microsatellite analysis (AmpF1 STR @Profiler Plus, Applied Biosystems, CA, USA) on separated PB CD34+ cells after sorting with immunomagnetics beads (MiniMACS, Miltenyi Biotec) on days +14, +28, and then every month. Of the 4 pts with <50% donor T-cells on day +14, 3 showed declining chimerism from day +28 versus 0/9 with >50% donor T-cell (p=0.014); two of the three (2 stage C CLL pts who received <5x10^6 CD34+ cells) eventually lost their grafts, whereas the third achieved full graft engraftment day +450 after repeated DLI. The primary objective of this ongoing UK pilot study was to determine the rate of engraftment in patients receiving i.v. Fludarabine phosphate (30mg/m2/day on days -7 to -3) and i.v. melphalan (140mg/m2 on day -2) followed by a filgrastim-mobilized PBSC infusion from an HLA-matched sibling donor. Patients in appropriate remission were eligible if considered unsuitable for conventional allogeneic transplant due to age or on clinical grounds. GVHD prophylaxis with cyclosporin was to be

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Allogeneic transplantation with ATG based reduced intensity regimen and bone marrow (BM) graft is associated with rapid donor chimerism, low GVHD rate and rapid CD8+ T cells recovery


Between May 98 and April 2001, 65 patients were transplanted in our center using a reduced intensity regimen. Inclusion criteria were: patient above 50 years of age, or with advanced disease, or with solid tumor, or ineligible for conventional conditioning. Among this series, we present here the data of 32 pts suffering from hematological malignancies who received a BM graft from an HLA identical sibling.

Patients and methods: Median age: 52 (25-60), M/F: 18/14, myeloid malignancy: 19 pts (AML: 13, MDS: 3, CML: 3), lymphoid malignancy: 13 pts (ALL: 2, CLL: 1, LG NHL: 3, HG NHL: 1; HD: 1, MM: 5). 20 pts were beyond CR1/CP1. The conditioning regimen included 6 days of fludarabine (25mg/m2/d), 2 days of busulfan (4mg/kg/d), and ATG (Thymoglobuline, Imtix Sangstat) 2.5mg/kg/d over 4 days (17 pts) or 3 days (15 pts). GVHD prophylaxis: CSA alone. All pts received a BM graft with a median of 2.3x10^6 CD34+ cells/kg (1.1-7.6), and 20x10^6 CD3+ cells/kg (12-43).

Results: Median time for ANC and platelet to reach 0.5 and 25x10^9/L was 12 (11-19) and 18 (9-26) days; 4 pts needed no plt. transfusion. During the 1st month, 7/10 (70%) analyzed pts reached a full donor chimerism (DC), and 3/10 pts had a mixed DC (75-95%). During the 2nd month, 16/21 (76%) reached a full DC, and 5/21 were still in mixed DC. Among the 31 evaluable patients, 9 (31%) presented grade 2-4 aGVHD (grade 2: 5, grade 3: 4, 4), at a median time of day 40 (28-86). Immune reconstitution was studied for 19 patients. Analysis of T cell subsets posttransplant showed a rapid and sustained CD3+CD8+ T cells and NK (CD3-CD56+) cells recovery. However, we observed a long-lasting deficiency of CD3+CD4+ T cells (less than 0.2x10^9/L beyond day +100). The latter could be correlated with the high rate of viral infections especially CMV, we already reported in this setting (Mohty et al, Bone Marrow Transplant. 26:251-256, 2000). 6 pts died from TRM (infections: 3, aGVHD: 3), while 13 pts died of relapse. With a median follow-up of 24 mo. (9-34), 13 pts (41%) are still alive, 11 of them in CR.

Conclusions: Based on these encouraging data in this high risk group, which will be presented in detail, we are now conducting a study of reduction of ATG based immunosuppression. Since immunosuppression modulation can help enhance the graft versus tumor effect, an additional 15 pts have already received only 1 dose of ATG and comparative data will be presented.

Additional abstracts to this topic

Fludarabine phosphate and melphalan - an immunosuppressive reduced intensity conditioning (RIC) regimen for allogeneic stem cell transplantation in patients with a range of hematological malignancies

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The primary objective of this ongoing UK pilot study was to determine the rate of engraftment in patients receiving i.v. fludarabine phosphate (30mg/m2/day on days -7 to -3) and i.v. melphalan (140mg/m2 on day -2) followed by a filgrastim-mobilized PBSC infusion from an HLA-matched sibling donor. Patients in appropriate remission were eligible if considered unsuitable for conventional allogeneic transplant due to age or on clinical grounds. GVHD prophylaxis with cyclosporin was to be
Nonmyeloablative allogeneic stem transplantation in patients with previously treated multiple myeloma


Allogenic stem cell transplantation (SCT) for patients (pts) with Multiple Myeloma (MM) offers a possibility of cure, but high transplant related mortality was reported. A reduced intensity regimen can establish a successful engraftment and induce a graft versus malignancy effect.

Ten pts with MM (median age 57 yrs, range 38-64) received a reduced intensity alloSCT from HLA identical siblings. All had a previously treated MM: 3 pts received a prior auto SCT and one pt received both auto and allo SCT. Disease status at time of SCT was PR in 5, minimal response in 2 and refractory disease in 3. Pts were grafted with G-SCF mobilized unmanipulated PBSC.

Conditioning regimens consisted of fludarabine 30 mg/m2 x 4 days (7 pts) or 3 days (3 pts) plus either thiopeta 5 mg/kg x 2 days in 7 cases, or melphalan 100 mg/m2 in 2 cases, or CTX 300mg/m2 in one case. All except one pt received GVHD prophylaxis: 7 pts received CSA alone, 2 received CSA plus short course of MTX. Median number of CD34+ cells/kg infused was 8.4 x 106 (range 3.5-20); median number of CD3+ cells/kg infused was 2 x 108 (range 1.7-8.8). Nine pts engrafted: median time to ANC>1000/ul was 15 days (range 0-26). Chimerism evaluated on day 15 days was 100% donor chimerism in 8 pts. One pt showed a mixed chimerism followed by relapse. Acute GVHD (grade >II) occurred in 2 pts and extensive chronic GVHD in 2. CR was achieved in 7/10 pts. Three pts are alive: 2 in CR at 12 and 48 months, one in relapse at 24 months. Seven pts died: 2 in relapse for disease progression and 5 of transplant failure 1 and pneumonia 1). In conclusion, although the considerable toxicity, the reduced-intensity alloSCT induces successful engraftment with complete chimerism in heavily treated MM pts. Long term disease free survival was observed in few pts; however, better results are probably achievable through the treatment of a more selected group of pts.

Autologous SCT followed by nonmyeloablative allogeneic SCT is highly efficacious in advanced stage patients with lymphoid malignancies


We analyzed efficacy of allogeneic stem cell transplantation (SCT) with dose-reduced conditioning after debulking therapy with autologous SCT in 9 patients with a median age of 58 (range 47 to 63) years.

Four patients had a non-Hodgkin's lymphoma (1 MCL, 3 DLCL), 5 a myeloma (MM). All these patients were at high-risk for transplant related mortality (TRM) due to age (n=8) or previous therapies (n=1). Furthermore, 3 patients with myeloma had previously received an ASCT. At the time of admission for ASCT 4 patients were in PR and 5 in refractory disease stage. As conditioning chemotherapy for ASCT patients were given melphalan (n=5), BEAM (n=1) or CBV (n=3).

Median interval between autologous and allogeneic SCT was 8.5 weeks (range 6 - 10). Prior to allogeneic SCT one patient was in complete remission (CR), 5 in partial remission (PR) and 3 in refractory disease. All patients underwent allogeneic SCT with dose-reduced conditioning, which consisted of fludarabine (90 mg/m2) and total body irradiation of 2 Gy, followed by infusion of peripheral blood stem cells from sibling donors (n=4) or unrelated donors (n=5). A median dose of 7 (0.8 - 12) x 10^6 CD34+ stem cells/kg body weight was infused. For GVHD prophylaxis all patients were given cyclosporine A (CSA) and mycophenolate mofetil (MMF). All patients had hematologic engraftment. Two patients developed acute GVHD and 3 of 8 chronic GVHD.
A sustained T cell mixed chimerism as the only evidence of donor cells 16 months after minitransplantation for acute myelogenous leukemia


A patient with AML minitransplanted in partial remission from a sibling suffering from Rheumatoid arthritis developed a sustained T cell mixed chimerism for 16 months as evidence of a maintained engraftment of only T cells after an initial engraftment in the myeloid lineage too.

High relative and absolute values of donor CD3+/HLA-DR+ and CD3+/CD8+ cells taken from the donor on the background of lymphopenia were measured. These lymphocyte populations examined in the recipient had even higher values with the CD3+/CD8+ cells representing 72% of total lymphocytes. It is speculated that the autoreactive donor T cells, after being activated, mounted an autoimmune reaction against own graft cells. The disappearance of the donor chimerism in the myeloid series after DLI given 3 months after SCT supports this possibility. Donor T cell activation may have occurred because of host specific antigens binding to MHC class I or class II or by superantigens implicated in the pathogenesis of Rheumatoid arthritis, which stimulate T cells based on the Vbeta gene segment, which is supported by the measured overexpression of certain Vbeta families in these polyglonal cells especially in the CD8+ population and/or by the immunotherapy given (IL-2, GM-CSF with DLI). The alloreactiveness of these T cells was clearly seen, as they produced an adoptive cellular response in the recipient leading to a hematological remission with MRD for 13 months without GVHD. The sustained mixed chimerism, which is thought to be associated with less GVHD, as it presents an immunological balance between donor and recipient cells, could explain the absence of GVHD. The patient relapsed with a CMML 13 months after SCT and was minitransplanted from an unrelated donor in November, 2001. As microchimerism can occur after organ transplantations and as only a few stem cells in the blood seem necessary for establishing the T cell repertoire in the Thymus, it is possible that T cell engraftment has occurred or at least was maintained there. We conclude that a sustained mixed T cell chimerism after minitransplantation is possible and may induce a GVL effect without GVHD.

Nonmyeloablative stem cell transplant (NMSCT) for hematologic malignancies and metastatic melanoma, and conventional transplant in a patient who rejected two NMSCT

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In our Center patients ineligible for standard allogeneic transplants because of age or medical contraindications, having an HLA-identical sibling available, were candidates for NMSCT. The conditioning regimen included fludarabine 30 mg/m2 on day -4, -3 and -2, and low-dose TBI (200cGy) and postgrafting immunosuppression was with a combination of mycophenolate mofetil (MMF) and cyclosporine (CSA). One patient with metastatic melanoma was conditioned with fludarabine 30 mg/m2 on day –8, -7, -6 and melphalan 50 mg/m2 on day –3 and -2 with same postgrafting immunosuppression as described above. Nine patients, median age 57 (range 46-67) years were treated. Diagnoses included MM relapsed after 2 tandem autologous stem cell transplant (n=1), refractory refractory MM (n=1), MM partial remission (n=1), AML 2nd CR (n=1), AML 1st CR (n=2), AML PR (n=1), refractory AML (n=1), Metastatic Melanoma (n=1). Follow-up ranged from 37 to 431 (median 107) days. The patients received unmodified PBSC grafts from HLA-identical sibling, a median of 6.4 x 106 CD34 cells/kg (range 5.0-9.5) were infused. Overall, transplants were well tolerated. Seven patients achieved >95% donor chimerism. No fatal graft rejection occurred. Grade II, III, IV acute GVHD occurred in 33%, 0% and 0% of patients, respectively, and responded well to treatment. Of three valuable patients 2 developed cGVHD. Currently 5 patients are alive (55%), 2 are in CR and 3 in stable PR. Three (33%) patients died of progressive disease (advanced phases), and 1 died of nonrelapse causes (CR). Overall response rate was 66%. One AML patient (2ndCR, 56 years old) received a conventional transplant after she rejected 2 NMSCT from 2 different siblings. The conditioning was busulfan based, the donor was the first sibling. Currently she is day +108 with 100% donor chimerism. In summary non-myeloablative conditioning allows allografting in patients not eligible for standard allogeneic transplants (older patients, heavily pretreated or with medical contraindications); regimen related toxicity is low, allowing in one patient a conventional transplant with low toxicity and successful engraftment after rejection of 2 NMSCT. No fatal graft rejection occurred; aGVHD was seen in 33% of patients. Although responses (CRs and PRs) have been seen longer follow up is needed to assess the role of nonmyeloablative allografts, especially for patients in advanced phases of disease at transplant.

Kinetics of chimerism, minimal residual disease, and development of GVHD in patients after allogeneic PBSC using a nonmyeloablative regimen containing busulfan, fludarabine, and ATG

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Objectives: Non-myeloablative regimens are used in patients not suitable for myeloablative regimens because of poor medical conditions or advanced age. This regimens have been proposed with the aim of reducing transplant related mortality preserving the immunological effects of the graft.

Methods: We used non-myeloablative regimen published by Slavin et al. (Blood 91, 1998, 756-763) consisted of fludarabine 30mg/m2/d (days –10 to –5), busulfan 4mg/kg/d (days –6 and –5), and ATG (Fresenius) 10mg/kg/d. On day 0 all patients received G-CSF mobilized PBSC from HLA-identical siblings. Intravenous CsA alone started on day –1 was used to prevent GvHD and graft rejection. Between March 1998 and July 2001 22 patients were transplanted with this regimen. The diagnoses were as follows: CML (8 patients), AML (7 patients), MDS (1 patient), HL (1 patient), NHL (2 patients), MM(2 patients), CLL (1 patient).

Results: The treatment was tolerated well. Engraftment with donor cells was demonstrated in all cases except one patient, who died soon after transplantation prior to engraftment because of bleeding. Donor chimerism was established early in most cases. 6 patients had aGVHD and 10 had cGVHD. Monitoring of MRD and chimerism is a good way for following patients with CML, but appears less conclusive in other disease.

Conclusion: This non-myeloablative regimen is very well tolerated, appears less conclusive in other disease.

Minimal Tbi-based conditioning followed by HLA-identical hematopoietic stem cell transplantation (HSCT) in patients with chronic lymphocytic leukemia (CLL)

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The main obstacles to expanding the use of conventional HSCT in CLL are the relatively high morbidity and mortality rates and the fact that many patients are deemed ineligible because of their age and/or clinical condition. The use of less toxic, non-myeloablative preparative regimens leads to a safer transplant procedure without affecting the development of a graft-versus-tumor effect, and the
efficacy of the combination of fludarabine (FMP) and cyclophosphamide (CTX) in advanced CLL can lead to the degree of immunosuppression required for stem cell engraftment. Seven patients (pts) with advanced CD5+ B-CLL were transplanted using G-CSF mobilized peripheral blood stem cells (PBSC) from HLA-identical siblings. At the time of transplant, 3 of the pts were in stage C and 4 in stage IIb, all of them refractory to alkylating agents and three unresponsive to FMP. The conditioning included two cycles of FMP 30mg/m2 day for 3 days and CTX 300mg/m2/day for 3 days (F-C), repeated every 28 days. Fourteen days after the second F-C cycle, TBI 200 cGy was delivered as a single dose, followed by the PBSC infusion (day 0). GVHD prophylaxis consisted of CSA 3mg/kg b.i.d. p.o. from day –1 to +90 and MMF 15mg/kg b.i.d. p.o. from day 0 to +27, and then tapered within 2 weeks. Full donor engraftment was achieved in 5 pts (one after repeated DLI under immunosuppression). The two pts experiencing rejections (28%) received the lowest dose of PBSC (<5x106/kg CD34+ cells) and successfully underwent a second non-myeloablative HSCT. Transplant related mortality was 0% and severe infectious episodes (2 cases of pulmonary aspergillosis, one associated with CMV pneumonia) were observed only the rejecting pts and completely resolved after treatment. Four pts (57%) developed grade II-IV GVHD (3 grade II) and all of whom were successfully treated. One patient died of disease progression (Richter’s transformation) 16 months after transplant. Three pts remain in CR (follow-ups: 13, 9, and 3 months) and 2 in PR (18 and 12 months) and one with stable disease (2.5 months).

The conditioning used in our patients can be considered minimal even in the context of non-myeloablative regimens, and so the achievement of a response mainly relied on the expected development of an effective graft versus leukemia (GVL) effect. One particular aspect in this series was the low incidence of acute GVHD and the fact that disease control was at least initially recorded in all cases.

Feasibility of allogeneic hematopoietic stem cell transplant after reduced intensity conditioning regimens in patients with advanced neoplastic disease

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Allogeneic transplantation after reduced intensity regimens can allow durable engraftment and mediate a graft versus malignancy effect possibly with reduced early morbidity and mortality, thus extending the eligibility of pts to alloHSCT. We report on 18 pts who received reduced intensity conditioning therapy, 11 from patient HLA-identical sibling (n=12) or matched unrelated donor (n=6) between Sept 99 and Nov 01. The median age was 51 years (range 24-59). 9 pts received BM and 9, G-CSF mobilised PBSC. Median time from diagnosis to transplant was 55 months (range 9-252). Six pts had undergone a prior autologous and one an allogeneic transplant. The diseases treated were AML –3, ALL –4, NHL –2, Multiple Myeloma –1, CML –5, MDS –1, MF –1 and Ewing’s sarcoma –1. At the time of transplant only 4 pts were in complete remission (CR1 = 1, beyond CR1 =3) and 14 pts had active disease. The conditioning regimens were: Fludarabine 30 mg/m2 x 5 days, Busulphan 4mg/kg x 2 days together with ALG 10mg/kg x 4 days or single dose TBI 200 Gy (n=14); Thiotepa 10mg/kg followed by Fludarabine 30 mg/m2 x 2 days and Cyclophosphamide 30mg/kg x 2 days (n=4). All except one received GVHD prophylaxis: CsA – MTX (n=9), CsA alone (n=4) and CsA + MMF (n=4). A median of 4.7 x 10^6/kg CD34+ cells/kg and 85 x 10^6/kg CD3+ cells were infused. All pts engrafted with a median time of 15 days to neutrophils >0.5 x10^9/L and 19 days to platelets >20 x10^9/L. Full chimerism was documented in 10 pts at a median of 32 days (17-82) and mixed chimerism in 4 pts. Three pts were not evaluable and one had an early autologous reconstitution. Acute GVHD (Gr.I, 2 x Gr.II, 1x Gr.III, 1x Gr.IV), despite only 2 patients received DLI. AML patients (N-10). 6 patients died. 4 patients due to relapse and 2 patients due to GVHD. Relapses were more common in the group of patients transplanted from HLA identical siblings (4/5 pts) than in the group of pts transplanted from MUD (1/5 pts). aGVHD (2 x Gr.I, 5 x Gr.II,) was observed in 7 patients, 4 patients survive in complete remission 16,10,9,9 months post BMT. CLL patients (N-2). Both these high risk CLL patients achieved complete remission. The patients survive 16 and 6 month post BMT in CR.

NHL patients (N-2). One patient died due to VOD and sepsis, one patient with mantle-cell NHL achieved complete remission and survives 4 months. MDS patients (N-3). All these pts died, 2 of them due to relapse of RA-IC and 1 due to RA-EB without RA died due to GVHD. One patient with relapsing Hodgkin disease survive without signs of disease 4 month post BMT and one patient with MM, who underwent 3 autologous BMT previously, survives 6 months post NSCT in PR.

Conclusions: NSCT in CML pts is curative procedure but is connected with high risk of GVHD or relapse. Safer manipulation with mixed chimerism post BMT is needed, STI 571 may be the solution. In AML pts we observed better outcome in the pts grafted from MUD due to lower relapse rate but GVHD and relapses remains the major problem. Our 2 CLL pts had excellent response to NSCT as had the patient with mantle-cell NHL. Our MDS pts with advanced disease had no profit from NSCT.
14. Chronic Leukemia

P617

Im proved disease-free survival after transplantation of PBSCs as compared to BM from HLA-identical unrelated donors in patients with 1st CP CML


Outcomes after PBSCS for 1st chronic phase CML (n=47) were compared to those of BMT (n=54) in the HLA-compatible unrelated donor setting. Median follow-up was 15 months after PBSC and 29 months after BMT. Both neutrophil and platelet recovery were faster after PBSCS (p<0.05). PBSCS was associated with improved immune reconstitution, with higher peripheral blood naive CD(4+CD(45RA+)) and memory CD(4CD(45RO+)) helper T cells at 3 months and 12 months post transplant (p<0.03). The cumulative incidence of acute (grade II-IV) and chronic GVHD were similar, but BMT was associated with a higher cumulative incidence of severe acute (grade III-IV) GVHD at 24% as compared to 8% with PBSCS (p<0.05). Molecular relapse, defined by two consecutive positive PCR assays for bcr-abl within a 4-week interval, occurred in 12 of 45 evaluable patients (27%) after BMT and in 5 of 47 patients after PBSCS (11%) (n.s.). Cytogenetic relapse occurred in 5 of 54 patients after BMT (9%), and in one of the 47 patients (2%) after PBSCS (n.s.) 17 of the 54 patients died after BMT (31%) as compared to 6 of 47 patients after PBSCS (13%). Deaths in the BMT-group were mainly associated with infections and severe acute GVHD. The estimated probability of transplant-related mortality and disease free survival at 1000 days after transplant were 30% and 59% in the BMT group and 13% and 86% in the PBSC group (p<0.03). Overall survival 1000 days after transplant was 64% after BMT versus 86% after PBSCS (p<0.05). In the multivariate analysis only acute GVHD significantly influenced treatment-related mortality (p<0.05).

P618

Interferon before allogeneic bone marrow transplantation for chronic myeloid leukemia: a GITMO retrospective analysis of 217 patients

R. Fanin for the GITMO

Background and Objectives. The influence of interferon (IFN) pretreatment on the outcome after allogeneic bone marrow transplantation (BMT) in chronic myelogenous leukemia (CML) is controversial. Between 1982 and 1996, 857 patients affected by chronic myeloid leukemia were transplanted in Italy from HLA-identical sibling donor and reported to the National Registry (GITMO). Of them, we retrospectively analyzed the outcome of the 217 previously treated by IFN.

Design and Methods. Median age at diagnosis was 38 years (10 – 58); male/female ratio 142:75; distribution according to Sokal index: <0.8 – 49 (22.5%) patients; 0.8 – 1.2 – 50 (23.0%); >1.2 – 19 patients (9.0%); IFN duration before transplant: 15 months (1 – 63). Phase at transplant: chronic phase (CP) – 174 (80.0%); accelerate phase 29 (13.5%); blastic phase (BP) – 13 (6.0%). Conditioning regimen: chemotherapy alone – 144 (66.5%) patients; chemotherapy and TBI – 70 (32.5%) patients. TCD vs not TCD: 33 (15.0%) vs 184 (85.0%) patients.

Results. 126 (58.0%) patients developed an acute GVHD (32 – 15 months after graft); grade III – IV); 128 (59.0%) patients are alive at a median follow-up from transplant of 33.5 months (1 – 149). Overall survival and DFS were respectively 55% and 39.0%. OS according to status at transplant were: CP – 60%; AP – 42%; BP – 15% (p value=.0004). DFS according to status at transplant were: CP – 44.0%; AP – 22.0%; BP – 15% (p value=.004).

If we considered patients previously treated with IFN vs those not treated with IFN, no statistically significant difference emerged neither for OS nor for DFS. Features analyzed in multivariate analysis for OS and DFS were: patients and donors gender, gender match, TCD; age at diagnosis and at transplant; TBI vs not TBI, interval time from IFN withdrawal and transplant (< / = 3 months vs > 3 months); interval time dx - BMT, IFN duration, aGVHD, phase at transplant, Sokal index. Features adversely affected OS were: TCD (p=0.05), age at diagnosis (p=0.02), aGVHD (p=0.07) and phase at transplant (p=0.0029). While features adversely affected DFS were: TBI conditioning regimen (.07) and IFN discontinuation < 3 months (p=.03).

Conclusions. These data confirms, as previously reported, that pretreatment with IFN does not compromise BMT outcome (no adverse OS and DFS); but clear candidates for early transplant should not be treated with IFN (better DFS if IFN discontinuation < 3 months).

P619

Allogeneic SCT for adult type CML (AT-CML) in children


SCT is the only treatment proven to cure AT-CML which is a rare disease in childhood. We report the outcome of SCT (289 HLA-matched siblings, 155 VUD) in 444 children (median age: 13 years) registered on the database of the CLWP of the EBMT since 1985. SCT was performed within one year of diagnosis in 74% of children with sibling donors and 42% of those with VUD. For the 224 children in CP1 transplanted from HLA-identical siblings the 5 year actuarial (OS) and EFS were 72% and 55% respectively. For 112 children transplanted in CP1 using VUD SCT the 5 year OS was 58% and EFS 46%. TRM at 40% was the principal cause of treatment failure in recipients of VUD transplant and significantly higher than after sibling SCT (21%; univariate HR: 2.0; 95% CI: 1.3-3.2; p=0.0013). Relapse of AT-CML occurred in 30% of CP1 patients receiving sibling donor SCT and 22% of VUD SCT (univariate HR: 0.6; 95% CI: 0.3-1.2; p=0.15). Outcome of SCT was inferior in the 108 children transplanted in advanced phase (CP2, accelerated phase and blast crisis): 5 year actuarial survival was 42%, EFS 30% with a relapse rate of 50% and TRM of 38%. In a multivariate COX model for OS, which included donor-relation, diagnosis to SCT interval, stage of disease and gender-mismatch: the first three parameters were significant. The Hazard Ratio for VUD versus sibs was 1.4 (95% CI: 1.0-2.0; p=0.03); for stage comparing advanced to CP1 the HR was 2.1 (95% CI: 1.5-2.9; p=0.01); and the HR for the interval showed an increased death rate for the period 6-12 months only. In the same model with relapse as an outcome, the significant risk factors were gender-mismatch and stage. For gender-mismatch, the Hazard Ratio was 0.5 (95% CI: 0.29-0.85; p=0.01) comparing male recipient/female donor to other gender combinations. The Hazard Ratio for advanced phase was 3.0 (95% CI: 2.0-4.5; p=0.01) comparing AP to CP1. This is the first large series to show that SCT from either a sibling or VUD confers long-term LFS in the majority of children with AT-CML. These data confirm the importance of performing SCT in CP1 and are important in evaluating the role of alternative treatment strategies, such as tyrosine kinase inhibitors, in children.
Long-term follow-up of allogeneic bone marrow transplantation after reduced-intensity conditioning in patients with chronic myelogenous leukemia in the chronic phase


Although allogeneic transplantation is a curative therapy for chronic myelogenous leukemia (CML), treatment-related mortality is still a major cause of death after transplant, especially in older patients. We investigated the safety and efficacy of a reduced-intensity conditioning consisting of low-dose total body irradiation (300cGy x 2/day), cytosine arabinoside (200mg/m2/day x 5) together with a continuous infusion of granulocyte-colony stimulating factor (5ug/day x 5), and cyclophosphamide (60mg/kg/day x 2) in patients with CML in the chronic phase. Fractionated splenic irradiation (5Gy) was also administered as part of the conditioning. Eight patients aged over 40 underwent allogeneic bone marrow transplantation from an HLA-matched sibling following this conditioning. Regimen-related toxicities (grade III or greater) were not observed. Rapid restoration of 100% donor chimerism was confirmed in 5 sex-mismatched transplant recipients by FISH. One patient died from severe acute graft-versus-host disease, and the other died from pancytopenia and pneumonia early in the course of transplant. A sustained engraftment was achieved in five long-term survivors; one rejected the graft but restored autologous Ph1-negative hemopoiesis. With a minimum follow-up period of 60 months, 6 patients are still alive and in remission including the patient with restored autologous hemopoiesis. One patient experienced a cytogenetic relapse 6 years after the transplant, which has been successfully treated with donor leukocyte infusions. We conclude that this reduced-intensity conditioning was effective with markedly reduced regimen-related toxicities in older patients with chronic phase CML. Further study is needed to confirm the advantage of this approach over conventional myeloablative conditioning regimens.

Long-term results of allogeneic bone marrow transplant (BMT) for chronic myeloid leukemia - A single center experience


One hundred and five patients (pts) who received allogeneic BMT for chronic myeloid leukemia (CML) from HLA-identical related donors between April 1981 and February 2000 were enrolled into this study. Eighty eight pts were in chronic phase (CP), 11 pts in accelerated phase and 6 pts - in blast crisis. Ten of these pts received a second BMT (BMT2). Patients were given cyclophosphamide (CY) plus single dose total body irradiation - TBI (CYTBI, n=38) or busulfan (BU) plus CY (BUCY, n=67). Overall 54 pts are alive and 52 of them are disease-free with a median follow-up of 11.3 (range 1.1-19.4) years. In CP pts the probability of disease-free survival (DFS) was better after BUCY - 61% than after CYTBI - 41% (P=0.07). DFS was significantly higher in CP pts who received transplant within 1 year after diagnosis (63%) or at the age less than 25 years old (73%). In multiple regression analysis CP (P=0.001), transplant within 1 year from diagnosis (P=0.019), age less than 25 years (P=0.023) and the absence of grade II-IV acute GVHD (P=0.012) predicted better survival. The probability of DFS after BMT2 was 60%. CP pts who received BMT after CYTBI had a higher probability of relapse (53%) than after BUCY (9%)(P=0.002). In these pts the use of CYTBI was the only factor which predicted relapse (P=0.004). The incidence of grade II-IV acute GVHD and moderate to severe chronic GVHD was 38% and 28% respectively. The probability of transplant-related mortality in CP pts was higher after CYTBI than after BUCY (58% vs 34%, P=0.08). Only 3 of 45 evaluable pts (6.6%) had PCR positivity beyond 1 year of BMT. All but six pts are currently on no medication and have resumed all activities without any limitation. This study confirms that allogeneic BMT is still the only curative approach for CML and should be offered to all younger pts with a suitable donor as soon as diagnosis as possible.

VUD transplants in CML with low dose rabbit ATG in the preparative regimen experience from a single center

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From Sept 95 to June 2001, 28 pts with CML underwent VUD transplants. 19 were males, 9 females. The median age was 35 yrs (20-50), the interval between diagnosis and transplant was 23 mos (range 9-116), 21 were in CP and 7 in acc ph. HLA typing was based on high-resolution molecular techniques; identity at the HLA,B, DQ B1 and DRB1 was requested , but in 8 cases there were one or more allele mismatches. The preparative regimen consisted of TBI, single fraction, 800cGy,from a linear accelerator, at a low dose-rate, EDX, 120mg/kg and rabbit ATG (Fresenius, Bad Homburg, D) 3 mg/kg/day from day -6 to -2. Pts in acc.phase also recived VP-16 or Ara-C. Source of the stem cells was the marrow in all cases; the median number cells infused was: NC, 4.1 x 108/kg; T-lymphocytes, 28 x 106/kg. A prognostically negative sex mismatch (donor F, recipient M) occurred in 9 cases. All pts engrafted. Acute GVHD, grade III-IV occurred in 5 pts. Chronic GVHD occurred in 9 pts ( limited form), 4 pts had an hematologic relapse and 1 a cytogenetic relapse. Causes of death were: relapse, 4, GVHD/infection, 4, other 2. At 5 yrs, OS is 61% (c.i. 42-81); for those transplanted in CP, OS is 76% (95%CI:58-84) and for those in acc.phase is 26% (85%CI:0-67%) at two years. The following parametrs were analyzed: sex, number of NC infused, number of T lymphocytes infused, interval diagnosis-tx, phase of the disease, donor-recipient sexmismatch. In univariate analysis , the common outcome-variables were found to be significantly associated with the following factors: a GVHD (age); cGVHD (phase of disease), cGVHS (age, number of NC infused); TRM (no factors); relapse (phase of disease). These data demonstrate that transplants performed in CP, with this preparative regimen, are associated with a good outcome; results are not satisfactory when the disease is in an advanced phase.

Study on optimisation of a dose of Fludarabine and ATG in nonmyeloablative regimen used in matched sibling and unrelated donors transplantation in chronic myelogenous leukaemia patients.

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This report presents our study on optimisation of Fludarabine and ATG dose to decrease toxicity not affecting the take. The whole study group consisted of 24 patients. All received Busulfan in a total of 8 mg/kg b.w. The doses of Fludarabine and ATG: Fludarabine 30mg/m2 x 6 days and ATG cumulative dose (c.d.) 40mg/kg b.w. [Group I:n=6], Fludarabine 30mg/m2 x 5 days and c.d. 20mg/kg b.w. [Group II,n=8]; Fludarabine 30mg/m2 x 4 days and c.d. 20mg/kg b.w. [Group III,n=12]. Patients characteristics in groups is as follow: Group I/FM/2/4, age 24-41 (median 30), source of transplanted cells (STC)-BM/PBPC 4/2, donor (volunteer unrelated donor (VUD)/matched sibling donor (MSD) 5/1); Group II/F/M 3/3, age 24-44 (median 38.5), STC-BM/PBPC 2/4, donor VUD/MSD 6/0; Group III/F/M 3/9, age 17-49 (median 35.5), STC-BM/PBPC 2/10, donor VUD/MSD 2/10. Results: transplant related mortality in groups I,II and III (fatal cases/number of patients in the group) was 2/6, 3/6 and 1/12 respectively. Causes of death: group I-2 patients died (271 and
496 days after transplantation) due to severe Candidiasis of central nervous system and CMV disease; group II-3 patients died (78, 101 and 187 days after transplantation) due to high organ toxicity grades (III and IV, liver and GI tract); group III-1 patient died (37 days after transplantation) due to septic complications. The range of CD34+ cells transplanted was 1.5x106-9.9 x106/kg b.w (median 3,3x106). Short tandem repeats technique performed on day +30 proved: group l-complete chimerism (cc) in all patients (6/6), maintained during a follow-up observation (9-29 months; median 23); group II-cc in all patients (6/6),mainained (follow-up 1-11 months; median 5); group III-cc in all but 1 patient (11/12), maintained (follow-up 18-33 months; median 21,9). One patient in this group recovered autologously probably due to the low number of CD34+ cells transplanted: 1.5x106/kg b.w. In all engrafted patients there was no Ph+ cells on day 30 post transplant. Therefore, it appears that the dose of Fludarabine may be decreased to 30 mg/m2 4 days and cumulative dose of ATG to 20 mg/kg b.w. And this is associated with better survival.

Fludarabine plus cyclophosphamide (F+Cy) or F+Cy plus autologous transplantation (Tx) in the treatment of CLL patients


Purine analogues, immunotherapy and autologous or allogeneic transplantation have been increasingly used in the treatment of CLL. Aims: 1. To verify the effect of F+Cy combination chemotherapy 2. To prove the feasibility of mobilization and the effect of subsequent Tx after high dose Cy conditioning 3. To compare the follow-up of the transplanted and non-transplanted group. Methods: CLL pts with at least one risk factor (stage, diffuse marrow infiltration, doubling time, cytogenetic) have been included. F 30 mg/m2 1-3. plus Cy 250 mg/m2 1-3. every 4 weeks until the normalization of lymph nodes, liver and spleen and peripheral lymphocytes less than 3x109/l plus two additional cycles were applied, followed by restaging. Pts in CR or PRnod underwent mobilization with Cy 3 g/m2 plus G-CSF and Tx after Cy 50 mg/kg/d -5,-4,-3,-2 conditioning. Patients not agreeing with the Tx and non-mobilizers (less than 2x106/kg CD34) were just observed. Results: Until September 2001 61 pts at the age of 35-64 years (median 56) finished the F+Cy. 60 pts could be evaluated, 1 pt has been lost. 23 OR (38%)-all IgH and FACS negative, 30 PRnod (50%), 4 PR (7%) were achieved, OR 95%, 2 pts (3%) were resistant and in 1 case (2%) the treatment had to be interrupted. 42 pts underwent the mobilization. 19 pts (45%) mobilized 2.25-11.52x106/kg CD34 cells (median 2.95) with 2-5 apheresis (median 2). 23 pts (55%) were non-mobilizers. 19 pts (5x CR, 14x PRnod) underwent the Tx with the restaging result 14x CR (all IgH and FACS negative) and 5x PRnod. With the follow-up of 5-43 months (median 24) after the Tx 2 pts relapsed and 2 patients died 2 and 6 months according to the EBV-LPD. 17 pts have been living, 2 and 3 years DFS 100% and 38%. The cohort of pts achieving CR or PRnod after F+Cy and non-transplanted consists of 36 pts (23x CR, 13x PRnod after the F+Cy restaging). With a follow-up of 3-38 months (median 18) 1 pts died due to an infectious complication. In non-transplanted patients released 18, 23 and 28 after the treatment and 22 pts remain in CR and 10 pts in PR nod, and 3 years DFS 97% and 53%. Conclusions: 1. F+Cy is a highly effective chemotherapy of CLL achieving OR more than 90%, including IgH and FACS negative CRs in some patients. 2. F+Cy compromises severely the mobilization in more than 50% of pts and excludes the possibility of the following Tx. 3. Tx with high dose Cy conditioning increases further the number of CR, including IgH and FACS negative CR.

Reduced intensity conditioning in elderly CML patients

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Chronic myelogenous leukemia (CML) is a classical indication for allogeneic bone marrow (BM) and stem cell (SC) transplantation. So far the procedure was limited to young patients in good medical condition. Recently the idea of reduced intensity conditioning has made this treatment an curative option also for elderly patients. Yet the results of these so called mini-transplants in CML patients have been of limited success. In order to reduce toxicity we established a conditioning protocol reducing the dose total body irradiation and adding Fludarabin. So far 27 patients, age 45 years and older, with BCR-ABL positive CML in chronic and accelerated phase were transplanted at our center. The regimen consisted of 2 x 4Gy TBI, 4 x 30 mg/m2 Fludarabin, 120 (80) mg/kg Cyclophosphamide (Cy) and 40 mg/kg ATG. The median age was 51,2 years. Stem cell source was BM (23) and SC (4). The donors were unrelated (11) and sibling (16). GvHD prophylaxis consisted of cyclosporin A (CsA) and a short course of methotrexate (MTX). Twenty three patients were transplanted in first chronic phase, 4 patients were in accelerated or 2nd chronic phase. The median Graftwohl-score was 3,5.

We compared the outcome of patients treated according to this reduced intensity conditioning (RIC) regimen with a historic control group of 67 elderly CML patients transplanted at our center using a standard intensity conditioning (SIC) consisting of 12Gy TBI/Cy/ATG or 16 mg/kg Busulfan/Cy /ATG. Patients with blast crisis were excluded. The results are shown in table 1.

At a median follow up time of 435 days 18/27 patients (66,6%) are alive. Engraftment in the RIC group was 100%. The median time of engraftment was 19 days. In the early phase post transplantation (until day 90) we observed a higher percentage of BCR-ABL positive nested RT-PCR in the RIC group. After 360 days PCR positivity in the RIC group declined to 36%. Furthermore is a trend towards lower incidence of cytogenetic relapse and superior overall survival in the RIC group. So far these differences are statistically not significant. We conclude that our RIC protocol was well tolerated by elderly CML patients. The reduction of TBI, substituted by Fludarabin, leads to reduced transplant related mortality, stable engraftment and a low rate of cytogenetic relapse.

Can STI571 replace DLI in CML patients with relapse or persisting disease after myeloablative as well as nonmyeloablative stem cell transplantation?

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STI 571 (Glivec) is a new drug, specifically targeting CML cells. We used STI in 6 patients suffering from relapse of CML after myeloablative BMT in 2 patients and relapse or persisting disease after nonmyeloablative BMT in 4 patients, to test this promising drug in these specific patients. Results of 4 evaluable patients: The first patient( UPN 50), transplanted in 1993 experienced hematologic relapse in 3/01. In 4/01 received DLI but his relapse progressed into blast crisis in 4/01. STI 571 was initiated (600 mg/d) at that time. Mixed chimerism changed to complete during 2 months and BCR/ABL positivity changed to negativity during 2 months of STI treatment, that was stopped 3 months later (9/01). The second patient( UPN 161), transplanted in 1997, experienced cytogenetic relapse in 6/01. STI (400 mg/d) was initiated in 9/01. His chimeric status has
Within two months from the achievement of CR with Glivec, the patient initiated STI treatment (400mg/d) in 8/01. The proportion of autologous hematopoiesis was reduced from 77% to 2% during 2 months of STI administration, although the patient has not achieved PCR negativity yet. The fourth patient (UPN Z71) transplanted in 4/01 from a nonmyeloablative conditioning regimen, had persisting disease influencing CML without risk of GVHD.

Conclusion: STI administration in our 4 evaluable patient resulted in significant reduction of autologous hematopoiesis in 2 patients, and in reversion from mixed to complete chimerism in 2 patients. All patients have also decline of BCR-ABL mRNA and in remission. We report our experience with Glivec in two patients treated for a blast crisis during BMT and subsequently submitted to a second transplant. These cases were enrolled in CML-003 protocol from Italian Co-operative Study Group (ICSG) on CML, with STI administered orally at daily doses of 600 mg.

Patient N.1, male, 38 yrs, developed a bifenotypic BC six years earlier in the course of their disease, from a stem cell harvest, because of persisting aplasia, at least in patients who benefited, due to the small number of normal stem cells left in their bone marrow. These long aplastic periods can lead to life-threatening complications. We have, in such a patient reinfused peripheral blood progenitors.

Within two months from the achievement of CR with Glivec, the patients underwent a second transplant, conditioned with standard Cy-TBI regimen (preparation regimen of the first BMT was BUCY-4). Both received PBSCs: patient N1: CD34+ = 5.83 x 10^6/Kg; patient N2: CD34+ = 3.2 x 10^6/Kg. The procedure was well tolerated; the days to engraftment were, respectively: for PMN >0.5 x 10^9/L +12 and +17; >1 x 10^9/L +13,+20; for platelets >20 x 10^9/L: +12,+20. The patients were discharged at the days +18 and +21. They are alive at days +174 and +48, respectively, the first relapsed at day +83.

Our experience indicates that STI571 in these two patients with CML-BC occurring after BMT was effective and safe. It did not preclude a second transplant. In fact, the engraftment was not delayed and the procedure was not complicated by major treatment related toxicity.

Successfull allogeneic BMT for CML in blast crisis with pre and post BMT imatinib mesylate (STI 571)

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CML in blast crisis carries a poor prognosis. Even with BMT, the survival rate is dismal. Different chemotherapeutic and biological regimens have been used with varying success. Imatinib mesylate (STI 571) is a novel tyrosine kinase inhibitor that has been breaking new ground in the treatment of CML. Its use in relation to BMT has not been established. We report a child with CML in blast crisis who was successfully transplanted together with STI 571 pre and post transplant.

NBA is a 13-year-old Malay girl who was diagnosed in June 2001 with Philadelphia-positive CML. Her presenting WBC was 219 x 10^9/L with 17% blasts and platelets 752 x 10^9/L. Bone marrow showed CML in accelerated phase with 14% myeloid blasts. She was treated with AML chemotherapy and went into morphological remission after 1 course of ADE. However, she went into blast crisis 4 weeks later with marrow blast count of 34%. She was re-induced with another course of ADE followed by STI 571 whilst awaiting matched allogeneic BMT. She was transplanted while in morphological remission in August 2001 with BUCY conditioning regimen, and engrafted on D+16. She had only grade 1 skin GvHD that was controlled with cyclosporine A. Her D+28 marrow was in cytogenetic but not molecular remission. She was restarted on STI 571 on D+30. She is currently D+101. Her bone marrow on D+84 is in both cytogenetic and molecular remission. She remains well and our plan is to continue STI 571 for at least 6 months post transplant.

Although the follow up is short, it is hoped that STI 571 will act as a “minimal residual disease eradicator” and maintain her remission. There is no consensus yet on the duration of STI 571 post BMT or the ideal way to incorporate its use into BMT. However it is encouraging that in our patient, she was converted from a cytogenetic remission to a molecular remission probably with the help of STI 571. The optimal treatment of CML in blast crisis may require the entire arsenal of chemotherapy, biological agents like alpha-interferon and STI 571, and BMT.
Monitoring of hematopoietic chimerism (HC) after stem cell transplants plays an important role in evaluation of engraftment and risk of relapse. HC was monitored in 30 CML recipients of alloPBSC transplants from HLA matched family donors. All patients were conditioned with Bu/Cy 120 protocol. In all cases CsA/Mtx regimen was used to prevent GvHD. 3 methods were employed for HC evaluation: 1/ semi-quantitative capillary electrophoresis to determine VNTR in peripheral blood and bone marrow, 2/ presence of bcr/abl transcript in the blood and bone marrow and 3/ examination of blood group antigens (using serological methods and additionally flow cytometry in 15 pts.). HC was studied on days 30, 60, 100, 180 and 270 posttransplant. Also the incidence of acute GvHD was analysed. On day 180 posttransplant 16 patients showed complete donor chimerism as determined by VNTR. The incidence of aGvHD (grade II-IV) in this subgroup of patients was 59%. In 3 of those both donor and recipient type erythropoiesis was detectable. In the remaining 14 pts. mixed chimerism in VNTR persisted beyond day 270. The incidence of aGvHD was 42%. Also in 8 of those pts. residual recipient type erythropoiesis could be detected. The two subgroups of patients were similar as to long-term presence of bcr/abl transcript. Our study confirms prolonged mixed HC in some CML patients after alloPBSCST. It may also suggest a correlation between the tempo of achieving full donor chimerism and the incidence of aGvHD. Further prospective studies of greater numbers of patients are warranted.

P631
CD38 expression has no prognostic influence after autologous stem cell transplantation (SCT) for chronic lymphocytic leukemia (CLL)
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Although published data are contradictory, CD38 surface expression might be a prognostic factor in patients with CLL treated conventionally: CD38+ CLL is prone to a progressive course and a dismal prognosis, whereas modest or missing CD38 expression should be correlated with stable disease and long overall survival. The purpose of this study was to investigate if the CD38 status has some prognostic influence after SCT. Therefore we studied CD38 expression in 55 patients with CLL who had undergone myeloablative radiochemotherapy with autotransplantation of immunomagnetically purified stem cells. CD38 expression was analysed by tricolor flow cytometry using fresh or cryopreserved diagnostic blood samples obtained at diagnosis or relapse post transplant. CD38+ and CD38- cases were defined as those with >=30% and <30% CD38-positive CD19+ CD5+ CLL cells, respectively. Results: >=30% CD38 expression was found in 25 patients (45%). The CD38 status was not correlated with the presence of other biological risk factors, such as short lymphocyte doubling time (p=0.28), 11q- deletion (p=0.21) and most importantly, VH gene mutational status (p=0.11). Accordingly, CD38 expression was not predictive for the outcome, long post transplant: Time to relapse and time to recurrence of molecular cloneity (IgH consensus CDR3 PCR/genescanning) were similar in patients with and without CD38 expression (2-year relapse probability 6% and 7%, respectively, p=0.93; 2-year cloneity probability 27% and 17%, respectively, p=0.71). Follow-up was 20 (3-92) months. A 10% CD38 cut-off yielded similar results. Conclusions: A significant prognostic influence of CD38 expression after autografting could not be detected in the present analysis. Thus, CD38 assessment is not an appropriate substitute for VH gene sequencing, which allows segregation of prognostic groups in terms of SCT for CLL.
treatment with temporary correction of haemopoiesis but with persistent fibrosis. Allogeneic transplant is a promising therapy for myelofibrosis in suitable patients. Pre-transplant splenectomy favours more rapid engraftment.

Additional abstracts to this topic

**Immunological short-term lymphocytes recovery after PBSCT for chronic lymphocytic leukemia**

L. Laurenti, N. Piccirillo, S. Cicconi, P. Chiusolo, F. Sorà, P. Piccioni, M. Garzia, S. Rutella, G. Leone, S. Sica (Rome, I) During last years PBSCT for Chronic Lymphocytic Leukemia (CLL) has been adopted for younger patients with high risk disease (A progressive, B or C stage). GIMEMA group developed a protocol for untreated younger patients (<60y) During the last two years 4 patients affected by Chronic Lymphocytic Leukemia (CLL), three of them previously pretreated, were submitted to autologous PBSC transplantation. All patients underwent chemotherapy with Fludarabine 4 cycles, Endoxan and PBSC harvest and transplantation after conditioning regimen with Mitoxantrone and Melphalan. Two patients were male, 2 female, median age was 47 years (range 46-53) all patients received G-CSF starting from day +7 until PMN >500 mmc). Median dose of CD34+ cells in the graft was 2.4 x106/kg (range 1.91-4.5) rapid and complete hemopoietic recovery was promptly achieved (PMN 500 mmc on day+13, PLT >20,000 mmc on day +14). Periodical immunological study was performed at day +7, +30, +60, +90, +120. We applied Simultest IMK that included CD3, CD4, CD8, CD4/CD8 ratio, CD19, CD3DR, CD16/56. Total lymphocytes count decreased after transplant at minimum values (220 mmc) at day +15, after than showed a sharp increased with a trend toward normalization by day +90 achieving value of 1005 mmc. CD3+ cells remained below the normal range during study period (591 mmc as maximum value at day +120). (vn: 1.185-1.540 mmc). CD4+ subset was costantly and markedly reduced in comparison with normal values (vn: 670-950 mmc) throughout the study period remaining below 176 mmc at day +120. CD8+ cells showed the same trend as CD4+ subset remaining costantly reduced although a sharp increase during the study period was noticed (404 mmc as maximum value at day +120) (vn: 505-695 mmc). As result a persistent reduction of CD4/CD8 ratio, except for day +15 in which ratio rose to 2.7, was observed until day 120 (vn:1.1-1.8). Effector cells as activated CD3DR+ were strongly increased before and after transplant in all checks achieving value of 1079 mmc at day +120 (vn:40-155). NK cells CD16/56+ were into the normal range by day +15 onwards (vn:70-190 mmc). All patients are in continuous complete remission at median time of 17 months after transplantation (range 7-30 months). In conclusion PBSCT in CLL was feasible in term of engraftment, but delayed total lymphocytes recovery (500 mmc on day +47) and in particular the reduction of CD4 subset must be monitored, with longer follow-up on a larger group of patients.

**Immunophenotypic remissions in patients with CLL after stem cell transplantation**

T.-T. Pelliniemi, M. Itäla, A. Rajamäki, K. Remes (Turku, FIN) CLL is an incurable disease with conventional chemotherapy. This study was designed to examine if stem cell supported high-dose therapy can induce durable immunophenotypic remissions in younger patients with advanced CLL. The immune phenotype of the CLL cells was defined as CD19+/CD20dim/CD5+/CD23+/CD79b- and was confirmed with the clonality of the CD20dim/CD5+ cells. For the analysis of minimal residual disease 1 x 10^6 CD19+ cells or 5 x 10^6 total bone marrow nucleated cells were analysed with FACSscan or FACScalibur (Becton-Dickinson) flow cytometry. Thirteen patients (9 M, 4 F) with median age of 50 years have received stem cell (9 autologous, 4 allogeneic) supported high-dose (9 HDCY + TBI, 2 BEAC) or reduced-dose (2 fludarabine-based) therapy at a median of 36 (7-122) months from diagnosis. The disease status at transplantation was CR in 4, PR in 4, stable in 4 and progressive in 1 patient after a median of 7 (4-14) chemotherapy cycles (1-15 different regimens) including fludarabine in 11 patients. The median numbers of CD34+ cells transfused were 2.7 (1.9-13.6) and 5.0 (4.5-9.4) x 10^6/kg in the autologous (4 CD34+ selected and 5 unselected blood grafts) and allogeneic setting, resp. The recovery was rapid with median time to ANC >1.0 x10^9/L of 12 (9-18) days, to PLT > 20 x 10^9/L 13.5 (7-26) days, and to discharge from hospital 17 (4-24) days. After transplantation, 9 of the 11 evaluable patients (two patients nonevaluable with only 1-month follow-up) achieved morphological and 8 patients immunophenotypic CR. Four patients have died, one with infectious complication at 7 months and three in fulminant relapse at 18 to 66 months. Seven of the evaluable patients are alive with a median follow-up of 51 (15-75) months. Four patients are in continuous immunophenotypic CR at 15+, 69+, 70+ and 75+ months, resp. One additional patient has remained in morphological remission with residual disease fluctuating between 0.1%-1% for 34+ months. In conclusion, this study demonstrates that long-lasting immunophenotypic remissions can be achieved with stem cell transplantsions even in patients with advanced CLL.

**Unrelated allogeneic hematopoietic stem cell transplantation (HSCT) for myelofibrosis with myeloid metaplasia (MMM)**

M. Koldehoff, N. Basara, G. Lentini, H. Pohl, M.G. Kiehl, A.A. Fauser (Idar-Oberstein, D) MMM is a clonal stem-cell disorder that leads to ineffective erythropoiesis, dysplastic-megakaryocyte hyperplasia, and an increase in the ratio of immature granulocytes to the total number of granulocytes. This clonal disease is accompanied by reactive myelofibrosis(MF) and by extramedullary hematopoiesis in the spleen or in multiple organs. The use of HSCT using related HLA-identical donor offers a potential to cure the patients(pts) with MF. However, so far only a few pts have been treated with unrelated donor(UD) HSCT. We discuss our pts with MMM successfully treated with HSCT from an UD. A 52years(y) old man with a history of a disease duration(hdd) of 144 months(ms), his bone marrow (BM) histology revealed severe MF without signs of osteosclerosis(Osc). The pt received a PBSCT with 4.2 x 10^6/kg CD34+ cells from HLA-identical UD. Neutrophil engraftment(NE) occurred on day +22, platelets engraftment(PE) on day +42 after PBSCT. MF in BM at day +100 and day +220 after PBSCT was documented. The pt is doing well 2y after unrelated PBSCT. The second pt is a 46y old woman with a hdd of 33 ms. BM histology showed MF with Osc. The pt received PBSCT with 3.3 x 10^6/kg CD34+ cells from HLA-non-identical UD. NE occurred at day +18 and PE at day +29 after PBSCT. MF in BM at day +100 was slightly decreased in comparison to the one prior to PBSCT. The pt is doing well one y after PBSCT. The third pt is a 39 y old women with a hdd of 47 ms. BM histology showed MF with extremely diffuse Osc and abnormaly megakaryocyte hyperplasia. The pt had a elective splenectomy 3 ms before HSCT. The pt received BM with 1.68 x 10^6/kg CD 34+ cells from HLA-non-identical UD. NE occurred at day +23 and PE at day +24 after BMT. After a transplant related complications i.e., staphylococcus sepsis, BK-virus associated cystitis with bleeding, CMV-reactivation, acute cutaneous GvHD, Grad II-III, treatment associated secondary transplantation failure the pt received a second PBCST with 3.2 x 10^6/kg b.w. CD 34+ cells from same UD at day +96. MF in BM at day +118 was slightly decreased in comparison to the one prior to HSCT. The pt engrafted and was discharged on day +124. However, the first outpatient follow up showed severe acute gut GvHD, and was admitted for the treatment of severe GvHD. The pt died on day +171 of severe multigang failure and progressive severe hepatic steatosis. In conclusion an allogeneic PBSCT from DU is a feasible and an effective treatment in selected pts with MMM.
15. Graft versus Host Disease

P634
Coexistence of high levels of ineffective erythropoiesis and functional engraftment after bone marrow transplantation for beta thalassemia
F. Centis, P. Tonucci, S. Rapa, R. Rossi, M. Battarra, E. Gueraccini, G. Tombari, M. Manna, F. Agostinelli, M. Andreani, G. Lucarelli (Pescara, I)

Beta thalassemia major is characterized by severe anemia mainly due to erythroid precursors intramedullary programmed cell death (PCD) whose amount directly correlate to the extent of erythroid hyperplasia (Centis et al. Blood 2000;96:3624). Bone marrow transplantation (BMT) is currently the only rational therapeutic modality for the definitive cure of beta thalassemia major. Persistence of residual host cells (RHC), referred to as mixed chimerism (MC), has been described in approximately 10% of long term survivors (follow-up 2 to 14 years) (Andreani et al. Blood 1996;87:8). In this report we compared the extent of erythroid precursors PCD in patients with increasing levels of RHC long after BMT (3-10 years), in transplanted patients with complete chimerism after BMT (CC), in beta thalassemic patients with increasing erythroid hyperplasia (EH Low, EH Medium, EH High) before BMT and in healthy controls (HC). Patients were evaluated for MC by DNA-based techniques (RFLP, PCR-VNTR) or FISH analysis for the Y chromosome. PCD was evaluated by FACS analysis using Annexin V (AnV) reactivity on the erythroid precursors (CD45-).

Figure shows that transplanted patients with a RHC percentage higher than 30%, which are completely transusions-independent, have an amount of apoptotic erythroid precursors close to beta thalassemic patients with a low erythroid hyperplasia before BMT. This biological observation make the healthy ex-thalassemics thalassemic patients with a low erythroid hyperplasia before BMT. In conclusion this method allows an accurate monitoring of the marker and the donor/recipient allelic configuration. This method can be rapidly performed with very low amount of DNA (20ng) allowing, in particular, detection and quantification of chimerism on cell sorted sub-populations. Except in one case where no allelic difference between the donor and the recipient could be detected, this assay has been successfully performed in 100 patients with HLA and sex-matched donor.

P635
Positive serum cross-match as predictor for graft failure in HLA mismatched allogeneic blood stem cell transplantation

Background: Evaluation of patient sera for complement-fixing anti-donor antibodies (serum cross-match, XM) before allogeneic blood stem cell transplantation (BSCT) is routine in most centers. However, in contrast to kidney transplantation, the predictive value of a positive XM for outcome of BSCT is still unclear and a positive XM is presently not regarded as an absolute contraindication to proceed to transplant.

Methods: To clarify the role of a positive XM as predictor for overall survival (OS) and graft failure (GF) after BSCT, a retrospective, single center, matched pair analysis was performed. Enrolled were all XM positive BSCT performed at our institution from 1985 until 2000 (n=30). Controls (n=30) were matched for disease, disease stage, patient age, period of transplant, conditioning regimen, protocol for prevention of graft-versus-host disease, and type of donor (related vs. unrelated, HLA identical vs. HLA mismatched). Results. Multivariate statistical analysis of adjusted and unadjusted 60 transplants revealed GF as the all dominating independent risk factors for low OS (RR:59.5, p < 0.0001). Univariate (Kaplan-Meier) analysis could attribute inferior OS and high incidence of GF to the subgroup of HLA mismatched, XM positive transplants (p = 0.01). Conclusions: A XM should always be performed in patients awaiting a BSCT from HLA mismatched donors, since a positive XM is a predictor for inferior OS due to GF in BSCT.

Transplantation - in press (ca.03/2002)

P636
Detection and quantification of hematopoietic chimerism using fluorescent STRs after allogeneic stem cell transplantation
J. Eliaou, O. Avinens, M. Moussiere (Montpellier, F)

Allogeneic bone marrow or peripheral blood stem cells transplanted to patients with lympho or myelo-proliferative disorders are known to efficiently eliminate tumor cells. The presence of complete donor-derived hematopoiesis is essential for sustained engraftment and prevention of relapse. Early detection and quantification of hematopoietic chimerism is of particular interest for clinical outcome.

Chimerism has been detected using agarose gel electrophoresis of PCR-amplification fragments of short tandem repeat (STR) markers. However, this assay is often limited by its sensitivity and do not allow a quantification of the donor/recipient hematopoietic cell ratio. We used a panel of 11 STR, 3 VNTR and 6 microsatellites markers. Fluorescence-based detection and quantification of PCR-amplified markers was performed on ABI 373 automated sequencer. Linear correlation (r=0.99) was always obtained between fluorescence peak ratios and donor/recipient cell mixture experiments performed for each donor/recipient pair. The limit of detection varies between 1% to 0.1% depending on the marker and the donor/recipient allelic configuration. This method can be rapidly performed with very low amount of DNA (20ng) allowing, in particular, detection and quantification of chimerism on cell sorted sub-populations. Except in one case where no allelic difference between the donor and the recipient could be detected, this assay has been successfully performed in 100 patients with HLA and sex-matched donor.

In conclusion this method allows an accurate monitoring of hematopoietic chimerism and is of particular importance for the adjustment of immune-suppressive therapy after stem cell transplantation.

P637
Endothelial cell markers (ECM) in acute graft versus host disease (aGvHD)
E. Bullorsky, C. Duboscq, C. Shanley, G. Stemmelin, J. Ceresetto, C. Rabinovich (Buenos Aires, AR)

aGvHD is the most common and serious complication associated with allogeneic BMT, characterized by an increase of endogenous cytokines and endothelial damage.

The aim of this study is to evaluate the endothelial cell markers FvW, PAI and E-Selectin in patients with aGvHD.

Patients: 22 consecutive patients who underwent BMT because of aplastic anemia (4), CML (5), AML (4), ALL (3), MDS (3), NHL(2)
and Hodgkin disease(1). GvHD prophylaxis was done with cyclosporine +
prednisone + MTX. The incidence of aGvHd was 45 % (severe in 6 pts), and none had associated microangiopathy.

Controls: 42 consecutive patients who underwent autologous transplants (ABMT) and 20 normal blood donors, all with similar age. ECM were determined by ELISA the day of admission, on day of BMT or ABMT before marrow infusion, and on days +7, +15 and +30 post-transplant. Results: ( * p < 0.01 )

<table>
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<tr>
<th></th>
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<tr>
<td>FvW</td>
<td></td>
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<tr>
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<td>110</td>
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<tr>
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<td>140</td>
<td>184*</td>
<td>211*</td>
<td>290*</td>
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<tr>
<td>PAIagg</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>14</td>
<td>10</td>
<td>38</td>
<td>27</td>
<td>25</td>
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<tr>
<td>E-selectin</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Normal 2-23</td>
<td>82*</td>
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<tr>
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<td>15</td>
<td>10</td>
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<td>25</td>
</tr>
</tbody>
</table>

Conclusions: 1) Patients who developed acute GvHD had significantly higher levels of FvW, PAI and E-selectin than patients without GvHD, ABMT or the normal controls. 2) The rise of these markers suggests an associated endothelial damage in patients with GvHD. 3) The early increase of E-selectin in pts who latter developed GvHD may reflect severe endothelial damage from intensive previous chemotherapy.

P638
Endogenous cytokines as markers of graft vs host disease (GvHD)
E. Bullorsky, C. Duboscq, C. Shanley, G. Stemmelin, J. Ceresetto, O. Rabinovich (Buenos Aires, AR)

Dysregulate cytokine production is responsible for many manifestations of acute GvHD, one of the most important complications of BMT. Aim of the Study: To investigate the levels of cytokines involved in inflammation response such as TNF alfa, IL-6 and also IL-10 in patients with GvHD. Patients: 22 consecutive patients who underwent allogeneic BMT because of aplastic anemia (4), CML (5), AML (4), ALL (3), MDS (3), NHL (2) and Hodkin disease (1). GvHD prophylaxis was done with cyclosporine + prednisone + MTX. 10/22 patients (45 %) developed aGvHd, confirmed by skin biopsy (Grade IV in 4 pts). Controls: 42 pts who underwent autologous transplant (ABMT) and 20 normal blood donors. Cytokines were determined by ELISA in the transplant cohort the day of admission (basal), in the middle of the conditioning regimen (CR), the day of BMT or ABMT before the infusion and on days +7, +15 and +30 post-transplant. Results: ( * p < 0.01 )

<table>
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<tr>
<th></th>
<th>Basal</th>
<th>CR</th>
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<td>11.5</td>
<td>6.5</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<td>12.3</td>
<td>10.8</td>
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<tr>
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<td>13.0</td>
<td>55.6*</td>
<td>48.3*</td>
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<td></td>
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<tr>
<td>Normal 1-9 pg/ml</td>
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<td>3.9</td>
<td>4.1</td>
<td>8.1</td>
<td>3.2</td>
<td>3.3</td>
</tr>
<tr>
<td>ABMT</td>
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<td>3.3</td>
<td>6.5</td>
<td>3.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Conclusions: 1) A significative increase of endogenous cytokines was observed on days +7/+15 in pts who developed GvHD. 2) During the CR there was an early increase of TNF in BMT pts compared with ABMT, more significative in pts who later developed aGvHd (early marker?), 3) pts without GvHd had a similar cytokine pattern than ABMT pts, with a difference in the mentioned early increase of TNFalpha.

P639
Analysis of effector cells involved in graft versus host disease (GvHD) in bone marrow (BM) graft recipients using an in vitro skin explant model

Objectives: To characterise effector cells involved in GvH reactions and predict the outcome of allogenic HLA matched BM transplants using a human skin explant assay. Materials and methods: Donor T cells from HLA matched siblings were sensitised in a primary MLC, using patient PBMC as stimulus cells in an in vitro skin explant assay. The levels of IFN-gamma, TNF-alpha and IL-10 in primary MLC and secondary MLC/skin supernatants were assessed by ELISA. T cell lines and clones derived from the model skin explant culture were characterised with respect to phenotype, intracellular cytokine profile (IFN-gamma, IL-4, IL-5, TNF-alpha, IL-10 and IL-13) and cytotoxicity. Results: In the primary MLC, donor cells with higher levels of TNF-alpha secretion correlated with patients developing more severe GVHD grades II-IV (158-2526 pg/ml, mean 968.85 + 886.04 pg/ml; n=3) compared with patients with grade 0-1. GVHD (61-221 pg/ml, mean 155.33 + 70.40 pg/ml; n=6). In the presence of the patient skin biopsy (secondary MLC) all the samples produced very low or undetectable levels of TNF-alpha (<35 pg/ml). High levels of IFN-gamma were produced in secondary MLC/skin supernatants and correlated (p<0.05) with patients who developed severe GVHD (155-279 pg/ml) compared to the patients with 0-1. GVHD grades (<31-153 pg/ml). Cytotoxicity of MLC expanded cells tested against patient, donor or K562 EBV lines showed in 4 out of 7 samples a response against patients' target cells. T cell cytotoxicity was increased in patients who developed severe GVHD (20.25 + 7.63 vs 3.77 + 4.90, p<0.02, for GvHd II-IV and 0-I grades, respectively). This may be due to a minor Histocompatibility antigen (mHag) specific response, not normally detected pre-transplant in vitro.

Conclusion: Our results imply that pre-transplant analysis of donor-patient responses (increased production of proinflammatory cytokines and cytotoxic responses in vitro) may predict the GvHD outcome of the recipients of HLA-matched alloBMT and aid in the further elucidation of mHags.

P640
Allsponses to HLA-DP detected by the measure of HTLP frequency before bone marrow transplantation could provide indications on the risk of development of graft versus host disease
A. Eljaafari, A. Farre, M. Michallet, C. Martin, D. Rigal, L. Gebuhrer (Lyon, F)

HLA-DP is considered as a transplantation Ag by certain authors, due to the isolation of HLA-DP specific T cell clones post-bone marrow transplantation (BMT) in patients suffering from GVHD. But for others, statistical analyses did not favour HLA-DP mismatch as a cause of GVHD. In this study, by measuring helper T lymphocyte precursor frequency (>HTLP) among blood donor pairs matched by high resolution typing for HLA class II molecules, we have shown that HLA-DP matching can give rise to high (superior to 1/50 000), intermediate (between 1/50 000 to 1/100 000) or negative (inferior to 1/100 000) frequencies of immunoreactive T cells, depending on the
combination pair tested. Moreover, when isolated T cells from a skin biopsy in a patient suffering from GVHD following BMT from an unrelated donor matched by high resolution typing for HLA-class I and class II molecules, except for HLA-DR at a single locus (A0401,1701), we found that the antigen sites were specific for HLA-DR Ag. This was assessed by the use of mAb against HLA-DR and of several APCs expressing HLA-DR 1701 or not. Mixed lymphocyte reactions and HTLPs performed in this donor/recipient pair before transplantation showed that MLR was weak, i.e. R =10%, whereas HTLP frequency was high, i.e. 1/5492. Altogether, our results support the notion that a single HLA-DR Ag mismatch alone is not enough to cause GVHD at the clonal level. This study was performed with the support of La Ligue Nationale contre le Cancer.

P641
Assessment of cellular permissivity between HLA-A*2402 and HLA-A*2403 at the clonal level
C. Martin, A. Farre, L. Gebuhrer, D. Rigal, A. Eljaafar (Lyon, F)

Detection of cellular permissivity should allow extension of donor registries and contribute to treat more patients. Therefore, in our department, limiting dilution assays are performed when a single HLA-antigen mismatch is found among donor/recipient pairs.

Methodology: Permissivity between HLA-A*2402 and HLA-A*2403 antigens was initially detected by the use of the cytolytic T lymphocyte precursor (CTLP) assay. Indeed, CTLPs were found negative in both directions among two different donor/recipient pairs expressing the HLA-A*2402/HLA-A*2403 Ag mismatch. To further assess this cellular permissivity, T cell clones specific for HLA-A*2403 Ag were generated. With this aim, peripheral blood mononuclear cells expressing HLA-A*2403 were used to stimulate HLA-A*2403-negative APCs sharing with them 5 out of 6 HLA class I antigens. Cytotoxicity was assessed by the 51-chromium release assay. Results: 4 T cell clones specific for HLA-A*2403 were obtained. Our results show that in addition to lysis of HLA-A*2403 expressing antigen presenting cells (APCs), these T cell clones were also able to lyse any APCs expressing HLA-A*2402. Supporting the specificity of the permissivity, HLA-A*2416 expressing APCs were not susceptible to lysis, neither nor APCs expressing full HLA class I and class II mismatched haplotypes. Conclusion: Here, by generating T cell clones, we have demonstrated cellular permissivity between HLA-A*2402 and HLA-A*2403 antigens. The methodology we used should allow acceptance of this mismatch in bone marrow transplantation. This work was performed with the support of La Ligue Nationale contre le Cancer.

P642
Genomic organization and HLA-restricted peptides of CD13 (aminopeptidase N)
B. Eiz-Vesper, D. Gottschalk, K. Müller, B. Hertenstein, R. Blasczyk (Hannover, D)

Minor Histocompatibility Antigens (mHags) are thought to be targets for graft versus host and graft versus leukemia reaction as well as graft rejection after stem cell transplantation. This study was de-signed to identify new mHags which are involved in the immunotherapeutically desired graft versus leukemia effect. Aminopeptidase N is a member of a family of membrane-bound metalloendoproteases. This Protease is expressed on the surface of normal and malignant human myeloid cells, fibroblasts, hepatocytes, and the epithelial cells that form brush borders of the small intestine and kidney. Cell-surface metalloendoproteases have specific functions, including potential roles in the control of growth and differentiation in hematopoietic and epithelial systems by participating in the final steps of digestion by cleaving peptide (peptide scavenging). An indirect consequence of genetic variation is the generation of polymorphic self peptides that may cause therefore histoincompatibility. By sequencing cDNA of Aminopeptidase N a 967-amino acid protein was predicted. We detected 17 single nucleotide polymorphisms, resulting in 13 amino acid exchanges. In order to develop a simple PCR-SSP strategy for these polymorphic sites we cloned DNA into a pcGR2.1 plasmid and sequenced. The gene coding for Aminopeptidase N has a length of 21,238 nucleotides and consists of 19 non-coding regions and 20 exons.

To further characterise immunodominant mHags of Aminopeptidase N we used the proteosomal cleavage prediction algorithm "PProC" to analyse the polymorphic regions for a problem of proteosomal processing. Here we present two Aminopeptidase N peptides (Q86R and I603M) predicted in the correct matter for peptide presentation in HLA-A*0201, HLA-B*0701, HLA-B*0801 or HLA-B*2705 molecules. Whereas the predictive value of the CTLP is 'broadly' accepted, the value of the HTLP is less clear. We hypothesized the HTLP to detect alloreactivity in a broader sense. So if CTLP and HTLP both are very low in value (below 10 in 1,000,000), could a stem cell donor with clear HLA-differences be acceptable? Moreover can we find donor/recipient combinations with these characteristics? Is it possible to search for such combinations? The answer seems to be yes!

P644
The incidence of acute GVHD is related to CD34+ content of the graft
I. Chamakh, R. Bélanger, R. Le Blanc, L. Busque, D. Fish, D.-C. Roy, C. Perreault, G. Sauvageau, M. Dumont, J. Roy (Montreal, CAN)

Introduction: There is no consensus on the ideal number of CD34+ peripheral blood stem cells that should be infused to allogeneic transplant recipients. Of concern is the observation made by Przepiorka et al., in 1999 that a high dose of CD34+ cells...
was associated with a higher incidence of aGVHD. We sought to determine whether the same observation could be made in a cohort of patients who received a homogenous prophylaxis with CSA and MTX. METHODS: We undertook a retrospective study of all patients who underwent allogeneic haematopoietic cell transplantation (HCT) patients performed between August 1995 and May 2001. Data collected included: demographics, diagnoses, conditioning regimen, D/R CMV status, number of doses of MTX received, incidence and grade of aGVHD, incidence of cGVHD, and graft cell components (CD34+, CD3+, CD4+, CD8+, CD19+ and CD56+ cells). All factors were studied with preliminary univariate analyses and Cox regression models. Results: A total of 139 patients (M/F: 85/54), age 23-57 years (median 45) received aGVHD prophylaxis with CSA and short course MTX: most patients (79%) received 4 doses of MTX, whereas 19% received only 3 doses. Diagnoses were CML (31%), lymphomas (19%), AML (16%), MM (11%), MDS (10%), ALL (9%), and others (4%). Conditioning included TBI-based regimens (40%), or chemotherapy only (60%). Incidences of aGVHD grade I-IV, II-IV and III-IV were 28%, 19%, and 9% respectively. Patients receiving 8x10^6 CD34+ cells/kg or more had an increased incidence of grade I-IV (48% vs 19%; p<0.001), II-IV (40% vs 9%; p<0.001) and III-IV (17% vs 6%; p=0.049) aGVHD. Similarly, patients receiving 6x10^6 CD34+ cells/kg or more also had an increased incidence of grade I-IV (38% vs 17%; p=0.007) and II-IV (29% vs 7%; p=0.01), but not III-IV (p=0.44). We nevertheless found evidence for the increased risk for morbidity in patients receiving greater than 6x10^6, or 8x10^6 CD34+ cells/kg. No other clinical or graft variables were associated with a higher incidence of aGVHD. Incidence of cGVHD was 48%, but in contrast to aGVHD, there was no increased incidence in patients who received higher doses of CD34+ cells. Conclusions: Our results confirm that higher doses of CD34+ cells/kg are associated with increased incidence of grade I-IV, and II-IV aGVHD. Whether doses of 6x10^6 CD34+ cells/kg or less should be targeted remains unclear at the present time since a higher incidence of aGVHD does not translate into a higher mortality rate.

P645

Risk factors for moderate-to-severe chronic graft-versus-host disease after allogeneic stem cell transplantation

M. Remberger, G. Kumlien, J. Aschan, L. Barkholt, P. Hentschke, P. Ljungman, J. Mattsson, J. Svennilson, O. Ringdén (Stockholm, S)

Among 810 consecutive haematopoietic stem cell transplantation (HSCT) patients, 679 survived more than three months and were evaluated for chronic graft-versus-host disease (GVHD). The aim of this study was to find predisposing factors increasing the risk to develop moderate-to-severe chronic GVHD. A majority of the donors were HLA-identical siblings or related (n=435), while 185 donors were HLA-identical siblings or related (n=435), while 185 donors were HLA-matched unrelated and 59 mismatched related or unrelated donors. Most of the patients had a haematological disease (32%) or malignancy (n=568), but 111 patients with a non-malignant disease were also included. Two-hundred and nineteen patients (32%) developed mild, 45 (6.6%) moderate and 18 (2.7%) severe chronic GVHD. The five years probability of moderate-to-severe chronic GVHD was 14%. We analysed 34 potential risk factors for chronic GVHD. In multivariable analysis acute GVHD grades II-IV (RR: 2.72, 95% CI: 1.56-4.74, p<0.001), recipient age >17 years (RR: 2.29, CI: 1.18-4.44, p=0.014), CML diagnosis (RR: 1.79, CI: 1.03-3.08, p=0.037) and absence of G-CSF treatment post HSCT (RR: 0.44, CI: 0.20-0.97, p=0.043) were independent risk factors for moderate-to-severe chronic GVHD. In patients with no risk factors, the 5-years probability of moderate-to-severe chronic GVHD was 0%, 7% with one risk factor, 17% with two, 26 with three and 47% with all four risk factors present. Conclusion: Independent risk factors for moderate-to-severe chronic GVHD were acute GVHD II-IV, recipient age >17 years, CML diagnosis and no G-CSF after HSCT.

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Cytokine gene polymorphism risk factor analysis in HLA matched sibling transplants


Recent single centre studies have demonstrated the association of the cytokine gene polymorphisms of IL-10, TNF, IFN gamma, IL-6 and IL-1Ra, with increased risk of GVHd if present in the patient or donor genotype. IL-6-174 associates with chronic GVHD. In this present study a cohort of 242 HLA matched sibling transplants were studied collectively from 4 BMT centres within Europe. This multi-centre cohort was diverse in structure, with a wide age range and prophylaxis regimen. Acute GVHD grades II-IV and III-IV associated with patient genotypes for IL-6-174 (p=0.001) and IL-10-1064(12-16), (p=0.004); donor genotypes for IL-10-1060(12-16) (p=0.003) and a trend observed for IFN gamma intron-1 allele 3 (p=0.085) by univariate analysis. Incidence of GVHD (0 versus I-IV) associated with patient genotype for IL-6-174 only. In this current heterogeneous population, after correcting these associations for multiple factors by binary logistic regression, a significant association for trend was still observed for GVHD III-IV with patient genotype for IFN gamma (p=0.042) and IL-10 (p=0.048), and for donor genotype with IL-10 (p=0.04). IL-6-174 had a significant association with GVHD II-IV (p=0.03) and also with a positive result in a skin explant assay. GVHR grade II-IV (p=0.03). Age, sex-mismatch and diagnosis were not significantly associated in this cohort. The results further suggest the need for individual centre analysis for patient GVHD risk assessment, as in a single centred paediatric cohort showed no association with cytokine gene polymorphism genotype. Similarly, a single centre effect of increased GVHD prophylaxis also appeared to alter the risk analysis, with TNF-308 showing a significant association with more severe GVHD. Interestingly population genotype also appeared to play a role in the risk factor analysis and requires further investigation in a larger cohort.

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Lack of interferon-gamma (IFN-gamma) induced indoleamine2,3-dioxygenase (IDO) expression in patients with severe acute GVHD (aGVHD) (grade III-IV)


IDO is an IFN-gamma induced enzyme, which is discussed to play an important role for prevention of allogeneic fetal rejection. IDO effects suppression of T cell activity by catabolizing the essential aminoacide L-tryptophan. It is thought that by expression of IDO, the mammalian conceptus defends itself against rejection. We studied the IDO-expression by RT-PCR in dendritic cells and by real-time RT-PCR in monocytes of patients undergoing allogeneic transplantation for leukemia, who developed acute GVHD (aGVHD), and compared the IDO-expression to that of pregnant women and healthy volunteers. A spontaneous IDO-expression was detected in monocytes of 20 pregnant women with an IDO/GAPDH ratio as a median of 1.0% (range 0.2-303.8%), whereas none of 15 healthy volunteers or patients who underwent allogeneic transplant had any detectable spontaneous IDO-expression (p<0.01). The IDO-expression increased by IFN-gamma stimulation in pregnant women (median 116%, range 0.2-544%), healthy volunteers (median 11.7%, range 0.1-172%) and patients with low grade of aGVHD (grade 0-II) 28 days after transplant (median 433%, range 25.0-846.7%), but not in patients with severe aGVHD (grade III-IV) (median 0%, range 0-3.1%), which was highly significant (p<0.01). IDO-expression was also measured in dendritic cells by qualitative RT-PCR, where a spontaneous IDO-expression was detected in 16 of 31 (52%) pregnant women versus none of 17 healthy
volunteers (p<0.01) and none of 62 studied patients after transplant. IFN-gamma induced IDO-expression was detected in all pregnant women, all volunteers and 47 of 49 patients after transplant with low grade aGvHD (0-II), whereas only in 2 of 13 patients with aGvHD grade III-IV (16%) IFN-gamma induced IDO-expression was found (p=0.01). These data suggest that IDO-expression might be involved in the development of alloimmune immune tolerance.

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Antithymocyteglobulin (ATG) in addition to dose-reduced busulphan and fludarabine does not reduce the incidence of graft failure, acute and chronic GVHD in HLA-identical sibling transplantation - results of a retrospective analysis


Introduction: It is not known whether the addition of 4 x 10 mg/kg ATG to dose-reduced busulphan and fludarabine (BU/FLU + ATG) is required in patients with HLA-identical sibling donors. Therefore, we analysed retrospectively the incidence of graft failure, acute and chronic GVHD after dose-reduced BU/FLU +/- ATG.

Patients & Methods: From January 1998 to April 2001 83 patients with HLA-identical siblings received BU/FLU + ATG (n=38) or BU/FLU (n=45) as conditioning regimen in six German transplant centers. Diagnoses were AML (n=25), CML (n=12), sAML/MDS (n=21) and lymphoma (n=25). Patients with lymphoma, CML in first chronic phase or AML CR1 were considered to have a low risk of relapse. All patients received stem cells. GVHD-prophylaxis was CSA mono (n=32); CSA/MTX (n=20) or CSA/MMF (n=31). Median age was 52 years (range 25-67). The median age in the BU/FLU + ATG-group was lower than in the BU/FLU group (46 versus 56 years, p=0.02). Distribution of low and high risk patients was comparable (p=0.12).

Results: One primary and two secondary graft failures occurred in patients who received BU/FLU + ATG, compared to two secondary graft failures in patients who received BU/FLU (p=0.656). Acute GVHD II-IV* occurred in 49% versus 52% of patients who received BU/FLU + ATG and BU/FLU, respectively. In univariate analysis of age, risk of relapse, conditioning regimen, number of CD34+ cells, GVHD-prophylaxis, donor sex and sex match, only donor sex was related to acute GVHD II-IV* (35% versus 65% in patients with male vs. female donor, p=0.006). Multivariate analysis of age, conditioning regimen, GVHD prophylaxis, risk of relapse and donor sex validated male donor sex to be associated with a lower incidence of GVHD II-IV* (p=0.003). Extensive chronic GVHD occurred in 23% versus 14 % of patients who received BU/FLU + ATG and BU/FLU, respectively. Univariate and multivariate analysis of the same variables was performed, but no significant risk factor was identified. Conclusions: In a retrospective analysis of 83 patients with HLA-identical siblings the addition of ATG to dose-reduced BU/FLU did not reduce the incidence of graft failure, acute GVHD II-IV* or extensive chronic GVHD. Patients with male stem cell donors had a lower incidence of acute GVHD II-IV*.

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Association of interleukin-1 levels with severity of in vitro graft-versus-host reactions as measured by the use of a human skin explant assay

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Interleukin-1 has been implicated in the pathogenesis of Graft versus Host Disease following allogeneic bone marrow transplantation (BMT). In this study we analysed the levels (by ELISA) of the three IL-1 proteins (IL-1 alpha, beta and Ra) in mixed lymphocyte cultures (MLC) of 25 HLA matched donor/recipient pairs. Results were correlated with a human in vitro skin explant assay for predicting GVHD. No significant correlation was observed in IL-1 alpha levels in the MLC supernatants and degree of GVHR. The level of IL-1 beta in the supernatant associated with increased GVHR (grade I vs II GVHR p=0.0137; GVHR grade I vs III p=0.0414 (Mann Whitney U Test)). IL-1 beta levels in MLC supernatants correlating with GVHR grade II and III were comparable. The level of IL-1Ra in the supernatant showed no significant difference between GVHR grade I and II, or grades II and III. A trend was observed between grade I and III (p= 0.1193, Mann Whitney U Test). When the results for GVHR grade I and II were combined and analysed against grade III a trend (p=0.062) was observed. These results are currently being correlated with patient and donor IL-1 genotypes. Furthermore, preliminary studies have shown that the use of anti-IL-1Ra antibody during the skin explant assay gives rise to increased GVHR. This preliminary study suggests that both IL-1 beta and Ra may play a role in the pathogenesis of skin Graft versus Host Disease.

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Risk factors for extensive chronic graft-versus-host disease (cGVHD) after allogeneic stem cell transplantation (Allo-SCT): prospective evaluation of 165 patients


In order to identify risk factors for extensive chronic graft-versus-host disease (cGVHD), we prospectively evaluated 165 consecutive pts with hematologic malignancies, submitted to allo-SCT from November '94 to October '00; the following parameters were analysed by uni- and multivariate analysis: donor/recipient age and sex, type of donor, recipient/donor CMV serology, diagnosis, stem cell source, dose of nucleated cells infused, conditioning regimen, acute GVHD and CMV infection after transplant. Furthermore, in pts without clinical evidence of cGVHD at day +100, the results of the following screening tests were studied: eosinophil count, total serum bilirubine, serum alcaline phosphatase and IgG levels, presence of autoantibodies or circulating immune complexes, skin biopsy, Schirmer test and pulmonary function study. One-hundred seventeen pts, alive and in complete remission at day +100, were included in the analysis: 63 pts with males and 54 with females. median age was 35 years (range: 18-55); the diagnoses were as following: 46 ANLL, 16 ALL, 44 CGL, 8 MDS, 2 NHL, 1 multiple myeloma. The donor was a HLA identical sibling in 92 cases and a matched unrelated donor in 25; the conditioning regimen consisted of TBI-Cyclophosphamide (Cy) (49 cases), Busulphan (BU)-Cy (52 cases), Bu-Etoposide-Cy (10 cases), Fludarabine-Thiotepa (6 cases). GVHD prophylaxis was performed with Ciclosporine A (CSA), short course Methotrexate (MTX) and Methylprednisolone (MP) in 101 pts and with CSA and MP in 16 pts. The median follow-up was 650 days (range: 150-2359); 79/117 pts (68%) developed cGVHD classified as limited (28 pts) or extensive (51 cases); the median time of onset was 134 days (range: 52-608). In univariate analysis, female donor (P=0.0003), prior acute GVHD grade > II (P=0.002), peripheral blood stem cells (PBSC) (P=0.005), bilirubine >= 1.2 mg/dL (P=0.007) and a positive Schirmer test (P=0.003) were associated with a higher risk of extensive cGVHD. Multivariate analysis identified four independent risk factors: acute GVHD grade > II (P=0.0005), PBSC (P=0.008), female donor (P=0.02) and CMV infection (P=0.03). These parameters were used to define a scoring system, based on the number of risk factors: the actuarial probability of developing extensive cGVHD was 25% in pts with 0-1 risk factors compared to a score > 2 (P=0.000001). Our study shows that the combination of four parameters provides a model to predict extensive cGVHD.
Expression of Sialyl Lewis x (CD15s) antigen on peripheral blood lymphocytes does not correlate with the occurrence of graft versus host disease (GVHD) after allogeneic peripheral blood stem cell transplantation (alloPBSCT)

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Objectives: Recently, Ishida et al. (Transplantation 2000; 69:59-63) reported that the expression of CD15s on peripheral blood lymphocytes is strongly increased in patients at the time of rejection after renal transplantation. Its use as an easy, helpful and noninvasive parameter for diagnosis and treatment of rejection after renal transplantation was proposed.

Our intention was to investigate whether the expression of CD15s on peripheral blood lymphocytes is also increased in patients with acute or chronic GVHD (aGVHD or cGVHD) after alloPBSCT.

Patients: 75 samples from 17 patients (pts) after alloPBSCT were examined (mean post tx day 100, range 15-569); 37 samples between d+15 and d+99, and 38 samples between d+100 and d+569.

Methods: The expression of CD15s on peripheral blood lymphocytes was examined by flow cytometry, using indirect immunofluorescence. We used murine anti CD15s antibody 2H5 (Pharmingen) unconjugated and goat anti mouse IgM FITC (DAKO) in a whole blood assay with erythocyte lysis (FACS Lyse, BD). Cells incubated with mouse IgM isotype kappa (Pharmingen) served as negative controls. Within the lymphocyte gate the mean fluorescence intensity (MFI) was determined. Results are expressed as MFI ratio (MFI value of lymphocytes incubated with 2H5 : MFI value of lymphocytes incubated with isotype control).

Results: Of the 37 samples between d+15 and d+99 seven samples were from pts with grade 1/2 aGVHD, of the 38 samples from day +100 25 samples were taken in the presence of cGVHD (22 limited, 3 extensive). Spearman correlation coefficients were calculated for the MFI ratio and presence or absence of aGVHD and cGVHD respectively. No correlation was found between MFI ratio and aGVHD (-0.08643, p=0.6110) and cGVHD (-0.41696, p=0.0992).

Conclusion: In our hands, the expression of CD15s does not correlate with the occurrence of low grade aGVHD or cGVHD after alloPBSCT. The value of CD15s expression in high grade aGVHD (3/4) could not be examined in our patient sample.

Chronic graft-versus-host disease after allogeneic blood transplantation with fludarabine-based immunosuppressive conditioning regimen

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The use of mobilized blood stem cells in transplantation has been associated with an higher risk of chronic GVHD (cGVHD), although this remain controversial. The incidence of cGVHD was evaluated in 33 consecutive patients who received HLA-identical sibling blood stem cell transplantation after immunosuppressive, fludarabine-based conditioning regimen. Diagnoses were Lymphoma=23 (HD=16; NHL=7), CML=2, MDS=2, Multiple Myeloma=1, Solid Tumor=5. Minimum follow-up was 12 months. Thirty-two patients received a conditioning regimen fludarabine 30mg/m2/d x 3 days with cyclophosphamide 300mg/m2/d x 3 days and one patient received fludarabine 30mg/m2/d x 3 days followed by TBI 200 cGy. In all patient GVHD prophylaxis consisted CSA/MTX. Median follow-up from transplant was 739 days (range: 394-1591). Thirty-two patients reached 100% donor engraftment while the last one was in mixed donor chimerism after 878 days. Limited chronic GVHD was observed in 14 patients (42%), while extensive chronic GVHD in 3 patients (9%). On univariate analyses, CD34+ cell dose, donor lymphocyte infusion (both for chimerism and progression) and previous acute GVHD were not associated with an increased risk of chronic GVHD. Interestingly, 13/14 patients with limited cGVHD are alive and progression free at a median of 678 days (range: 395-1422). On the contrary 1/3 patients with extensive cGVHD and 7/16 patients that did not developed cGVHD are alive and progression free (X2, p = 0.022). No deaths were observed between patients with and without cGVHD. One interesting phenomenon of both progression and cGVHD; in the group without cGVHD, 4 patients died for progression and one patient for heart failure. Although long-term complications incidence of chronic cGVHD is similar to what reported for conventional myeloablative allografting, cGVHD did not affect outcome in these long-term patients, probably because of the higher rates of limited cGVHD compared to extensive. Interestingly, progression free survival was higher in patients with limited cGVHD, suggesting a possible association with disease control. Increasing number of patients will help to better evaluate these preliminary results.
Use of peripheral blood stem cells (PBSC) is likely to be associated with higher incidence of lung involvement in chronic graft vs. host disease (GVHD) following allogeneic hematopoietic stem cell transplant (AH SCT)

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665 patients (M: 396, F: 269; median age: 26 yr. range:0-55) who survived at least 100 days post allograft for haematological malignancies were analyzed to evaluate the incidence of lung involvement in chronic GVHD. Transplant were done for acute leukaemia (n=481), chronic leukaemia (n=109) or other diagnosis (n=75) and the disease was good risk in 282 and poor risk in 383 cases. Donor was HLA identical sibling in 580, matched unrelated in 63 and mismatched family member in 22 cases. Patients were conditioned with TBI (n=566) or chemotherapy only (n=99). GVHD prophylaxis was with CyA alone (n=330), CyA/methotrexate (n=307) and other measures (n=28). Source of stem cells was BM (n=604) or blood stem cells (n=61). The incidence of chronic GVHD was 304/665 (45.7%) and it developed at a median of 39 months post transplant. Of these, 30 patients (M: 21, F: 9; median age: 31 yr., range: 6-55) had evidence of lung involvement at a median of 7.7 mo (1.3-78). Probability of developing lung GVHD was 6% in the whole group and 9.5% in patients with chronic GVHD. In univariate analysis lung GVHD was more common with use of peripheral blood stem cells (9/61 vs. 21/624, p=0.001), diagnosis other than acute leukaemia (13/184 vs. 17/481, p=0.05), CyA/Mtx as GVHD prophylaxis (20/307 vs. 10/358, p=0.021), and trend with age >26 yr. (19/310 vs. 11/325, p=0.073) In multi-variate analysis only use of blood stem cells was independently associated with higher incidence of lung involvement (RR: 5.99, 95%CI: 2.7-13.3, p=0.0001). Patients without chronic GVHD had significantly better survival than those who developed grades II to IV AGVHD, and 9(21%) developed grades III to IV AGVHD. In univariate analyses, advanced stages, patient's HSV seropositivity and the days of MTX were significant risk factors for developing grades II to IV GVHD. Class I and/or Class II HLA allele mismatch(es) was not identified as a risk factor for developing AGVHD. In analyses using repeated measures ANOVA, the mean plasma concentration of FK506 during the first three weeks was significantly lower in those who developed grades II to IV AGVHD than that of patients who did not (17.2 vs. 15.4 or 14.5 ng/ml). Based on these findings, we conclude that the combination of FK506 and MTX was effective in preventing AGVHD after allogeneic stem cell transplantation from an HLA allele matched or mismatched unrelated donor. Serum level of FK506 should be closely monitored to maintain the level above 15 ng/ml during the first three weeks after transplantation.

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In vitro immunosuppressive activity of new anti-CD25 antibodies

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Humanized or chimeric monoclonal antibodies (mAb) directed against the interleukin-2 (IL-2) receptor alpha-chain, CD25, are promising new immunosuppressive agents due to better pharmacokinetic profiles and less toxicity. These mAbs have been efficiently used in prevention and/or treatment of rejection in solid organ transplantation and are currently under investigation for prevention/treatment of graft-versus-host disease (GVHD) in stem cell transplantation (SCT). The in vitro activities of the chimeric mAb basiliximab and the humanized mAb daclizumab were analyzed in different test systems for allogeneic response and T cell activation in comparison to cyclosporine A (CsA).

Anti-CD3-induced T cell proliferation was dose-dependently decreased by both anti-CD25 mAbS. Basiliximab at a concentration of 1 µg/ml and CsA at 0.1 µg/ml, concentrations which are achieved in vivo, significantly decreased proliferation from 100% (without drugs) to 34±22% and 7±2% (p<0.001, respectively). The reduction was less when the drugs were added during the culture period. In contrast, basiliximab at 1 µg/ml and CsA at 1 µg/ml inhibited phytohemagglutinin-induced T cell proliferation only to 69±15% and 35±6% (p<0.006 and p<0.001, respectively). The most impressive effects were seen in mixed lymphocyte cultures. A concentration of 1 µg/ml basiliximab reduced the alloantigen-induced proliferation to 17±6% and 0.1 µg/ml CsA to 18±3% (p<0.001, respectively). Even in the presence of exogenously added IL-2 (100 U/ml), a concentration which stimulates T cell proliferation, 1 µg/ml basiliximab inhibited alloantigen-induced T cell proliferation to 57±11% and 0.1 µg/ml CsA to 27±1% (p<0.0032 and p<0.0115 against inhibition without IL-2, respectively). Similar results were obtained with daclizumab. This in vitro study demonstrates a strong immunosuppressive activity of both chimeric and humanized mAbs against CD25 which is comparable to that of CsA. These data justify further evaluation of their activity in prevention and treatment of GVHD in human SCT.

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Pre-transplant serotherapy with campath 1H or ATG is effective in preventing GVHD in patients undergoing allogeneic PBSC transplantation from matched unrelated donors

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We have previously shown that a short course of pre-transplant serotherapy is highly effective in preventing acute and chronic GVHD in patients undergoing UD marrow transplants. We have now evaluated this strategy for patients receiving PBSC transplants from unrelated donors. Since May 1999 we have transplanted 21 patients using G-CSF mobilised PBSC from HLA-matched volunteer donors. Eight patients had CML (7 in CP and 1 in AP), 11 had acute leukaemia (6 in 2nd CR, 3 in 1st CR with poor risk features and 2 had refractory disease) and 2 had MDS. The median age was 38.2 years (range 18-51 yrs). All patients received standard conditioning comprising TBI (14.4 Gy in 6 fractions) and Cyclophosphamide (120 mg/kg) and serotherapy from days -5 to -1 inclusive using Thymoglobuline (Sangstat) 150 mg/day for the CML patients and Campath anti-CD52 antibody for the AL patients. The first 2 AL patients received Campath 1G antibody 10 mg/day and the remaining patients received Campath 1H antibody 10-20 mg/day. Post-transplant GVHD prophylaxis consisted of CSA alone to 6 months in 5 patients considered to be at particularly high risk of relapse and CSA combined with a short course of MTX (10 mg/m2 on days +1, +3 and +6) for the remaining 16 patients. The median dose of PBSC infused was 6.8 x 10^6 CD34+ cells/kg (range 2.0-13.58). All patients engrafted...
except one who received a higher dose of Campath 1H 20 mg/day following the switch from Campath 1G and subsequent patients received a reduced dose of 10 mg/day. The median time to ANC > 0.5 and platelets > 20 was 14 and 16 days respectively compared to a similar cohort of 60 patients transplanted at our centre with the same protocol using bone marrow. There have been 5 deaths from transplant related cases (1 rejection and 4 infections) and 4 patients have relapsed. The incidence of GVHD has been low with only 5 cases II grade I acute GVHD (3 of whom received no MTX) and 2 cases of Grade II GVHD (both of whom did receive MTX). Of the 10 patients evaluable at 6 months post-transplant, only 2 patients had a chronic GVHD. Overall incidence of acute GVHD for this group of patients is 59.6% and although larger numbers are required this data confirms that the use of pre-transplant serotherapy is effective in preventing GVHD in the setting of MUD PBSC transplants even when MTX is omitted.

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G-CSF given after HSCT with HLA-identical sibling donors increases the risk of acute GVHD II-IV

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The effect of granulocyte-colony stimulating factor (G-CSF), given after transplantation, was studied in 155 patients transplanted with hematopoietic stem cells (HSCT) from an HLA-identical sibling donor at Huddinge University Hospital between 1993 and 2001. There were 95 males and 60 females with a median age of 37 years, range 1-60. Diagnoses were AML in 58 cases, CML 38, ALL 32, lymphoma 11, MDS 6 and other malignancies in 10. 96 patients were in first CR/CP and 59 in later stages. Conditioning consisted of total-body irradiation in 118 and busulfan in 37. All patients were given a short course of methotrexate combined with cyclosporine as GVHD prophylaxis. Bone marrow was given to 108 patients and 47 stem cells from peripheral blood (PBSC). Sixty-six of the patients received G-CSF after HSCT, while 89 did not.

Patients receiving G-CSF had significantly shorter time to neutrophil engraftment (p<0.001) and needed less platelet transfusions, platelet engraftment and infections. However, we found that G-CSF treated patients had significantly higher incidence of acute GVHD grades II-IV, 34% vs 9% in not G-CSF treated patients (p<0.001). G-CSF was independent of other known risk factors for acute GVHD grades II-IV as shown in the multivariate analysis. The incidence of grade III-IV acute GVHD was 8% among G-CSF treated patients and 2% among non-treated (p<0.012). Death in GVHD occurred in 4 and 2 cases (p=0.06) in the two groups respectively. Cumulative incidence of transplant-related mortality (23% vs 25%), survival (68% vs 58%), relapse (25% vs 29%) and relapse-free survival (58% vs 55%) at three years was similar in both groups.

Conclusion. G-CSF given after HLA-identical sibling HSCT increases the risk of acute GVHD grades II-IV, but not the transplant-related mortality.

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Gene polymorphisms of molecules involved in immune reactions in recipients of MUD transplants: relationships with GVHD


Recent reports suggest that polymorphisms of genes encoding for cytokines potentially involved in the pathogenesis of GVHD may be linked to an increased risk of both acute (IL-10, TNF-a, IFN-gamma, IL-1RA) and chronic GVHD (IL-6, IL-1RA) GVHD after HLA-matched sibling hematopoietic stem cell transplantation (HSCT); this hold true both for patient’ and donor’ alleles. Determination of these gene polymorphisms may be useful for patient monitoring and treatment, but also for donor selection whenever more than one sibling donor is available; however, this might be even more indicate in case of HLA-matched unrelated donors (MUD), where the risk of severe GVHD is exceedingly increased and several potential volunteers are often identified. We report our experience in 70 pts subjected to an HLA-matched MUD transplant and their donors. The following polymorphisms were analysed: IL-1beta (nt 3,953 C>T), IL-1RA (VNTR intron 2), IFN-gamma (dinucleotide repeats within first intron), IL-10 (microsatellite nt –1,064), CD14 (nt 1344G>C), MTHFR (nt 677 C>T and nt 1,298 A>C), CTLA-4 (nt +49 A>G). We failed to observe significant associations of these gene polymorphisms with acute GVHD, although there was a trend for both MTHFR (recipient and donor) and donor IL-1RA (p= 0.06). On the other hand, we found that development of cGVHD was associated with specific recipient and donor gene polymorphisms. These were: for recipient, IL-1RA allele 2, p=0.039, and CTLA4 allele A, p=0.028; for donor, CD14 allele A, p=0.025. They were entered in a multivariate analysis for acute and chronic GVHD together with other known risk factors: advanced age and F donor to M recipient were risk factors for acute GVHD, with CMV serology borderline; for chronic GVHD, development of previous acute GVHD, advanced age were risk factors in association with the above mentioned gene polymorphisms. These data suggest that gene polymorphisms of molecules potentially involved at different points in the pathogenesis of chronic GVHD might be used as an additional selection factor when multiple HLA-matched unrelated donors are available in an attempt to decrease the incidence of chronic GVHD; on the other hand, HLA-linked features appear to be mostly responsible for the development of acute GVHD so that analysis of other gene polymorphisms is dispensable in this setting.

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Isolation of CD4/CD8 double positive T cells that are specific for a putative Mhag, not presented by HLA molecules

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Minor histocompatibility antigens (mHag) are classically described as self-peptides derived from cellular proteins that are presented by MHC molecules and recognized by MHC restricted T cells. We have isolated T cells from a skin biopsy in a patient suffering from GVHD after HLA-identical BMT. Two distinct populations were characterized. The former was CD8+ and specifically recognized recipient APCs but two weakly to assess MHC restriction. The second was a double positive CD4/CD8 population which very specifically recognized recipient APCs in proliferative assays, but without any need of MHC molecules, as assessed by blocking experiments with the use of mAb against class I and class II HLA molecules. Following stimulation with recipient APCs, the cytokine profile of these latter cells showed high levels of TGF beta and IL-10, intermediate levels of IL4, but no secretion of IL-2. Moreover, these cells expressed CD25, CD69, and CTLA-4, 7 days post-stimulation in the absence of IL-2. In conclusion, we show herein the first case of CD4/CD8 double positive T cells that are specific for a putative mHag. Interestingly enough, the Ag recognized by these cells is unlikely to be presented by MHC molecules, as classically described. Whether these cells are regulatory T cells is under investigation, at present.

This work was performed with the support of the Association for Research against Cancer, and the Etablissement français des greffes.
In this study we analysed the number of lymphocytes, monocytes and dendritic cells type-1 (DC1) and type-2 (DC2) in the graft and 1, 3, 6 and 12 mo after transplant in 32 patients (7 CML-CP, 8 Acute Leukemia in CR1, 1 MDS, 11 MM, 5 NHL) receiving unmanipulated allogeneic PBSC transplantation from G-CSF mobilized HLA-matched related donors, and addressed whether circulating DC may correlate with development of acute and chronic GVHD. All patients were evaluable for aGVHD and had a median follow-up >150d, while patients evaluable for cGVHD (n=21) had a median follow-up of 1 year. DC are identified by flow cytometry as negative for lineage markers (CD3, CD14, CD16, CD19, CD20, CD34, CD56) and positive for HLA-DR, whereas DC1 are CD11c+, while DC2 are CD123+. Three groups of patients were initially identified according to the degree of aGVHD: 10 patients grade 0, 13 patients grade I and 9 patients grade II-IV. These groups were comparable for: age of the patients, diagnoses, conditioning regimens, GVHD prophylaxis, number of DC1, DC2, CD34+ cells, T, NK and B lymphocytes and monocytes in the graft. One month after transplant DC2 median number in patients without aGVHD was 5.1x10^6/L (range=0.7-12.4), significantly higher than in patients with aGVHD grade I or II-IV: 1.4(range 0.1-13.3) and 0.9 (range 0.3-1.8) x10^6, respectively (p<0.05), while no significant differences were observed in lymphocytes and DC1 numbers among the three groups. Three months after transplant patients with aGVHD grade II-IV still had lower numbers of DC2 than patients without aGVHD: 1.2 (range 0.1-6.6) vs 3.5 (range 1.7-7.6) x10^6/L (p<0.05). Also, we analysed patients who developed no cGVHD (n=10) vs patients with limited/ extensive cGVHD (n=11). The two groups were comparable for patients characteristics, as well as for CD3+ cells, lymphocytes, monocytes and DC1, while patients with cGVHD showed a trend toward higher DC2 numbers in the graft (3.3x10^6/kg, range 1.6-6, vs 2.2x10^6/kg, range 2.77, 2.6-10.4, x10^6/L, respectively, p=0.05), whereas numbers of DC1, monocytes, B, NK and T cells were comparable in the two groups. Our results suggest that development of acute and chronic GVHD might be associated with selective loss of DC2 circulating in peripheral blood.

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**Quantitative assessment of immune transcripts in diagnosing GVHD**

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After allogeneic SCT, donor T cells are primarily responsible for the antithost activity, resulting in graft-versus-host disease (GVHD). Three effector pathways have been described for cytotoxic T lymphocytes: one requires perforin and granzyme B, the second Fas and its ligand (FasL) and the third pathway consists of secreted molecules (e.g., TNF-a, IFN-g). The aim of the present study was to utilize competitive reverse transcription (RT)-PCR as a sensitive approach to evaluate the pattern of immune activation gene expression after SCT. Gene expression of granzyme B, perforin, FasL and TNF-a in peripheral blood was analysed using a semiquantitative RT-PCR method. Eight patients who underwent allogeneic SCT for CML (n=3), AML (n=4) and SAA/MDS (n=1) were included in the study. Cell source was mobilized peripheral blood stem cells (n=5) or bone marrow (n=3) from HLA-identical siblings (n=3) or matched unrelated donors (n=5). Conditioning therapy consisted of Cy+Bu (n=5) or TBI+Cy (n=3). The patients were in general analysed on day 1, 14, 21, 24, 28, and every second week up to 3 months after transplantation.

Six of 8 patients are alive. Acute GVHD was diagnosed in 6/8 patients – grade I (n=2); grade II (n=2); grade III (n=1) and grade IV (n=1). Increased gene expression during acute GVHD was detected for granzyme B, perforin and FasL in all patients with GVHD whereas TNF-a showed a more diffuse correlation. Up-regulation of FasL was detected before GVHD was diagnosed whereas granzyme B and perforin was up regulated at the time of diagnosis. The median increase of gene expression was for granzyme B 6 times (range 2-200), for perforin 6 times (range 2-50) and for FasL 100 times (range 100-190). The results showed no correlation between the gene expression levels and grade of GVHD. GVHD treatment with steroids decreased the expression about 3 times for granzyme and perforin. The decrease of FasL gene expression was not correlated to GVHD therapy. In 5 cases the up-regulation of the gene expressions was not due to GVHD. The increase was caused by bacterial septicemia (n=2), hemolysis (n=1), pericarditis (n=1) and unknown reasons (n=1).

In conclusion, there is a dramatic up-regulation of immune activation genes at the onset of acute GVHD. Although not specific for acute GVHD, quantitative assessment of immune transcripts may be of value in diagnosing and monitoring GVHD treatment.

**P663**

**A comparative study of Glucksberg criteria and IBMTR severity index for acute graft versus host disease (aGVHD) grading in allografted patients**

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A-GvHD severity has been graded for the last 20 years by the standard %Glucksberg –Seattle% criteria. However, in practice the outcome of patients (pts) with same stage but distinct patterns of organ involvement had sometimes been different. Recently, International Bone Marrow Registry (IBMTR) proposed a new staging system (A to D) on the basis of organ involvement only. We compare the predictive value of the two grading systems for treatment-related mortality (TRM), relapse (R), treatment failure (TF, relapse or death), and TRM related to GvHD (G-TRM) in a series of 151 consecutive pts allografted for malignant or non-malignant diseases. One hundred twenty two pts received allografts from siblings and 19 from volunteer (VUD) donors, aged 29 (1-54) yrs. All received non-TBI, BU-based conditioning regimen and the standard combination of CSA and MTX as a- GVHD prophylaxis. aGvHD developed in 96 (64 %) pts and was staged as gr I: 40 (26%), gr II: 35 (23%), gr III: 8 (5%), gr IV: 13 (9%) and A: 19 (13%), B: 30 (20%), C: 34 (22 %) and D: 13(9%), according to GCS and IBMTR criteria, respectively. Three out of eight pts with gr III (GCS) were graded as B according to IBMTR Severity Index because of stage <= skin involvement and <= liver or/and gut involvement. Compared to pts without aGVHD (reference group), increasing relative risks (RRs) for TRM, G- TRM, TF were associated with more advanced stage in both systems (gr I – IV/ A to D); however, statistically significant RRs were found only for gr IV and D (p: 0.001, 0.001, 0.03, respectively for both systems). Moreover, a statistical significance for TRM (p: 0.04) and G-TRM (0.02) was observed for gr III pts according to GCS criteria. On the other hand, decreasing relapse rate was associated (although not significantly) with more advanced stage in both systems. In our series of sibling, mismatched related and VUD transplants, GCS and IBMTR aGvHD grading systems are equally predictive for relapse, treatment failure, TRM and a-GvHD related mortality. Glucksberg’s criteria appeared more predictive than IBMTR Severity Index for TRM and G-TRM for pts with GR and C index.
T cells recognizing endogenous peptides presented by HLA class I molecules on the surface of hematopoietic progenitor cells are believed to play an important role in the graft-versus-leukemia Effect after stem cell transplantation. Such alloantigens may be derived from leukemia-specific proteins or polymorphic self proteins which behave as minor histocompatibility antigens (mHags). To identify new mHags, which can be used to generate leukemia-specific T cell response we looked for genetic polymorphisms in the CD33 gene, as CD33 is expressed by human monocytes, promyelocytes, myeloid blasts, some acute undifferentiated leukemias, and occasionally by acute lymphoblastic leukemias. The alignment of cDNA sequences of CD33, obtained from the original reports, indicated four poly-morphic amino acids at positions I198M, L257V, S296R and N313K. In order to develop a simple PCR-SSP strategy by the use of genomic DNA we cloned and sequenced the non-coding regions (introns) of the whole gene. CD33 gene consists of 14,571 nucleotides. The predicted protein sequence of 364 amino acids has the typical features of a transmembrane protein. The gene could be divided into 7 exons and six non-coding parts. By sequence analysis we detected a new polymorphism at amino acid position G69R. To further examine whether the polymorphisms of CD33 could lead to the expression of peptides binding to HLA class I molecules we used the proteasomal cleavage prediction algorithm "PAPRoC" and HLA peptide binding analysis "SYFPEITHI". Nonapeptides from the polymorphic region G69R were predicted to bind with high affinity to HLA-A*01 and HLA-B*2705. Peptides derived from I198M alleles were predicted to bind to HLA-A*0201, HLA-A*2601 and HLA-B*2705. For peptides derived from polymorphic regions L257V, S296R and N313K high affinity to HLA-A*0301 molecules was determined. These binding predications are weaker than the binding of HLA-1 peptide to HLA-A*0201, but may be sufficient for the in vitro induction of peptide-specific T cells.

Identification of donor-recipient disparity for minor histocompatibility antigen HA-8 using fluorescence-labeled oligonucleotides

Minor histocompatibility antigens (mHag) are major target structures of graft-derived T lymphocytes and, therefore, can play a causative role in the development of graft-versus-host disease (GVHD) after allogeneic HLA-matched hematopoietic stem cell transplantation. Recently, the new human mHag HA-8 has been identified that is presented by HLA-A0201 and is derived from KIAA0020, a gene of unknown function. The immunogenic HA-8 peptide, RTLDKVLEV, is recognized by cytotoxic T lymphocytes and is generated by a single base substitution (G to C) that results in a proline to arginine exchange at amino acid position 1. Until now, there are only few data available about the frequency of this mHag among HLA-A0201-positive individuals. We therefore developed a RealTime-PCR assay for HA-8 subtyping on genomic DNA using site-specific fluorescence-labeled oligonucleotides and a LightCycler device. This approach allowed the rapid and reliable identification of patients with a homozygous or heterozygous HA-8 status in a single PCR run without the need for further post-PCR-processing and sequencing procedures. Parallel sequencing analysis showed an absolute concordance of results obtained with the LightCycler assay or standard sequencing techniques. Our data on normal HLA-A0201-positive individuals demonstrated an almost equal distribution of both HA-8 alleles in the caucasian population. Ongoing work on HLA-A0201-positive donors and recipients of allogeneic hematopoietic stem cell transplants will identify pairs with HA-8 disparity and will follow their clinical course after transplantation. Our approach should ultimately help to find out whether donor-recipient pairs with HA-8 disparity are prone to the development of GvHD or graft rejection.

Protosomal cleavage analysis of polymorphic proteins to predict presentation of minor histocompatibility peptides

Minor histocompatibility antigens (mHags), derived from polymorphic proteins, are thought to be targets for graft-versus-host disease and graft-versus-leukemia reactions after allogeneic stem cell transplantation. To achieve a correct size for an optimal binding to MHC class I molecules, proteins have to be fragmented by proteasomal processing. We used the proteasomal cleavage prediction algorithm recently developed by Nussbaum et al. to analyze the known aa sequences of the autosomal mHags HA-1, HA-8, HB-1 and of the Y-chromosomal mHags SMCY, DFFRY and UTY for a properly proteasomal processing. Strikingly, the immunodominant HA-1H nonamer was correctly predicted at both the N- and C-terminus. By replacing histidine by arginine at position 3, an epitope-destroying cleavage site is inserted into HA-1. For the HA-8R nonamer and the HB-1H decamer slightly prolonged peptides at the N-termini were predicted which could be further N-terminally processed by cytosolic or ER-trimming proteases, but the C-termini of these mHags were correctly predicted. The replacement of arginine by proline in HA-8 as well as of histidine by tyrosine in HB-1 led to epitope destroying cleavage sites. For the Y-chromosomal mHags derived from SMCY und UTY, the algorithm predicted correct C-terminal cleavage sites but the predicted peptide fragments were shorter than the HLA-restricted CTL epitopes described for HLA-A2, B7, B8 and B60. The proteasomal cleavage analysis provides evidence that a part of mHag peptides predicted to bind to HLA class I molecules will never be generated by proteasomal cleavage and thus are without any clinical relevance in transplantation.

Idarubicin induced impaired gut integrity and acute GVHD in allogeneic HSCT recipients

Introduction: Myeloablative regimens for allogeneic HSCT induce gut mucosal barrier injury (MBI) which may trigger the development of acute GVHD. We describe changes in gut permeability and absorption among recipients of an HLA-matched, MLC-negative T cell depleted sibling bone marrow transplant and their relation to acute GVHD. Study population: 16 subjects were given idarubicin 42 mg/M2 by continuous infusion for 48 hours on day –12 then cyclophosphamide 60 mg/kg i.v.on days -6 and -5 and total body irradiation with 4.5 Gy on days -2 and -1. GVHD prophylaxis consisted of i.v. cyclosporine (3 mg/kg/d continuous infusion day –1 to +14, 2 mg/kg/d thereafter) until the patient was able to take 6 mg/kg/d orally. Methods: Gut permeability was determined by the uptake of 5 g lactulose, 1 g L-rhamnose, 0.2 g D-methylglucosamine and 0.5 g D-xylose dissolved in a 100 mL isotonic solution which was drunk by the patient on day -12, -7, 0, +7, +14, +21 after an overnight fast and after having emptied their bladders. Urine was collected over 5 h, the total output was recorded and an aliquot was stored at - 80°C. The sugars were detected using high-performance liquid chromatography and the fluorescence label 9-fluorenylmethyl
chlooroformate hydrazine. Lactulose/thammose (L/R) ratios were used as an index of gut integrity. Acute GvHD was classified according to Glucksberg et al. and considered important when the overall grade was 2 or more. Toxicity prolonging admission or leading to readmission (TRC) and mortality within 3 months after HSCT unrelated to the underlying disease was considered to represent TRM. Antimicrobial prophylaxis and therapy were employed according to a standard protocol.

Results:

<table>
<thead>
<tr>
<th>L/R ratio</th>
<th>Baseline</th>
<th>Maximum</th>
<th>GvHD TRC</th>
<th>TRM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<tr>
<td>0.1 ± 0.1</td>
<td>6.2 ± 5.0*</td>
<td>4</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

*p<0.05 L/R maximum (= HSCT day =7) with respect to baseline (t-test). The mean L/R ratio was higher on days 0, +7 and +14 than on day -7 and +21 (p<0.05) with respect to baseline. TRC were one case each of thrombotic-microangiopathy-postHSCT, interstitial pneumonitis, CMV-encephalitis, renal failure requiring haemodialysis, veno-occlusive disease (VOD), coma, invasive aspergillosis. TRM was due to CMV-encephalitis, invasive aspergillosis, renal failure, VOD, coma.

Conclusion: Gut integrity is significantly perturbed in allogeneic HSCT recipients receiving idarubicin and may trigger acute GVHD, TRC and higher TRM.

P668
Randomized multicenter prospective clinical trial on the efficacy of mycophenolate mofetil in comparison to methotrexate for the prophylaxis of acute GvHD in stem cell transplant recipients

Mycophenolate mofetil is a widely used immunosuppressive drug in solid organ transplantation inhibiting T and B cell proliferation. Data on mycophenolate mofetil (MMF) for the prophylaxis as well as the treatment of acute GvHD after bone marrow or peripheral blood stem cell transplantation are still limited. We now report the results of an interim analysis of an ongoing randomized multicenter trial. Patients (n=30) were randomized to receive either MMF 2g/d (2 x 1 g/d; n=11) or 3 g/d (2 x 1.5 g/d; n=11) or a short course of methotrexate (n=8) in addition to cyclosporine for aGvHD prophylaxis. In addition to clinical monitoring plasma levels of MPA were analyzed by an HPLC method.

Thirty patients were eligible for analysis at this interim analysis. Patients suffered from ALL (13), AML (8), MDS (1) and CML (8). 5 patients had a matched related donor (1 MTX, 4 MMF), 5 a matched unrelated donor (5 MTX, 16 MMF) and 4 a mismatched related donor (2 MTX, 2 MMF). Conventional conditioning was TBI based in 19 patients (5 MTX, 14 MMF) and busulfan based in 11 patients (3 MTX, 8 MMF). Significantly (p=0.02) more patients with advanced disease were randomized for MMF. Most patients received peripheral blood stem cells (21, 5 MTX, 16 MMF). Engraftment as defined by neutrophils > 500/µl (14.4 vs 17.6 days) and platelets > 50.000/µl (18.3 vs 25.2 days) was significantly (p=0.043) faster in the MMF group without any dose related effect. 5 patients from the MTX group and 8 patients from the MMF group suffered from GVHD grade 0 – 1, whereas 3 patients from the MTX group and 14 patients from the MMF group evolved > grade 1 GVHD (p=0.15). Mucositis was significantly reduced in the MMF group with 5 patients developing grade 2 and 3 mucositis, whereas 7 patients of the MTX group developed grade 2 – 4 mucositis. 4 patients of the MTX and 13 patients of the MMF group (p=0.024) are still alive despite more advanced disease patients in the MMF group. From these preliminary data we conclude that MMF seems to be an effective tool in GvHD prophylaxis. The optimal MMF dose needs further pharmacokinetic data.
hematopoietic cells (BM vs. PB, p=0.01). Group A patients had significantly higher incidence of both aGVHD and chronic GVHD (70% vs 50% p=0.02 and 88% vs 71% p=0.01, respectively); however, both groups had similar overall treatment related mortality (TRM) and GVHD TRM rates (p=0.81 and deriving respectively). Relapse rate and 11-year overall survival (OS) and disease free survival (DFS) of the two groups did not differ significantly (p values: 0.81 and 0.92, respectively). On multivariate analysis, with the exception of haemopoietic recovery no significant association was detected between either of the two MTX regimens and response variables (TRM, relapse rate, DFS, OS). Table 1 illustrates the following variables: age, underlying disease, disease stage, donor/recipient sex disparities, cell dose and source of transplant, acute and chronic GVHD. In conclusion, compared to the standard four-dose regimen, a three-dose MTX/CyA anti-aGVHD prophylactic regimen favors faster engraftment, regardless of graft source, without compromising the outcome of matched related allo-HCT.

P671
Late-onset non-infectious pulmonary complications after allogeneic stem cell transplantation (SCT)
Despite the success in treating otherwise fatal diseases, allogeneic stem cell transplantation was associated with various complications. Among them, late-onset non-infectious pulmonary complications (NIPC) have critical influence on morbidity and mortality after SCT. We have prospectively evaluated a total of 174 consecutive patients with malignant and non-malignant diseases who received SCT at Keio University Hospital from January 1990 to April 2001 and survived at least 70 days after transplantation. Clinical, radiological, pulmonary function, and pathologic examinations were performed, and a total of 35 patients (20%) were diagnosed with NIPC between 54 and 779 days after transplantation. Based on the pulmonary processes, NIPC was classified into four subgroups: bronchiolitis obliterans (BO)(n=4), BO with organizing pneumonia (BOOP)(n=21), lymphocytic interstitial pneumonia (LIP)(n=8), and diffuse alveolar damage (DAD)(n=2). NIPC was diagnosed in 6 patients in the absence clinically active chronic GVHD, however, the rest of patients had extensive chronic GVHD at the time of diagnosis of NIPC. Risk factors for the development of NIPC except for extensive chronic GVHD could not be identified. The use of allogeneic peripheral blood stem cells or recipient Ôs age was not significantly associated with a higher incidence of NIPC. 81% of patients with BOOP and 50% of patients with LIP obtained a complete response with steroids and/or other immunosuppressive agents. A complete response was maintained in the majority of patients with BOOP and immunosuppressants were successfully tapered off. However, a flare-up was observed in 50% of patients with LIP. In patients with BO, disease activity was stabilized in two, and two died on disease progression. All patients with DAD died on disease progression or infections. We conclude that there is a strong association between chronic GVHD and late-onset NIPC, and the clinical subtype of NIPC has a significantly impact on their treatment outcome.

P672
Relation between CsA trough blood concentration and severity of a GVHD in pediatric allogeneic BMT
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Objectives: CsA efficacy in GVHD prophylaxis is inconstant, because of an important pharmacokinetic variability. As the optimal CsA blood levels remain unclear, the aim of this study was to establish a relation between CsA trough blood concentrations (TBC) and the grade of aGVHD. Methods: 67 children (3 months to 18 years old) received an allogeneic BMT (unrelated donors (n=34) with 22 mismatch donors and related donors (n=33)) for leukaemia (n=36), severe aplastic anaemia (n=12), hemoglobinopathies (n=4), inborn errors (n=11) and immunodeficiencies (n=4), between 1997 and 2000. GVHD prophylaxis consisted in CsA alone (n=16) or associated to MTX (n=48). ATG were given if the donor was unrelated. CsA was measured in whole blood by an EMI assay. CsA TBC in the first month post-BMT were estimated by a Bayesian method (USCPACK software) and correlated with aGVHD occurrence by logistic regression (SPSS software).
Results: The incidence of aGVHD was 41.8% (grade I : 23.9%, II : 9.6%, III : 0%, IV : 8.1%) and the grade of aGVHD was significantly associated with the risk of aGVHD (first week post-BMT: p=0.002, second week: p=0.002). When TBC were > 85 ng/ml, only 3 patients developed aGVHD (grade I) while 18 did not. When TBC were < 85 ng/ml, 23 patients developed aGVHD (grade I: 11, grades II-IV: 12) and 18 did not. CsA trough blood concentrations for the first 2 weeks of treatment for each grade of acute GVHD were: no GVHD : 111 +/- 10 ng/ml, mild GVHD : 75 +/- 11, moderate GVHD : 61 +/- 13, severe GVHD : 49 +/- 15. Moreover, our results showed a linear relation between TBC of CsA and grade of acute GVHD (p=0.009). Conclusion: CsA efficacy in GVHD prophylaxis appears to be strongly dependant of TBC in the 2 first weeks post-BMT: 85 ng/ml seemed sufficient to prevent aGVHD in children. This low concentration may probably leads to a lower incidence of infections and nephrotoxicity. In contrary to previous studies, CsA trough blood concentrations were not significantly associated with aGVHD. This could be partially explained by the use of Bayesian pharmacokinetic monitoring and the precision of the CsA assay used. To improve long-term survival in children transplanted for leukaemia, a pharmacokinetic monitoring of CsA to reach TBC established in this study could be more specific than a standard decrease of CsA doses. Our results suggested that TBC of CsA monitored between 85 ng/ml for the first weeks, whatever is the dosage regimen, could favour mild and moderate GVHD without increasing severe GVHD.

P673
A retrospective comparison of oral versus intravenous administration of Busulfan: Preliminary results show similar toxicity but less relapse
Busulfan-cyclophosphamide combination is one of the most widely used conditioning regimens in myeloablative allogeneic transplants. Busulfan can be administered in two different regimens: I/VBU or by oral route (POBU). Intravenous formulation (Busulfex, Orphan Medical, Inc, Minnetonka, MN) has been shown to be both efficient and safe in clinical Phase I/II trials. In this study we aimed to analyze effects of I.V. formulation in comparison to the historical case-matched group of patients with similar characteristics but who have received conditioning with POBU. POBU and IVBU groups received 16mg/kg p.o. BU and 12.8mg/kg i.v. BU (calculated per adjusted IBW), respectively. Both groups received the same dose of cyclophosphamide 120mg/kg i.v. conditioning regimen. Nineteen patients were assigned to each group by matching transplant characteristics like recipient age, diagnosis and stem cell source. The IVBU group had a median age of 33 (14-48) years, diagnosed as AML/CML/MDS/ALL=11/5/2/1, M/F ratio=14/5, and 50% of patients with LIP obtained a complete response with steroids and/or other immunosuppressive agents. A complete response was maintained in the majority of patients with BOOP and immunosuppressants were successfully tapered off. However, a flare-up was observed in 50% of patients with LIP. In patients with BO, disease activity was stabilized in two, and two died on disease progression. All patients with DAD died on disease progression or infections. We conclude that there is a strong association between chronic GVHD and late-onset NIPC, and the clinical subtype of NIPC has a significantly impact on their treatment outcome.

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S178
respectively. Log-rank analysis of the overall survival curves (Kaplan Meier) of IVBU and POBU patients revealed no significant difference for overall survival 18.2±1.3 vs 48.5±8.4 mo.s , respectively. Although relapse seem to be observed less frequently among the IVBU patients further follow up is necessary. IVBU is an easy to use prescription with similar and acceptable toxicity in regard to POBU but the cost-effectiveness and its impact on DFS and OS warrants longer follow-up and further prospective controlled studies.

P674

Cyclosporin interaction with grapefruit juice in BMT patients - an experience from Iran

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Diet is one of the major modulating factors in altering drug efficacy in humans. Recently, grapefruit juice has been found to enhance the bioavailability of several clinically important drugs such as nifedipine, cyclosporin, terfenadine, ethinylestradiol, midazolam and triazolam. Cyclosporin is an immunosuppressive agent that used in transplantation for prevention of chronic rejection. Grapefruit juice causes an increase in Cmax and AUC of drug mediated by suppression of Cyp 3 A4 in small intestine wall. To the best of our knowledge, this study is the first double-blind placebo controlled study, has been performed in twenty allogeneic bone marrow transplant recipients at Shariati Hospital in Tehran-Iran. Subjects were co administrated oral cyclosporin and grapefruit juice and water simultaneously and again two hours later. Blood samples were collected at 11.5 hours following drug administration. Cmax, Cmin and diastolic and systolic blood pressure were obtained. Cyclosporin concentration was analyzed by specific monoclonal radio immunoassay (RIA) technique. Grapefruit juice significantly increased (42%) cyclosporin Cmax. (3.5h), compared with water, (p = 0.001). However the blood level obtained at (11.5h) Cmin was only increased about 20% (p = 0.316). The average systolic and diastolic blood pressure, decreased significantly in grapefruit juice group compared with water group (p = 0.001 vs. p = 0.012). This study demonstrated that administration of grapefruit juice could increase cyclosporin Cmax in bone marrow transplant recipients. Because grapefruit juice is a safe and effective means to enhance absorption of many therapeutic agents, it is an appropriate means in terms of reducing dosing requirements of cyclosporin in BMT patients, therefore may reduce some of the side effects of cyclosporin.

P675

Secondary myelosuppression in recipients of allogeneic stem cell transplantation - incidence and risk factors

K. Jobst, A. Erdmann, J. Hahn, R. Andreesen, E. Holler (Regensburg, D)

Secondary myelosuppression occurring after uneventful engraftment is frequently observed in patients (pts) receiving allogeneic stem cell transplantation (SCT) and causes significant morbidity. In order to analyze the exact incidence and identify risk factors we evaluated 45 consecutive pts receiving either bone marrow (n=23) or peripheral blood stem cell (n=22) grafts from HLA-identical (n=30) or matched unrelated (n=15) donors. Secondary myelosuppression was defined as a decline of neutrophil counts < 1000/mm3 (ANC<1000) or a drop of platelet counts below 20000/mm3 (Plt<20) for at least 3 days after primary engraftment and in the presence of complete neutropenic risk factors such as stem cell source, CD34 cells in the graft, donor, CMV status of donor and recipient, documented viral infections, use of virostatic drugs, GvHD prophylaxis with MMF and occurrence of moderate to severe acute and chronic GvHD were addressed.

ANC<1000 were observed in 51% of pts at a median of 75 (range 29-182) days whereas Plt<20 occurred in 30% of pts at a median of 83 (range 33-175) days following allogeneic SCT. In univariate analysis, CMV seropositivity, use of virostatic drugs. GvHD grade III/IV as well as extensive chronic GvHD were associated with ANC<1000, while GvHD grade III/IV and GvHD prophylaxis without MMF were risk factors for Plt<20. In multivariate analysis, severe acute and chronic GvHD were the only factors predicting secondary myelosuppres-sion.

Our data indicate a high incidence of this major complication of allogeneic SCT. The strong association with GvHD suggests apart from expected risk factors such as viral infections and virostatic drugs direct involvement of myelosuppressive cytokines or cellular effects.

P676

Bronchiolitis obliterans (BO) or Bronchiolitis obliterans organizing pneumonia (Boop) after allogeneic hematopoietic stem cell transplantation (HSCT): clinical features and courses in 28 patients

M. Koldehoff, M.G. Kiehl, A.A. Fauser, N. Basara (Idar-Oberstein, D)

BO is defined clinically by an obstructive defect on pulmonary function testing (PFT) and histologically by obliteration of the small bronchioles. BOOP is a clinicopathologic syndrome distinct from BO characterized by Epler. The aim of our study was to determine the incidence, management and clinical evolution of BO or BOOP in a population of long-term HSCT survivors followed up in our center (IO). Between 7/94 and 1/01, 410 pts underwent an allogeneic HSCT in IO. Of the 187 pts who had survived for at least 120 days (d) after HSCT and were followed in IO. 28 pts developed BO or BOOP over this time. The most important clinical data are given in this Table: gender:23M,5F; diagnosis:12CML,6AML,5ALL,3MDS,2thoters; preparative regimen:161TBI/CY,10BU/CY,2others; match-grade:18HLA-ident,6HLA-DR,1HLA-A,1HLA-B,1HLA-A+B,10thers; HRST:17BM,11PBSCT,18TBI,5BM,11PBSC.

10 pts were smokers before HSCT, 3 pts continued smoking after HSCT. The average time between HSCT and diagnosis of BOOP was 458 d (range 125 - 1282 d). Serologic data for CMV matching were available in all donor recipient pairs (DRP). Relatively few (n=5) DRP were serological negative for the CMV. 23 pts had a risk of reactivating CMV. In 3 pts CMV infection developed before the onset of BOOP. An infection episode requiring treatment was preceding the onset of BOOP in 18 pts, of which 14 pts had respiratory tract infectious or sinistitis, 1 pt had gut infection and 3 had CMV infection. Grades II to IV acute (a)GvHD occurred in 14 pts (50%), 5 pts had grade I aGvHD and no signs of aGvHD were found in 9 pts Limited chronic (c)GvHD occurred in 10 pts while extensive cGvHD was detected in 10 pts. The most common predominant pattern in BOOP was air-space consolidation. PFT indicated severe restriction combined with moderate obstruction in most pts. Histopathological studies of the lungs were performed in 14 pts. The most common abnormalities were found in and around the bronchioles and were characteristic of constrictive bronchiolitis. All 3 BO and 25 BOOP pts responded initial to a treatment with prednisolon, anti-obstructive agents and anti-microbiological agents. The follow up of all pts showed a minimal efficiency in the pts with progressive BO (3/3) and moderate efficiency in the pts with recurrent BOOP (2/5). We concluded that BO or BOOP are
severe complication after HSCT with an incidence of approximate 15% of all HSCT in IO and more frequently in active cGvHD.

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A prospective randomized trial of marrow versus mobilized peripheral blood in HLA-matched related allogeneic transplants using a three-day short course methotrexate and cyclosporin as prophylaxis for acute graft-versus-host disease

Despite a 4- to 10-fold increase in T-cell numbers, pilot studies reporting on the use of allogeneic mobilized PB did not show higher incidence or severity of aGVHD. These unexpected results can probably be explained by the immunomodulating properties of M-PB. Based on these results, we prospectively tested the use of an abbreviated (3-day) short course MTX (day+1,+3,+6), combined with CSA, as aGVHD prophylaxis in a randomized study where patients received BM or PB. Our aim was to evaluate if deletion of one dose of MTX could: 1. accelerate time to ANC and PtT recovery. 2. minimize regimen related morbidity and toxicity, without increasing the incidence or severity of aGVHD, especially in the PB arm. Primary endpoints were: time to ANC and PtT recovery greater than 0.5 and 20x109/L respectively, incidence and severity of aGVHD, morbidity and mortality. We report on the first 59 patients (30 BM, 29 PB) with a minimum 100- day follow-up. Median age at the time of transplant in the BM and PB arms was 22 (range 15-45) and 23.5 (range 14-45) years respectively. All were HLA-matched related transplants for hematological malignancies. According to disease status prior to transplant, 37 (63%) were categorized as high risk (ALL and AML in CR2, CML in CP2, and MDS). Median follow-up is 371 days (range 104-925). In comparison to BM, median time to ANC and PtT recovery was significantly shorter in the PB arm (p less than 0.001 and less than 0.005 respectively). The cumulative incidence of aGVHD grade II-IV at day 100 was 28% in both arms. Despite a high incidence of grade III-IV aGVHD (75% BM, 71% PB), treatment related mortality remained low (12%) for the whole group. Three of 56 patients (5%) assessable for aGVHD died of the event (1 BM, 2 PB). Organ toxicity greater than grade II, according to Bearman's criteria, was observed in 22% of all patients. In summary, in comparison to previously published randomized trials using a 4d MTX regimen, deletion of one dose of MTX in this study did not result in an increased incidence of aGVHD in either arms, and was associated with a low incidence of treatment related morbidity and mortality. However, it did not contribute to enhance engraftment. In view of the young age of this cohort of patients, these results should be interpreted with caution. The apparent high incidence of severe aGVHD should be further addressed in a randomized trial using PB as the sole source of stem cells.

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Direct evidence of donor T-cell origin of massive resistant ascites in patient with acute GvHD (aGVHD) after nonmyeloablative allogeneic PBSC for CLL
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Stable engraftment of allogeneic stem cells can be achieved after non-myeloablative (NM) conditioning but GvHD remains a serious obstacle. It is not clear yet if features of GVHD after NM regimens are the same as after conventional grafts. We performed a NM allograft in a patient (52 F) with resistant CLL IV. The regimen was Fludara 180 mg/kg, BU 8 mg/kg, Thymoglobuline 2.5 mg/kg. She received G-CSF mobilized PBSCs from HLA-id brother (8.2x106CD34+/kg, 8.9x108CD3+/kg). Engraftment was fast but PB lymphocytosis increased rapidly with 40x109 lymph/L on D23. BM demonstrated 52% of CLL infiltration but CD3+ chimerism (FACS sorting+XYFISH) was 45% donor. Because of GvHD persistence CSA was stopped on D+63 to induce GVl effect. Seventeen days after (D70) the patient developed acute skin IV and gut III GVHD. On D80 she started complaining of abdominal distension and tenderness. Echoscan on D84 confirmed the appearance of 2 l ascites. Usual causes of effusion (VOD, infections, cardiac insuff, autoAb) were excluded. The appearance of ascites was associated with persistence of gut and skin, but not liver GVHD. Doppler revealed normal portal vein flow and CT scan showed normal hepatic parenchyma structure. During next 68 days (D80–D148) the patient was drained of 2.4 l of ascites on four occasions. The parallel specimens were made from BM, PB, ascitic liquid and liver. Ascites cellularity was presented with 83 +/- 14% lymphocytes. 83% of them were CD3+ and XYFISH confirmed their donor origin (95%XY). On the contrary, BM showed heavy infiltration with CLL B-cells (56%) and PB contained 11.7x109 WBC/L with 80% mononuclear CLL B-cells. Transjuglar liver biopsy on D134 revealed 90% of CD19+ infiltration. Therapy with CSA, steroids, anti-IL2-r Ab and MMF was applied. Only after 2 months of treatment the patient improved GVHD symptoms and simultaneously ascites disappeared. Actually she is doing well at D220 with minimal signs of gut GVHD and 95% of donor’s CD3+ in PB and BM. Unexplained effusions have been previously reported after BM. Sperm of sex mismatch graft in our patient we were able to confirm that ascites could be one of the AGVHD manifestations. There was significant discrepancy between heavy disease infiltration in BM, PB, liver and almost pure population of donor’s T-cells in ascitic fluid. It is not clear if ascites was a secondary peritoneal reaction of intestinal GVHD or developed due to direct interactions between activated donor’s T-cells and recipient’s mesothelium.

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Immunohistochemical study of acute graft versus host disease (aGVHD)
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The histologic alterations of gastrointestinal (GI) and skin aGVHD are of particular interest due to their frequent diversity and overlap with those secondary to the conditioning regimen. The aim of this study was the immunohistochemical investigation of the lymphocyte population and the target tissues in GI and skin aGVHD. Eleven colon and 5 skin biopsies from transplanted pediatric patients with a suspicion of aGVHD were reviewed. The Streptavidin-Biotin immunohistochemical method was performed on paraffin sections for the detection of B-(CD20/L-26), T-(CD1a,CD3,CD4,CD8) or NK-(CD56,CD57,Leu7,CD16) cell markers and HLA-DR (TAL-1B5). The presence of cytotoxic T cells was pursued by the use of TIA-1 and Granzyme B. The neuroendocrine differentiation marker Chromogranin A was also applied. In all cases of enteric GVHD a CD3+-predominant lymphocytic population with prevailing CD8+ and partial CD4+ involvement in early-phase aGVHD was found with only rare CD56+NK cells present. Granzyme B was identified in isolated intracytoplasmic lymphocytes. In all cases of histologically classic aGVHD, there was HLA-DR expression in cryptic epithelial cells, unlike their detection in stromal pericytotic fibroblasts and macrophages in biopsies of non-specific lesions. In skin biopsies of patients with aGVHD a CD3+-lymphocytic population with predominant CD8+ intraepithelial lymphocytes and variations in the CD4 involvement in the dermal infiltrate. In all cases a variable expression of HLADR in keratinocytes and variations in the CD4 involvement in the dermal infiltrate. In all cases of histologically classic aGVHD, there was HLA-DR expression in cryptic epithelial cells, unlike their detection in stromal pericytotic fibroblasts and macrophages in biopsies of non-specific lesions. In skin biopsies of patients with aGVHD a CD3+-lymphocytic population with predominant CD8+ intraepithelial lymphocytes and variations in the CD4 involvement in the dermal infiltrate. In all cases a variable expression of HLADR in keratinocytes and variations in the CD4 involvement in the dermal infiltrate.
biopsies it is associated with the immunohistochemical identification of Chromogranin A+ neuroendocrine cells in the residual crypts. In skin biopsies it is combined with decreasing Langerhans histiocytes with a reduced CD1a expression.

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The influence of graft monocytes on the outcome of allogeneic bone marrow transplantation


The influence of graft monocytes on graft-versus-host disease (GVHD) has not yet been established in clinical trials. To further understand this association, we evaluated the influence of bone marrow graft monocytes, primarily, to analyse the correlation with acute (a-GVHD) and chronic GVHD (c-GVHD), and secondarily with engraftment and survival. Eligibility criteria were: age <60 years; patients with primary malignant or non-malignant hematological disease receiving BM from an HLA-identical sibling; availability of enumeration of CD 34+ cells, T cell subsets, B cells and monocytes in the graft. We analyzed 83 patients. Conditioning was mainly BuCy2 and GVHD prophylaxis CSA-MTX. The median day to reach peripheral leukocytes ≥0.5x10⁹/l and platelet count ≥20x10⁹/l was 20 (11-34) and 18.5 (10-60) respectively. In univariate analysis, any parameter was correlated with a faster engraftment. The frequency of a-GVHD grades 2-4 was 12/83 (14.5%). In univariate analysis, total nucleated cells (TNC) ≥2.3x10⁸/Kg and CD14+ cells ≥4.78x10⁶/Kg were correlated significantly with lower rates of a-GVHD (p=0.04, p=0.02, respectively). Furthermore, patients >27 years old and donor gender mismatch had higher rates of a-GVHD (p=0.03 and p=0.04, respectively). In a multivariate analysis, both TNC and age maintain significance in a lower risk of a-GVHD. The probability was 3.2% when age <27 years and TNC infused ≥2.31 x 10⁸/Kg. A higher risk of a-GVHD was found (51.5%) when age >27 years and TNC infused ≤2.31 x 10⁸/Kg (P<0.001). The number of CD14+ cells showed a correlation with TNC (R=0.48 Spearman correlation). This interaction might be the cause for the loss of significance for monocytes in the multivariate analysis. Clinical c-GVHD of all grades developed in 31/77 (40%) available patients. It was extensive in 20 cases and limited in 11 cases. In univariate analyses there was a correlation between previous a-GVHD and a higher risk of c-GVHD (p=0.001). CD14+ cells did not influence c-GVHD. The estimate of 6-year overall survival (OS) was 66% (95% CI: 55%-79%). In univariate analyses, the absence of a-GVHD was correlated with a higher survival (p<0.001). There was a trend for a better survival in patients receiving more CD34+ cells (p=0.06). The CD14 cells had no impact on overall survival. These preliminary data suggest that monocytes may have a protective effect in allogeneic BMT.

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Autologous transplants with low intensity conditioning to treat GVHD; an attempt to reverse donor type chimerism

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Few treatment options are available in patients with graft-vs-host disease (GVHD) refractory to steroids and lymphocyte cytotoxic agents. Reverting donor type chimerism by autologous re-transplant might be a consideration. Recent development of low-intensity transplantation may open such an approach to patients with GVHD, hitherto considered unsuitable transplant candidates because of poor performance status.

Case 1: A 27-year old patient with a Ph+ CML in accelerated phase, refractory to STI, received an allogeneic stem cell transplant (SCT) from a 1-antigen mismatched unrelated donor after conditioning with VP-16, Cy and TBI. She engrafted well with 0% allogeneic donor chimerism in blood and marrow and continued remission of ALL. Reversal of positive t(9;22), marrow involvement and hemolytic uremic syndrome (HUS). On day +48 he underwent conditioning with fludarabine and 2 Gy TBI and received an autologous re-transplant, previously harvested in chronic phase. Bone marrow aplasia ensued, 10% of remaining cells were of recipient origin. GVHD improved temporarily after conditioning but progressed thereafter and the patient died of progressive GVHD 25 days after the attempt at transplant reversal.

Case 2: a 23-year-old patient with a Ph+ positive ALL received chemotherapy followed by a syngeneic SCT after conditioning with VP-16, Cy and TBI in CR1. At relapse, she was treated with further chemotherapy and an allogeneic SCT from an HLA-identical sibling to induce graft-vs-leukemia. She developed cutaneous GVHD on day 60 progressing to involve intestine, lung with severe bronchiolitis obliterans, and HUS. She became unable to walk due to a progressive chronic inflammatory demyelinating polyneuropathy. All these manifestations were considered to be GVHD. She then underwent a second syngeneic SCT after conditioning with fludarabine and 2 Gy TBI 9 months after the allogeneic SCT. She engrafted well with 0% allogeneic donor chimerism in blood and marrow and continued remission of ALL. After temporary improvement of GVHD, possibly due to conditioning, bronchiolitis obliterans progressed further. In a bronchial lavage sample, 15% allogeneic donor cells were detected at a time when marrow and blood had completely reverted to patient chimerism. The patient died of progressive GVHD 60 days after the attempt at transplant reversal.
In conclusion, treatment of steroid refractory aGVHD with Inolimomab shows high initial response rate, but might be accompanied by an increased rate of invasive fungal disease. This association needs further investigation and intensification of antifungal prophylaxis might be considered when Inolimomab is applied.

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Anti TNF-alpha antibody in the treatment of severe acute graft versus host disease

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Acute graft-versus-host disease (aGVHD) is a serious complication of allogeneic peripheral blood stem cell transplantation (PBSCT). Patients with severe aGVHD not responding to the treatment of steroids have a poor prognosis (after treatment with anti-thymocyte globuline about 90% lethality). Here we report 6 cases (4 female, 2 male) of severe steroid refractory aGVHD (grade III-IV) following non-myeloablative (n=5) and myeloablative (n=1) PBSCT for AML (n=2; 1 relapsed), CML (n=2), ALL (n=1) and Hodgkin’s lymphoma (n=1). Three patients had intestinal aGVHD (2 grade III; 1 grade IV), two suffered from hepatic aGVHD (grade IV), one patient had intestinal and hepatic (grade IV) aGVHD. aGVHD was in all patients refractory to a course of immunosuppressive therapy with Cyclosporine A (Sandimmune®), mycophenolate mofetil (CellCept®) and steroids. All patients were treated with 10 mg/kg body weight infliximab, a chimeric anti TNF-alpha antibody, once a week for a time period of four weeks. Infusion of infliximab within 60 minutes was tolerated well and no allergic reactions were observed. Four patients received 4 cycles, 2 only 2 cycles of infliximab. Patients treated with 4 cycles clinical improvement of aGVHD could be observed after the second or third cycle. One of the 4 patients did not respond to infliximab at all and died of a severe intracerebral bleeding. Two patients died of severe infectious complications (enterococcus sepsis, viral pneumonia) at 13 days / 51 days after beginning of infliximab therapy. One patient is still alive and in good clinical health without developing severe infection (160 days after infliximab therapy). Two patients who received only 2 cycles of infliximab died, one (female, 43 years, ALL) of an intracerebral bleeding, one (male, 19 years, AML) of multiorganic dysfunction syndrome (MODS). At time of death there was no sign of improvement of aGVHD. In conclusion the use of infliximab may lead to clinical improvement of steroid refractory severe aGVHD, but high rates of lethal infectious complications must be considered. Although there are no guidelines for dosage of infliximab and the number of cycles to be administered. Anti TNF-a antibody should only be used carefully in selected patient groups.

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Comparison of two doses of thymoglobulin (rabbit ATG) as rescue therapy of steroid resistant acute GVHD

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The results of treating steroid resistant acute GVHD are usually poor. The French society for HCT (SFHM) carried out a multicenter prospective randomised trial comparing two doses of Thymoglobuline (rabbit ATG, SangStat, Lyon) infused for 4 consecutive days to see if optimising the dose to achieve a balance between control of the donor alloreactivity and the risk of over-immunosuppression would improve outcome: 5 mg/kg in the high dose arm “A”; 1.25 mg/kg in the low dose arm “B”. Preliminary analysis on 11 and 9 patients in arms A and B respectively showed: the initial aGVHD occurred after a median time of 25 days in arm A (15-77) and 20 days in arm B (11-97). Following non-response to steroid treatment, all but one aGVHD in each arm were grade III or IV. The median time between the start

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Inolimomab treatment of steroid refractory acute GVHD - Association with invasive aspergillosis?

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Background: Acute graft versus host disease (aGVHD) remains a significant complication after allogeneic stem cell transplantation. Patients with > grade II, steroid refractory aGVHD have a poor prognosis with survival rates less than 20%. Preliminary studies suggest a role of interleukin -2-receptor (CD25)antibody in the treatment of these patients. We report on five patients who received Inolimomab for treatment of steroid refractory aGVHD.

Patients and Methods: All patients had advanced hematologic malignancies (NHL (n=1), MM (2), CLL (1), MDS (1)) and had developed relapse after high-dose chemotherapy. Conditioning regimens were non-myeloablative in four patients and myeloablative in one followed by transplantation with peripheral blood stem cells from matched unrelated (n=4) or related (n=1) donors. All patients showed sustained engraftment after a median of 16 days (range 11-22). Despite standard prophylaxis in all patients grade III-IV aGVHD with predominant intestinal involvement was diagnosed at a median of 21 days after transplant. At day 14 post transplant GvHD was refractory to steroids and anti-thymocyte globulin (ATG).

Inolimomab infusions, the other four patients were only treated for 5 mg/kg/d for five days. (One patient received three series of Inolimomab infusions, the other four patients were only treated once).

Results: In 3/5 patients complete resolution of diarrhea and significant improvement of skin and liver disease could be achieved and no relapse occurred. In one patient only a partial response could be achieved and treatment was repeated, whereas in another patient aGVHD did not respond and the patient died within a few days. Although all patients received anti-fungal prophylaxis with fluconazole followed by empiric therapy with amphoterin C, three patients developed invasive aspergillosis and died thereof. All three patients had invasive pulmonary and/or cerebral aspergillosis diagnosed by positive bronchocele lavage, CT-scan or on autopsy.

In conclusion, treatment of steroid refractory aGVHD with Inolimomab shows high initial response rate, but might be accompanied by an increased rate of invasive fungal disease. This association needs further investigation and intensification of antifungal prophylaxis might be considered when Inolimomab is applied.
Role of micophenolate mofetil (MMF) in the treatment of cGVHD

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Chronic graft versus host disease (cGVHD) still represents a major complication after allogeneic hematopoietic stem cell transplantation. In this study 26 pts with extensive cGVHD following an allogeneic PBSC (n = 21) or bone marrow (n = 5) transplant from an HLA-matched donor were treated with Micophenolate Mofetil (MMF) at the dose of 2 g/die, and patients who were treated for at least 1 month were evaluated for their clinical response. So far, 21 pts (8 CML, 7 AML, 6 MM) were evaluable. Median age was 42 yrs (range: 19-56) and 11 pts were males and 10 females. All the pts were prepared with standard conditioning regimens, 6 including unfractiioned TBI, and 5 pts received also rabbit ATG treatment (15 mg/kg) before transplant. GVHD prophylaxis was CsA and short MTX. The median duration of MMF treatment was 5 months (range: 2-15) and median follow up is 5 months (range: 3-28). In 12/21 pts (57%) a partial response was observed. Particularly, in responder pts an improvement of cGVHD appearance was more evident than low dose daily 1.25 mg/kg tested in this trial. However, if treatment is started promptly after failure of steroids (as was initially planned in this study), it may be that low doses would be as effective as high doses, with fewer risks. A study with a larger cohort and longer follow-up is warranted to see if a better response rate translates into an improved survival rate.

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Inolimomab (Leukotac) in the treatment of acute graft versus host disease following allogeneic hematopoietic stem cell transplantation

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From July 1998 to November 2000, 25 patients were evaluated retrospectively for the safety and the efficacy of inolimomab given for the treatment of acute GvHD following allogeneic stem cell transplantation.

Initial disease was congenital immune deficiency (nine patients), acute or chronic leukemia (thirteen patients), non-Hodgkin lymphoma (one patient), ovarian cancer (one patient), and myelodysplasia (one patient).

Acute GvHD was diagnosed within an average of 39 days after bone marrow transplantation (grade I for two patients, grade II for ten patients, grade III for six patients and grade IV for seven patients). For most of the patients the initial treatment of the acute GvHD consisted of corticosteroids from 1 mg/kg to 7 mg/kg. Inolimomab was administered in the event of uncontrolled steroid-resistant GvHD. Patients received this treatment within an average of 24 days after the onset of acute GvHD at a dose of 0.3 mg/kg/day during a period of 28 days. No adverse event has been notified to date.

The results were as follows: seven complete responses, thirteen partial responses (two quoted as very good partial responses), and five non responses. The overall response rate was 80%. Only one relapse of acute GvHD was notified and was successfully treated with an additional course of inolimomab. We also noted that five of the seven complete responses were obtained with doses of inolimomab >/= 0.4 mg/kg/day.

Four months after transplantation, sixteen patients were alive, four had died of infectious complications, three of uncontrolled GvHD, one of relapse of the disease and one of multiple organ failure. Nine patients survived for more than twelve months.

This analysis shows that prolonged treatment with inolimomab is well tolerated. According to the results, inolimomab seems to be effective in steroid resistant GvHD both in pediatric and adult populations. The best results are obtained with high doses and early institution of inolimomab treatment after the diagnosis of corticosteroid resistance. These results deserve to be confirmed by a prospective randomized controlled study which is already scheduled for early 2002.

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Good effect of quinine on dystonias in chronic graft-versus-host disease

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Background: Clinical features of cGVHD may comprise neuromuscular manifestations such as dystonias, eg carpal spasms, muscular cramps. Pts symptoms are transient, but sometimes painful and incapacitating. There is no accepted treatment for this overlooked condition.

Objective: Based on the report of one pt (#1 below) a preliminary dose-finding study of the prophylactic effect of quinine, or a quinine-containing beverage, on cGVHD-related dystonias was undertaken. Pts started with increasing volumes of Tonic Water® (TW; quinine content 68 mg/L) that if necessary were replaced with quinine tablets. Pts were observed for the safety and the efficacy of quinine.

Pts and results. Pts #1: 57 yrs, male, CML, 5 yrs post-Tx (MUD) with steroid-dependant (10 mg/d) mucosal cGVHD and, most prominently, dystonias.

Pts reported that his dystonias completely vanished after intake of TW and they reappeared if he stopped taking the beverage. After a series of dose changes, including a period with quinine tablets, the lowest effective dose was found to be TW 250 mL (17 mg bid).

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Treatment of steroid resistant acute GvHD with daclizumab and etanercept  
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Steroid resistant acute GvHD (aGvHD) following allogeneic hematopoietic stem cell transplantation (HSCT) is associated with high morbidity and mortality. In the past, treatment consisted mainly of anti T-cell antibody therapies. Here we report the treatment results of steroid resistant aGvHD with the humanized IL-2 receptor antibody Daclizumab in combination with the TNF-receptor fusion protein Etanercept.  
Patients: Seven patients (age 43-57 years) with steroid resistant aGvHD (grade III n=3, grade IV n=4) after myeloablative HSCT were treated with Daclizumab and Etanercept. Steroid resistance was defined as progression or no response of aGvHD after at least 7 days of Methylprednisolone (MP) 2mg/kg/d. All patients had underlying hematological malignancies and the donor types were HLA-matched related (n=2), HLA-matched unrelated (n=4), and 1 HLA-mismatched (n=1). The median time of onset of aGvHD was 27 (range 14-95) days post HSCT. The median time interval from time of aGvHD diagnosis to the initiation of antibody treatment was 17 (range 12-34) days.  
Methods: Treatment consisted of Daclizumab 1mg/kg given IV on days 1, 4, 8, 15, 22 and Etanercept 16mg/m2 given SC on days 1, 8, 15, 22. All patients had underlying hematological malignancies and the donor types were HLA-matched related (n=2), HLA-matched unrelated (n=4), and 1 HLA-mismatched (n=1). The median time of onset of aGvHD was 27 (range 14-95) days post HSCT. The median time interval from time of aGvHD diagnosis to the initiation of antibody treatment was 17 (range 12-34) days.  
Results: Treatment resulted in 4 complete remissions and 2 partial remissions. One patient died 5 days after initiation of treatment and was not evaluated for response. The two patients with partial remission died due to infectious complications (bacterial sepsis and aspergillosis) on days 75 and 146 after completion of antibody treatment. All patients with complete remissions of aGvHD are currently alive with a median follow up of 219 (range 86-253) days. Three patients remained in complete remission concerning their aGvHD and one experienced relapse of aGvHD after complete withdrawal of immunosuppression. Toxicities of treatment were minimal beside the described infectious complications and CMV reactivation in five patients successfully treated with virostatic treatment. One patient developed hemorrhagic cystitis of unknown origin on day 48 of his treatment.  
Discussion: The data demonstrate feasibility of combined treatment with Daclizumab and Etanercept for steroid resistant aGvHD. No treatment failures have been observed and the mortality rate of 43% is in line with published results. For further evaluation a phase II study of the combination of Daclizumab and Etanercept was initiated.

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Combination therapy using daclizumab (Anti-CD 25) and sirolimus in steroid refractory acute GvHD  
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Steroid refractory acute GvHD is a major cause of morbidity and mortality after allogeneic bone marrow or stem cell transplantation. New strategies have been developed using a variety of cytokine and receptor blocking antibodies. This evaluation presents our experience with a combination of Sirolimus and Daclizumab (anti CD25) in heavily pretreated patients with acute GvHD grade III and IV. Daclizumab is a novel blocking antibody directed against the high affinity receptor of the Interleukin-2 receptor (CD25) while Sirolimus inhibits the intracellular signalling pathway of IL-2 resulting in a synergistic inhibition of T-cell activation and proliferation. We treated twelve patients with steroid refractory acute GvHD of the intestine grade III and IV. GvHD had been diagnosed clinically and histologically via total colonoscopy. In addition six patients had concurrent GvHD of the liver grade III-IV. All but one patient had already received and not responded to OKT-3. Daclizumab was administered on days 1, 4, 8, 15, 22 and 28 at 1mg/kg. Sirolimus was given orally, at serum levels of 10-15 ng/ml. The average age of the patients was 34 years (range 14-50). Ten patients had received PBSC, one BM. The underlying diseases were Myeloma (2), NHL (1), CLL (1), ALL (4) and AML (3). Overall response to Sirolimus/Daclizumab was 54 % (6/11) with 5 complete remissions and 1 partial remission. Four patients are alive more than 120 days after Sirolimus/Daclizumab therapy. The two other responders died of relapse and aspergillosis. A major response in severe liver GvHD was observed in only one patient. The 5 patients that did not respond died of progressive GvHD or infectious complications with a median of 37 days after start of Daclizumab/Sirolimus therapy. Severe adverse events related to the infusion of the antibody were not seen. Viral reactivations were diagnosed in 5 cases (1 CMV, 2 HHV6 and 2 EBV). We conclude that Daclizumab and Sirolimus is a promising combination therapy especially for isolated steroid refractory intestinal GvHD. The overall response rate of 54% in these heavily pretreated patients may justify an earlier treatment with Daclizumab plus Sirolimus. Concomitant steroid refractory severe GvHD of liver showed poor response and remains difficult to treat. Prospective trials are necessary to clarify the role of Daclizumab plus Sirolimus steroid refractory acute GvHD.

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Treatment of acute graft versus host disease with methylprednisolone with or without antithymocyte globulin (thymoglobulin): a preliminary analysis of a randomized GITMO study  
M. van Lint on behalf of the GITMO  
In a previous study we have shown that patients not responding to 5 days of 6-methylprednisolone (6Mpred) given as treatment of acute graft versus host disease (GvHD) have a transplant related mortality of 46%. GITMO has opened a prospective trial to test the efficacy of antithymocyte globulin (thymoglobulin Sangstat) in association with 6-methylprednisolone (6Mpred) 5 mg/kg in the treatment of acute graft versus host disease (GvHD). Primary end point of the study is transplant related mortality in patients not responding to 5 days of 6Mpred 2 mg/kg/day. Patients are registered at diagnosis of GvHD grade and received 6Mpred 2 mg/kg/day for 5 days. On day 5 patients the dose of 6Mpred is supposed to be reduced by 25%: patients who are considered eligible for dose reduction are also considered stable or responding to 6Mpred and are only followed up. Patients who are not considered eligible for dose reduction are randomized to received 6Mpred 5mg/kg/day for 10 days with or without Thymoglobulin 1.25 mg/kg/day on alternate days x5 (total dose 6.25 mg/kg). One hundred and seventeen patients entered the study as of 30.sept.2001. 87 reduced the dose on day +5 and 30.

Pt #2: Female 42 yrs, CML, 5 yrs post-Tx (MUD) without treatment for a mild non-progressive sclerodermatous cGvHD. Severe dystonias in small and large muscle groups. No effect of TW up to 500 mL/d. Prompt and almost total disappearance of symptoms on quinine 100 mg bid.  
Pt #3: 40 yrs female, AML, 3 yrs post-Tx (MUD) with extensive sclerodermatous cGvHD, on treatment with steroids, CyA. No effect of TW but marked improvement after quinine 100 mg bid.  
Pt #4: 34 yrs male, MPS, 3 yrs post-Tx (RD), extensive sclerodermatous cGvHD, treated with CyA, steroids. Widelypread dystonias interfering with work and car-driving. Symptoms disappeared on quinine 200 mg/d with the exception of occasional facial dystonias.  
Pt #5: 56 yrs female, CML, 6 yrs post –Tx (MUD) with no GvHD-symptoms, apart from dystonias. Symptom-free on TW 140 mL, (10 mg), bid.  
Summary. In five pts with dystonia, as part of their cGvHD, quinine – in some cases in very low doses - exerted a dramatic effect on the neuro-muscular symptoms. These findings, to our knowledge not previously described, should be confirmed in a larger study.

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were randomized. Transplant mortality was 14% in day+5 responders and 25% in patients randomized to receive or not ATG. The study is still enrolling patients. With a baseline 2 year TRM rate of 46% and with a 20% expected reduction of TRM we need to randomized 74 patients each arm (power 80%, alf error 0.5).

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Treatment of refractory chronic graft versus host disease (cGVHD) with MMF: preliminary results

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Refractory cGVHD is the main cause of morbidity and mortality after allogeneic cell transplantation. MMF blocks T and B cell proliferation through inhibition of de novo synthesis of guanosine nucleotides. MMF has been successfully used in organ transplantation and in the management of aGVHD and was recently introduced in the treatment of cGVHD. We examined the efficacy and safety of MMF in 25 patients with steroid-refractory extensive cGVHD. Median age was 29 (13-48) years. Nineteen patients had undergone identical sibling and 4 VUD transplant; there were 12 donorrecipient sex mismatches. All but one patient were conditioned with BUCY2±ATG, the remainder FluCy4+ATG. aGVHD prophylaxis was CSA+MTX in 24 and CSA in 1 patient. Clinically significant preceding aGVHD developed in 13/25, while high-risk cGVHD in 8/25 patients. Anti-cGVHD therapy prior to MMF consisted of steroids in varying combinations with CSA, Thalidomide, Imuran and PUVA for a median duration of 6 (3-22) months. MMF (10-30 mg/kg) was administered combined with either PDN/CSA or PDN-CSA in 14/25 patients, PDN-Thalidomide in 6/25 and in other combination in 5/25 patients, for a median duration of 4 (1-21) months. Twenty patients (80%) attained clinical response. Complete regression of clinicolaboratory findings occurred in 16/25 (64%); no cGVHD reactivation), partial in 4/25 (16%) and failure in 5/15 patients (20%). MMF had no significant effect on B and CD4 cell counts. Grade 3 WHO hematologic toxicity was seen in 3/25 patients, necessitating MMF discontinuation in 2/3; 10 patients required anti-CMV pre-emptive treatment after 2 consecutive PCR positive results; there were 4 systemic fungal infections. During MMF treatment, 4 relapses of the underlying disease were observed. At the end of study, 17/25 patients are alive, 16 with cGVHD remission (complete in 14, partial in 2 patients). Death was due to refractory cGVHD in 4/8 and disease relapse in 3/8 cases; one patient off anti-cGVHD treatment died of myocardial infarction. In conclusion, MMF has an acceptable efficacy/safety profile; thus it can be used as an alternate approach for the treatment of refractory cGVHD.

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Extracorporeal photopheresis (ECP) in graft-versus-host disease (GVHD) treatment: single centre experience in chronic and acute GVHD


Extracorporeal photopheresis (ECP) has been shown to be effective in the treatment of GVHD. The aim of this study was to assess the efficacy of ECP in chronic graft-versus-host disease (cGVHD) and his role in aGVHD.

Patients: 2 grade III liver aGVHD pts and 22 affected by extensive cGVHD, (skin 13/22 pts, bowel 5/22 pts, oral sicca 6/22 pts, liver 7/22 pts, lung 2/22 pts, ocular sicca 5/22 pts, myositis 1/22 pts, urogenital 1/22 pts, thrombocytopenia 2/22 pts); 12 de novo cGVHD and 10 chronic evolution of cGVHD were studied. The two aGVHD pts were steroids resistant, all cGVHD pts had to respond to conventional immunosuppressive protocols: ciclosporine 22/22, steroids 22/22, azathioprine 10/22, thalidomide 5/22, tacrolimus 2/22, micofenolate mofetil 5/22, Cyclophosphamide 2/22. All had received HLA-matched stem cell transplants for haematological malignancies, from sibling (IS) (n = 18) or unrelated (UD) (n = 6) donors, 12 bone marrow transplants and 12 peripheral blood stem cell transplants. Treatment procedure was performed as elsewhere described. GVHD prophylaxis was done in all patients with Ciclosporine and Metotrexate, plus ATG in UD transplants. ECP was performed using UVAR apparatus (Therakos) repeated on two consecutive days at two week intervals for the first three months and then every four weeks until GVHD resolution.

Refractory cGVHD: one patient was dead after the first ECP cycle, another patient relapsed after two cycles and all 2 were unevaluable. Of the 20 evaluable pts, after a mean of 17 cycles of ECP (range 2-35), cGVHD resolved completely in 7/20 (35%) and partially in other 10/20 (50%). A further benefit gained from the study was that all responding pts were able to reduce the immunosuppression, that has been discontinued in 2 cases. Three pts (15%) did not responded: two experienced stable disease and one died because of progressive cGVHD. Karnofsky performance score improved from 67% (range 40-90 %) to 86 % (range 70-100%) in responders pts.

aGVHD: One patient experienced progressive GVHD and died; the other promptly resolved. No severe side effects were documented and no infectious episodes occurred throughout the course of the treatment except Herpes Zoster Virus in two cases. Our results suggest that ECP is a safe therapy for extensive cGVHD resistant to conventional treatment. ECP could be employed in aGVHD steroid resistant pts too.

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Extracorporeal photopherochemistry: an innovative treatment for graft-versus-host disease. A monocenter retrospective pediatric study

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Acute and chronic GVHD are the main causes of transplant related morbidity and mortality. Aim of our study is to assess the role of extracorporeal photopherochemistry (ECP) in the cure of resistant GVHD in a pediatric population. Since January 1992 to December 2000, 33 patients (26 males, 7 females) affected by resistant acute or chronic GVHD underwent ECP in the Pediatric Department of the University of Padova. The patients received bone marrow transplant from HLA id. sibling n=11, or unrelated n=14, familiar HLA id. n=3, haploidentical n=1, unrelated 1 ag n=7, 1 MSD n=2. The median age at the transplant was 9.3 (1.4-17.9) years. The median time from onset of GVHD to start ECP therapy was 3.2 (0.2-97.2) months. The median body weight was 30.5 (10.5-85) Kg. Acute GVHD:14 patients underwent ECP because of aGVHD at the median time of 1.5 (0.4-3.2) months from the transplant. All patients presented skin involvement, 10/14 patients had gastrointestinal GVHD, 7/14 children had liver involvement and 6/14 had pancitopenia. The median Lansky/Karnofsky play performance scale was 60% (range 30-90). Results: 11/14 (78.6%) children showed significant improvement. At the end of the cycles of ECP no GVHD was present in 7/14 patients (50%), 4 were out of any immunosuppressive therapy (28,5%), 7/14 were unevaluable. Of the 20 evaluable pts, after a mean of 17 cycles of ECP (range 2-35), cGVHD resolved completely in 7/20 (35%) and partially in other 10/20 (50%). A further benefit gained from the study was that all responding pts were able to reduce the immunosuppression, that has been discontinued in 2 cases. Three pts (15%) did not responded: two experienced stable disease and one died because of progressive cGVHD. Karnofsky performance score improved from 67% (range 40-90 %) to 86 % (range 70-100%) in responders pts.

aGVHD: One patient experienced progressive GVHD and died; the other promptly resolved. No severe side effects were documented and no infectious episodes occurred throughout the course of the treatment except Herpes Zoster Virus in two cases. Our results suggest that ECP is a safe therapy for extensive cGVHD resistant to conventional treatment. ECP could be employed in aGVHD steroid resistant pts too.
was present in 5/19 patients (26.3%), 4 were out of any immunosuppressive therapy (21%), 7/14 were able to reduce the dose (52.6%). The median Lansky/Karnofsky score improved from 60 to 85%.

Conclusions: our result demonstrate that ECP is an effective and well tolerated therapy for patients affected by acute and chronic resistant GVHD. Prospective randomized trial are needed to assess the impact of an early introduction of ECP on the outcome of patients with GVHD.

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**Extracorporeal photopheresis (ECP) in the management of steroid-refractory (SR) or steroid-dependent (SD) extensive cutaneous chronic graft-versus-host disease (cGVHD) after allogeneic stem cell transplantation (ASCT)**

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We conducted a retrospective analysis of all ASCT patients started on ECP for SR/SD cutaneous cGVHD during a 27 month period (9/98-12/00). SR patients failed to respond to standard doses of systemic corticosteroids, SD patients had an initial response followed by a cGVHD flare upon steroid taper. Only patients who were treated after Day 100 and who received at least 4 weeks of ECP were considered evaluable. The Therakos UV-A/ER/ULTA systems were employed. Primary endpoints for the analysis were ECP feasibility, complete (complete resolution of skin rash and/or clinically inactive skin involvement), partial (improvement in skin rash and/or skin involvement on at least 50% of body surface area) and steroid-sparing (50% or more decrease in steroid requirement) response rates, as well as overall and cGVHD-related mortality. Thirty-two ASCT patients were evaluated. Their median age was 43 years (range 5-70). Their diagnosis was acute/chronic leukemia (n=18), lymphoma (n=10) and others (n=4). Fifteen (47%) had received a marrow transplant vs. 17 (53%) had received a blood stem cell graft. The ASCT was from matched sibling donors (n=22;69%), matched unrelated donors (n=7;22%) or other (n=3;9%). Among steroid-treated patients, eleven (34%) were SR and nineteen (59%) were SD. Cutaneous cGVHD was extensive in 30 (94%) and 20 (63%) had visceral/hepatic, gastrointestinal cGVHD as well. Patients developed cGVHD at a median of 181 days after ASCT. Thirty patients had received other therapies before ECP, including tacrolimus, infliximab, thalidomide and mycophenolate mofetil (MMF). Patients received a median of 39 ECP sessions (range 12-100+) over a median of 5.4 months (range 1 -28), with a median of six sessions per month. The CR rate was 34% (n=11) and the steroid-sparing response rate was 63% (n=19). Of 8 CRs, 6 are ongoing. One patient is lost to f/up. Eleven (35%) patients have died after ECP, with all cases due to cGVHD or cGVHD-related infectious complications. Of the 20 surviving patients, 19 (95%) remain on at least some immunosuppressive therapy (21%), 7/14 were able to reduce the dose (52.6%). The median Lansky/Karnofsky score improved from 60 to 85%.

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**Extracorporeal photopheresis (ECP) in acute and chronic GvHD: is there a relationship between the number of treated and infused MNC and clinical responsiveness?**

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Background:ECP proved to be effective in GvHD, either acute or chronic, after failure of conventional immunosuppression. The mechanism of action of ECP, the optimal schedule and a possible relationship between number and, possibly, phenotype of collected, treated and infused MNCs and clinical outcome are still controversial.

Patients and methods:5 pts with grade 2-3 aGvHD and 11 with extensive (10) or limited (1) cGvHD underwent ECP for unresponsive GVHD or severe side-effects of heavy immunosuppression. Overall, 11 had mucosal and/or skin involvement, 5 had visceral or combined involvement. In our Institution ECP is performed as a two-step procedure: i) 8-MOP (200 ng/mL) is added to MNC cells collected by using a Cobe Spectra cell separator and ii) UV-A irradiation (2 J/sq cm) is performed through a Vilber-Lourmat device. WBC count and differential were performed on the yield. CD19 and CD16-56 determination in peripheral blood was performed before and after 7, 12, 16 and 20 procedures. Clinical response was assessed as CR, PR and NR. Data are shown as median and range.

Results:221 ECP were performed (12/pt, range 6-28), without clinically relevant side-effects. 3 pts (2 acute, 1 chronic) received a MNC dose x106/Kg/ECP of 50.4 (9.7-164.4) and showed NR after 12 (6-28) ECP; 4 pts (1 acute, 3 chronic) received a MNC dose of 121.5x106/Kg /ECP (15.4 – 296.0) showed PR after 15 (9-17) ECP; 9 pts (2 acute, 7 chronic) received 107.6 x106/Kg/ECP showed CR after 12 (8-23) ECP, 10/11 pts with mucosal and/or skin GvHD responded (3 PR, 7 CR), while 3/3 pts with visceral or combined GvHD responded (1PR, 2 CR). Among the 9 evaluable for flow cytometry up to now, all responding pts (2 PR and 6 CR) had a decrease in CD8+ cells; on the opposite an increased in CD8+ cells was observed in the NR patient. CD16+ cells increased in 4 pts with CR and in 1 with NR, while decreased in 2 pts with PR and in 1 with CR.

Conclusions: In the attempt of elucidating the mechanism of action of ECP, our study suggests a possible correlation between the MNC dose treated for each procedure and the clinical outcome. In conclusion, the valuable information provided by flow cytometry suggests that a higher MNC dose is more effective in terms of response rate and duration. The role of MNC in the immune response and clinical outcome of ECP treated patients should be further investigated.
was 23.9 (range 4-46) and the mean follow-up was 27 months (range 2-37).

Results: 18 patients (85%) showed a satisfactory response, especially with skin, mucosal and liver involvement. Furthermore, 81% of patients were able to reduce or suspend IST. No relevant side effects were observed, making ECP not only effective but also safe and well tolerated. Finally, no increase in infectious complications were documented.

Conclusions: In our experience, ECP resulted a new effective therapeutic approach to treat cGvHD in adults. The lack of adjunctive infectious episodes strongly supports the hypothesis of a direct immuno-modulatory action of this treatment. The encouraging results obtained lead to the possibility of an early (first line) employing of ECP in cGvHD management.

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Home care during the pancytopenic phase after allogeneic hematopoietic stem cell transplantation using unrelated or related donors

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After myeloablative treatment and allogeneic stem cell transplantation (SCT), patients are kept in isolation rooms in the hospital to prevent neutropenic infections. During a three-year period, patients living within one hours' driving distance from our SCT unit were offered to be treated at home after SCT. Thirty-six patients intended to treat at home (2 never went home) were compared to 18 patients who were offered home care, but who chose hospital care (control group 1). The home care patients were also compared to 36 matched controls treated in the hospital (control group 2). In the home care patients, the median age was 42 (range 14-58). All had hematological malignancies and 20 were in first remission or first chronic phase. Donors were 25 unrelated donors, 10 HLA-identical siblings and one identical twin. Prognostic factors like diagnosis, disease stage, age, sex, type of donor and source of graft were comparable in the study group and the controls. The patients spent a median of 16 days at home (0-26). Before discharge, they spent a median of 4 days in the hospital (0-39). The home care patients were discharged on median day 19 to the outpatient clinic, which was significantly faster than day +30 and +25 in the two control groups, respectively (p<0.01, p<0.05). In multivariate analysis, the home care patients had a shorter time to discharge (RR 0.30, p=0.016), less sepsisemia (RR 0.35, p=0.037), fewer days with i.v. analgesics (RR 0.33, p=0.021), fewer days with total parenteral nutrition (RR 0.21, p=0.001), less acute GVHD grades II-IV (RR 0.27, p=0.017), lower transplant-related mortality (RR 0.19, p=0.015), and lower costs (RR 0.28, p=0.01), compared to the controls treated in the hospital. Two-year survival was 76% in the home care group vs. 54% (p=0.007) and 60% (p=0.06) in the two control groups, respectively.

To conclude, this study suggests that home care during the pancytopenic phase after SCT is superior to isolation in the hospital. A prospective randomized study is warranted.

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The effect of IgM enriched human immunoglobulin and rabbit anti-thymocyte globulin on the stimulation of mononuclear cells

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Whether IgM-enriched intravenous immunoglobulin (Pentaglobin) is a useful adjunct treatment for GVHD prophylaxis in allogeneic stem cell transplantation is unclear. Clinical data with the use of a five-agent GVHD prevention regimen including Pentaglobin and ATG are encouraging. In vitro both have been reported to modulate alloreactive T cells. We compared their inhibitory effect on the phytohemagglutinin-induced lymphocyte proliferation. ATG blocked proliferation of lymphocytes at lower doses and much stronger than Pentaglobin. The combination of both was not different from ATG alone. In Pentaglobin, glucose used as stabiliser, caused the effect. Starting at a concentration of 40 mg/dl glucose, glucose alone showed a dose-dependent inhibition of PHA-induced proliferation. For the in vivo application of Pentaglobin, the results suggest that Pentaglobin does not inhibit the proliferation of T cells.
unrelated \( (n=24) \), mismatched \( (n=3) \), or identical family donors \( (n=1) \). Prior to transplantation 13 pts underwent 12 Gy fractionated total body irradiation and 120 mg/KG body weight cyclophosphamide, one pt additional etoposide, and 14 pts a reduced conditioning regimen consisting of Fludarabin, Busulfan, and ATG, respectively. Standard GVHD prophylaxes consisted cyclosporine \((\text{CsA}) \) 3 mg/KG as a continuous infusion started on \(+1\) and methotrexate \((\text{MTX}) \) 15 mg/m\(^2\) on day +1 and 10 mg/m\(^2\) on days +3, +6 and +10, respectively. In case of the reduced condition regimen MTX was omitted. Starting on day +10 until day +45 after transplantation pts received a dose of 1 g MMF iv. every twelve hours and changed to oral medication when tolerated. All pts engrafted in a median of 12 days \((\text{range} \ 9-22)\), became independent of platelet transfusions in a median of 14.5 days \((\text{range} \ 10-61)\), and were discharged from hospital on day 35 \((\text{range} \ 15-72)\). Twenty out of 28 pts developed acute GVHD including 18% GVHD grade III or IV \([4 \text{ pts} \ (14\% ) \], 11 \text{ pts} \ II (39\%), 2 \text{ pts} \ II (7%) \), and 3 \text{ pts} \ Ivo \( (11\%) \). Subsequently, eight pts developed limited and one pt extensive chronic GVHD. With a median follow up of 17 month \((\text{range} \ 3-30)\) overall survival is 64.3% and disease free survival 60.7%. Only two deaths were due to GVHD Ivo. Ten out of 13 pts being CMV IgG positive became positive for pp65 indicating reactivation of CMV. However, CMV reactivation was independent from the donor status. MMF-related side effects were moderate \((\text{e.g.} \ \text{nausea and vomiting})\). In conclusion, MMF seems to be safe and feasible in the prophylaxes of severe acute GVHD for high risk pts but combined with a high risk for reactivating CMV in CMV positive pts.

**MabThera: an optional treatment for chronic graft-vs-host disease (GVHD)**

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Mabthera \( \text{(Rituximab)} \) is an anti-CD20 antibody whose target is CD20 molecule, constituted and selectively expressed by mature cells. Although it has been approved for relapsed or refractory CD20+ follicular B cells NHL, its use can be extended to other diseases such as autoimmune diseases. Few reports indicate efficacy in autoimmune disorders such as idiopathic Thrombocytopenic Purpura \((\text{I.T.P.})\), autoimmune hemolytic anemia, IgM neuropathies, etc. Chronic GVHD \( \text{(CGVHD)} \) is often considered an autoimmune disease because of distinctive similarity to various autoimmune disorders, especially collagen vascular disease. In this report, we describe two patients with extensive CGVHD of the skin like collagen vascular disease, who were previously resistant to immunosuppressive treatment. They received Mabthera at a dose of 375mg/m\(^2\) weekly. Treatment was well tolerated. The first patient was a 65-year-old male who underwent non-myeloablative transplant \((\text{NST}) \) from his sibling, due to NHL follicular predominant large cells, at first relapse, in 1996. After two cycles of treatment, we stopped therapy, due to progression of signs of GVHD. The second patient was a 25-year-old male who underwent allogeneic BST in 1998 from his sibling due to NHL diffuse large cells in a stage of refractory disease. After 4 cycles of treatment, there was major objective improvement in the status of CGVHD. Skin became less dry and softer, secretion of saliva that was absent prior to the treatment appeared, and improvement of CGVHD of the eyes was noted. The patient almost stopped the use of eye drops and Karnofsky score increased from 60 to 80. This effect lasted for one month. We are considering renewing the treatment. We conclude that Mabthera treatment appears feasible for CGVHD. More clinical trials will be needed to define the role of Mabthera in CGVHD.

**Prevention of graft versus host disease with Ig M enriched immunoglobulins: a preliminary analysis of a randomized study**

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In a previous study Ig M enriched immunoglobulins demonstrated to have a greater immunomodulatory capacity in vitro than standard IgG by immune cell activation and regulation of release of cytokines such as IFN gamma, IL2, IL6. We have opened a prospective trial to test the efficacy of Ig M enriched immunoglobulins \( \text{(Pentaglobin)} \) in prevention of acute and chronic graft versus host disease \( \text{(GVHD)} \) in patients undergoing bone marrow transplant \( \text{(BMT)} \) with alternative donor. Primary end point of the study is reduction of GVHD and transplant related mortality \( \text{(TRM)} \). The patients are randomised at BMT to receive Pentaglobin 200mg/kg b.w. or Sandoglobulin 400mg/Kg b.w. weekly from \( +1 \) to \(+100\) post BMT. Until 25 November 2001 patients enrolled are 89. Diagnosis are: chronic myeloid leukemia \( \text{(CML)} \) \( (n=42) \), acute myeloid leukemia \( \text{(AML)} \) \( (n=20) \), acute lymphoblastic leukemia \( \text{(ALL)} \) \( (n=15) \), myelodysplastic syndromes \( \text{(MDS)} \) \((n=5)\), lymphoproliferative disorders \( \text{(LPD)} \) \((n=2)\). Median age is 34 years. The percentage of TBI in the conditioning regimen, cells dose at BMT, type of donors \((\text{mismatched unrelated donors vs related non HLA identical})\) is not different in the two groups of patients. The cumulative acute GVHD III – IV grade is 10%, chronic GVHD extensive is 30%, TRM 40% and overall survival is 52%. Causes of death are GVHD (10%), infections (21%), relapse (4%), other (11%). The study is still enrolling patients.

**The role of busulfan at post transplant pulmonary complications in allogeneic hematopoietic cell transplantation**

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Respiratory complications are one of the major causes of non-relapse morbidity and mortality who underwent allogeneic hematopoietic cell transplantation \( \text{(AHCT)} \) and affect 40-60% of the patients. Obstructive airway disease \( \text{(OAD)} \) partly as a result of bronchiolitis obliterans occurs at 2-13% of transplant recipients and mostly in association with GVHD. The unpredictable serum level because of its variable bioavailability is one of the major disadvantages of high dose oral busulfan \( \text{(BU)} \) conditioning. We aimed to find any role of administered busulfan dose calculated by patients’ actual body weight \( \text{(ABW)} \) versus ideal body weight \( \text{(IBW)} \) (IBW). We analyzed retrospectively 24 patients affected by post transplant OAD among 308 allogeneic hematopoietic cell transplant recipients \((\text{HLA identical sibling transplants, Bu-Cy conditioning})\) between years 1994 and 2000 in a single center. We matched 22 historical controls \((\text{without OAD})\) according to their age, diagnosis, sex and stem cell source to 24 patients with OAD. The administered dose of BU was higher in OAD group than control group but it was not significant. We did not find any significant difference between calculated dose \((\text{IBW adjusted})\) and administered dose according to actual body weight in both groups \((\text{Table})\). There was also no difference between doses given according to ABW versus calculated body weight \((\text{IBW})\). We did not find any influence of BU dose on OAD. But determination of serum levels of BU is obligatory to show any correlation between the total BU dose given and OAD. Further analyses of factors except busulfan is necessary to find out any predisposing condition that contribute to OAD post AHCT.
Hemorrhages following allogeneic HSCT: incidence and impact on transplant outcome

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We have analyzed the incidence of hemorrhagic complications in 807 patients undergoing an allogeneic hemopoietic stem cell transplant (HSCT) between 1991 and 2001 in our Unit. Median age was 35 years (16-66) and the donor was an HLA identical sibling (n=570), a family mismatched member (n=74) or an unrelated donor (n=183). At least one hemorrhage was recorded in 238 patients (29%), 23% vs 45% for HLA identical sibling and alternative donors (p=0.0001). The proportion of patients with bleeding episodes was significantly higher in the presence of acute GvHD grade III-IV (55%) as compared to grade II (31%) and grade 0-I (23%)(p<0.0001). It was comparable in patients receiving bone marrow grafts (24%) or peripheral blood grafts (18%) (p=0.2). The average duration of the episode was 6 days (range 1-90) and occurred as a hemorrhagic cystitis in 38 patients, a gut bleeding in 26 and in other sites in 174. Patients with hemorrhages spent significantly more days in Hospital (median 42 days) as compared to patients without bleeding (median 42 days, p=0.0001) and had significantly higher transplant mortality (35% vs 26%, p=0.009). The impact on TRM was particularly significant in patients with or without gut bleeding (63% vs 28%, p=0.0001). We conclude that hemorrhages are a frequent complication of allogeneic HSCT and correlate with acute GvHD. The significant impact on the duration of Hospital admission and on transplant mortality, warrants prospective clinical trials, testing the efficacy of recombinant human activated coagulation factor VII.

16. Solid Tumors

P702

The EBMT Solid Tumors Working Party (STWP) Registry: 2001 report

T. Demirer (Ankara, TR)

By November 2001, the EBMT has included 19086 patients with solid tumors, two thirds (66%) being female; mean age is 32.54 years +18.30. To date, 5037 patients received marrow, 13905 PBSC and 144 received both. Overall toxic death rate in 1991 was 6% but reduced to 2% in 2000. HD MITO given at 60 mg/m2 on day –4; 2) HD MEL given at 160 mg/m2 on day –2; 3) APSC infusion with a median of 8.5 x 106 CD34+kg (range 3-16) on day 0; 4) G-CSF 5 mcg/kg on day +3. The median number of days in hospital was 23.5 (range 15-35). The median duration of severe neutropenia and thrombocytopenia was 9 (range 8-13) and 16 (range 9-29) days, respectively. The median number of units of erythrocytes and platelets transfused was 4.5 (0-11) and 5 (1-9), respectively. 24.3% patients did not develop fever during severe neutropenia. Of the remaining, 75.7% developed fever for a median of 3 (1-8) days (FUO 53.7%, Microbiologically Documented 25%, CVC-related 17.8%, Clinically Documented 3.5%, according to EORTC criteria). No systemic fungal infection was documented and there were no infectious deaths. Mucositis was observed in 81.5% of cases (WHO grade 3-4 in 26.3%). No acute and late cardiac toxicity was observed and left ventricular ejection fraction didn’t show any difference during follow-up. Only one patient died because of transplant-related cause (cerebral thromboembolic disease). At a median follow-up of 28 mo. (range 5-80), median PFS and OS were 18 and 34 mo, respectively. Furthermore, 32% of pts were predicted to remain alive and 20% progression-free at 64 mo. We conclude that a conditioning regimen with HD of MITO + MEL fits well within the HDC program. It implies good tolerability and no cardiac toxicity.

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High-dose mitoxantrone (Mito) + melphalan (Mel) with autologous peripheral stem cells (Apsc) infusion in metastatic breast cancer (Mbc) patients


High-dose chemotherapy (HDC) with APSC rescue has been most frequently used in breast cancer (BC), even it has not yet proven its worth in randomised controlled trials. Anthracyclines are among the most active agents in advanced BC. MITO demonstrated a different toxicity profile when compared to doxorubicin. Haematological and extra-haematological toxicity of MBC patients, median age 38 years (range 33-54). Autograft was the final part of a HDC, including a induction phase with 3 courses of conventional chemotherapy with Epirubicin 120 mg/m2 and Cyclophosphamide (CTX) 600 mg/m2 and a first course of myeloablative regimens including CTX 600 mg/m2, Thiotepa 500 mg/m2 and Carboplatin 800 mg/m2. Autograft phase included: 1) HD MEL given at 60 mg/m2 on day –4; 2) HD MEL given at 160 mg/m2 on day –2; 3) APSC infusion with a median of 8.5 x 106 CD34+kg (range 3-16) on day 0; 4) G-CSF 5 mcg/kg on day +3. The median number of days in hospital was 23.5 (range 15-35). The median duration of severe neutropenia and thrombocytopenia was 9 (range 8-13) and 16 (range 9-29) days, respectively. The median number of units of erythrocytes and platelets transfused was 4.5 (0-11) and 5 (1-9), respectively. 24.3% patients did not develop fever during severe neutropenia. Of the remaining, 75.7% developed fever for a median of 3 (1-8) days (FUO 53.7%, Microbiologically Documented 25%, CVC-related 17.8%, Clinically Documented 3.5%, according to EORTC criteria). No systemic fungal infection was documented and there were no infectious deaths. Mucositis was observed in 81.5% of cases (WHO grade 3-4 in 26.3%). No acute and late cardiac toxicity was observed and left ventricular ejection fraction didn’t show any difference during follow-up. Only one patient died because of transplant-related cause (cerebral thromboembolic disease). At a median follow-up of 28 mo. (range 5-80), median PFS and OS were 18 and 34 mo, respectively. Furthermore, 32% of pts were predicted to remain alive and 20% progression-free at 64 mo. We conclude that a conditioning regimen with HD of MITO + MEL fits well within the HDC program. It implies good tolerability and no cardiac toxicity.

P704

High-dose chemotherapy with autologous peripheral blood progenitor cell transplantation in patients with germ-cell tumors


Autologous peripheral blood progenitor cell rescue was provided in our center, from January 1998 to September 2001, to 22 patients with relapsed or refractory germ-cell tumors. Median age was 27 years and tumor markers were elevated - HCG in 6 and AFP in 5 patients at the time of high dose chemotherapy. Stem cell mobilization was performed after the 3rd cycle of 5 x 106 combination with G-CSF. The amount of CD 34-cell/kg b.w. was between 2.0 - 10.3x106. High-dose conditioning regimen CARBOPEC (carboplatin 1600 - 2 200 mg/m2 , etoposide 1800mg/m2 , cyclophosphamide 6400 mg/m2 ) was used. The treatment was well tolerated without transplant - related mortality. Engraftment was rapid, recovery of hematopoiesis in neutrophils over 1.0x109/l and platelets over 50x109/l was reached on average on days +10 and +13 respectively, using filgrastim 5 ug/kg beginning the day +6. Using WHO criteria , hematological toxicity was grade IV, non-hematological toxicity of the therapy was predominantly of grade II. The follow-up period is from 3 to 41 month. At present, 12 (55%) out of 22 patients are alive ( 10 pts
are in CR, 2 pts are in SD ), 10 (45%) pts died. Among 12 surviving pts two pts after autologous transplantation had retroperitoneal lymphadenectomy, five pts received 3rd line chemotherapy with Paclitaxel and Gemcitabin for relapse or progression of the disease. Patients are reevaluated every 4 months during the first 2 years.

In conclusion, high-dose chemotherapy with autologous PBPC rescue in patients with advanced germ-cell tumors is feasible and promising method of treatment in this group of patients. Results of multicentric randomized EMBT study IT 94 are needed to exactly evaluate the role of this method in the therapy of patients with germ-cell tumors.

P705

Genomic imbalances predict outcome in patients with high-risk breast cancer treated with high-dose chemotherapy and autologous stem cell transplantation


Breast cancer development and progression is associated with the accumulation of genetic aberrations that have been proposed to determine tumour phenotypes and clinical evolution of patients (pts). We used comparative genomic hybridization (CGH) to perform a genome-wide screening of DNA copy number variations on 34 frozen and paraffin tumors from pts with high risk breast cancer (HRBC), stages II-III (10 or more lymph nodes affected), treated with high dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT). With a median follow-up time of 74 months, 15 pts (44%) have relapsed. Using CGH, a total of 383 genomic imbalances was found (median per tumor: 11, range 2-24), including chromosomal gains/amplifications (median: 4) involving 1q (59%), 17q (38%), 16p (35%), 8q (35 %), and 20q (32%), and losses (median: 6) involving 9p (41%), 18q (41%), 11q (38%), 8p (38%), 18p (38%) and 17p (32%). In univariate analysis there was not a correlation between the number of genomic imbalances and the outcomes of pts. However pts with 4 or more genomic losses had a shorter disease free survival (DFS) than those with <4 (p=0.03). Pts with gain/amplification of 17q12 affecting the HER2-neu locus (8 cases) had a longer DFS than those with <4 (p=0.003). Other characteristics of pts such as age, menopausal status, number of lymph nodes affected, tumor size, EP receptors and histologic grade were not associated with differences in DFS. These data suggest that the pattern of genomic imbalances may represent a novel marker for predicting the response to therapy in patients with HRBC.

P706

Combined surgery, sequential double high-dose chemotherapy with autologous peripheral stem cell transplantation and intrathecal radiopeptide brachytherapy for relapsed medulloblastoma of the cauda

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We report a single case of a now 13-year-old boy with a medulloblastoma (Initial stage: Chang T3B). After craniotomy and incomplete tumor resection the boy was treated with chemother presentation with acute urinary retention. Spinal MRI showed a subarachnoid space metastasis (LS-S4). Since upfront surgery and additional external radiotherapy did not represent an option a sequential double high-dose chemotherapy (HDC) followed by autologous peripheral stem cell transplantation (aPBSCT) was performed. Conditioning regimen of course # 1 consisted of thiotepa and etoposide and of course # 2 of cyclophosphamide and melphalan. Time interval between courses # 1 and # 2 was 16 weeks. A leukocyte count of > 1,0 x 109/L was achieved at day 11 for both cycles. HDC was well tolerated. Between the first and second aPBSCT, the remaining tumor was removed by a laminoplasty (L4-S4). Subsequently the treatment was completed by 4 courses of brachytherapy intrathecally injecting Yttrium-90 labelled modified dotatoc (DOTATOC). Median persistence of the disease was 1 ½ years after completing therapy, the boy is still in complete remission. The most compromising deficit remains the neurological bladder dysfunction with the need of a permanent external drainage. In conclusion this case illustrates the potential of a combined approach in individual complex situations such as relapsed medulloblastoma. The poor prognosis of relapsed brain tumors in children allows the search for new treatment modalities. Further clinical trials are necessary to establish timing, intensity and indication of HDC with aPBSCT in relapsed medulloblastoma. Intrathecal brachytherapy using radiolabelled peptidic vectors might represent an additional therapeutic option for relapsed medulloblastomas.
P708
Long-term hematologic recovery following high-dose chemotherapy with autologous peripheral stem cell transplantation (PSCT) or autologous bone marrow transplantation in children with solid tumors
Objective: BMT from autologous sources has been employed to reconstitute the bone marrow following chemo-radiotherapy-conditioning regimens for several years. We performed PBSC collection safely on an outpatient basis. Rescue with PBSC after myeloablative therapy results in a significantly reduced neutropenic period.
Patients: Since May 1989 we performed leukapheresis in 132 pts with solid tumors using a Baxter CS-3000 Plus. Patient age ranged from 6 months to 19 years, weight from 6 to 65 kg. 363 PBSC collections were performed with an average of 2 collections per pt.
98 of 132 pts (neuroblastoma 29, Wilms’ T. 14, Ewing S. 13, CNS 15, PNET 9, others 18) were subsequently given the thawed product; 5/98 pts had a double transplant. The treatment regimen at all 98 of 132 pts consisted of Etoposide 200 mg/m² days 1, 2 and 3, Thiotepa 250 mg/m² days 1, 2 and 3, Cytoxan 60 mg/kg days 4 and 5 (ETC).
Results: Median recovery time defined equal or greater than 500 PMNs and 50,000 PLTs/mm³ was 16.5 days (range 12-22 days) for PMNs and 19 days (range 8-25 days) for PLTs. Nine pts received PBSC support after submyeloablative therapy; median time of recovery in this patient group was 6.5 days (range 5-13 days) to PMNs and 10 days (range 8-15 days) for PLTs. The introduction of CSF allowed for a significantly shorter recovery time for PMNs. Both G-CSF and GM-CSF were given at 5 mcg/kg each. PMNs greater than 1.000/mm³ and PLTs greater than 50,000/mm³ occurred after 12.5 days (range 5-15 days) and 19 days (range 13-25 days) respectively. 5/98 pts underwent two BMT with a different treatment regimen. To reduce the number of procedures we monitored the CD 34+ count, collecting PBSC when count was above 20 cell/ml. Up to date 44 (45%) pts are in CR, 8 (8%) are AWD and 46 (47%) DOD.
Conclusions: The results of this experience suggest that PBSC can be successfully collected in small children and can be used to reconstitute the bone marrow after myeloablative therapy. The ETC regimen is well tolerated and efficacious in producing prolonged event free survival in high-risk patients.

P709
Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes (CTL) for the treatment of patients with EBV-positive relapsed nasopharyngeal carcinoma
Nasopharyngeal carcinoma (NPC) is an EBV-related epithelial neoplasm. Currently, the elective form of treatment for NPC is radiotherapy, alone or combined with chemotherapy, which cures a high percentage of cases presenting with early stage tumor. However, as the prognosis of patients with advanced stage disease remains poor, there is a need to develop additional forms of treatment. It has recently been shown that infusion of EBV-specific CTLs expanded in vitro can safely and effectively control EBV-positive posttransplant lymphoproliferative disease. NPC cells are EBV-antigen-positive, and therefore they could also be suitable targets for specific immunotherapeutic treatment. Having been shown that EBV-specific T cell immunity can be significantly reduced in NPC patients compared with that in healthy virus carriers, we explored the feasibility of developing salvage therapy of NPC by an adoptive immunotherapy approach employing HLA-identical family donors as the source of PBMCs to induce and expand EBV-specific CTLs. Our pilot clinical study has concerned patients with advanced stage NPC, for whom HLA-identical donor CTLs were expanded. Immunophenotyping showed that CTLs were on average 99% CD3+, 70% CD8+, 20% CD56+, and contained 10% cells that were CD3+CD4+CD56+ and 15% cells of NK phenotype. EBV-specificity of the CTL lines was indicated by the strong lysis observed against autologous EBV-LCL, with little or no reactivity against HLA-mismatched targets. In addition, donor CTLs showed a HLA class I-restricted lysis of patients’ tumor cells. One of the patients received 4 weekly doses of EBV-specific T cell lines. CTL transfer was well tolerated, and a TC scan performed after the last infusion showed a partial reduction of the intracranial portion of the tumor mass. A tumor biopsy was also performed, and immunocytochemistry analysis demonstrated a marked increase of CD8+ lymphocyte infiltrate in comparison to a pre-infusion sample. Studies are in progress to determine the origin of the lymphocyte infiltrate. Preliminary data obtained in this patient indicate that EBV-specific CTLs can be used safely, are able to exert specific killing of autologous tumor cells in vitro, and have antitumor effect in vivo. Further studies on a larger cohort of patients are required to assess the respective role of donor or autologous EBV-specific CTLs in the control of EBV-positive NPC, and the possibility to implement the clinical results by employing LMP-2 specific CTLs.

P710
Transplantation of a megadose of full-haploidentical hematopoietic stem cells (HSCT) as a treatment of metastatic relapse of Ewing sarcoma - Report from the Committee on High Risk Sarcoma of the Cooperative Soft Tissue Sarcoma Study (CWS)
The prognosis for patients (pts) with bone and/or bone marrow metastasis (B/BM) metastases of solid tissue sarcoma (STS) remains very poor. The 5 yrs EFS of pts registered in the CWS Studies 81-96 was < 5%. Pts with metastatic STS who relapsed had no chance of being cured. Immune reconstitution after HDC is very poor with prolonged CD4 depletion. We hypothesize that the immune system may play a role in the control of the residual sarcoma cells. Therefore we initiated a prospective phase I/II study on HSCT from mismatched related donors as a consolidation therapy for pts with B/BM metastases of STS. The conditioning consisted of busulfan, (4mg/kg/day x 3), Thiotepa (10mg/m²/day), cyclophosphamide (50mg/kg/day x 4), fludarabine (30mg/m²/day x 2) and orthoclone (day – 4 till + 13). No posttransplant immunosuppression was given. Mobilized peripheral CD34+ cells were selected with CliniMACS device. We report on a 13 year old girl with a disseminated Ewing sarcoma (thorax wall, lung and B/BM) diagnosed in April 1998. The patient was treated according to the CWS-96 Study with 6 drugs combination (CEVAIE) + local irradiation + tandem HDC until Mai 1999. In Nov. 1999 a tumor relapse was diagnosed (thorax wall, vertebra and skull). We decided to treat the patient on the protocol for haploidentical HSCT. Before conditioning, two 10 days courses of low dose oral therapy of trofosfamid and idarubicin were administered. Cryopreserved cells from the mother were reinfused on day 0 (CD34 8x10E6/kg and CD3 4835/kg). Additional retransfusions of fresh donor cells were given on days +4 and +5 (CD34 11,1x10E6/kg and CD3 5163/kg). On day +9 the leucocytes were 1600/µl and a chimerism analysis revealed complete donor type. During the posttransplant period a slow regression of the measurable metastatic sites in the skull, vertebra and thorax wall was observed. The patient is alive, tumor progression free, 21 months after HSCT. We conclude that: 1. Tumor regression was initially due to myeloablative conditioning but further regression was possible due to the graft vs. tumor effect 2. Myeloablative conditioning could be advantageous in young patients when tumors are still chemosensitive as a means of reducing the tumor.
burden. 3. Megadoses of CD34 cells from haploidentical family donors present a very suitable source for haematopoietic and immune reconstitution in young patients. 4. No posttransplant immunosuppression may facilitate the development of graft vs. tumor effects

P711
Treatment results of children with high grade brain tumors treated with high-dose chemotherapy cycles with stem-cell rescue - A single center experience
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Objectives: To assess efficiency and toxicity for children older than 3 years with high grade brain tumors treated with the SJMB 3/97 protocol (whole craniospinal irradiation with concomitant vincristine and 4 cycles of submyeloablative chemotherapy - cyclophosphamide, cisplatin, vincristin with PBSC rescue).

Methods: Between 1999 and 2001, 18 children (11 boys and 7 girls) were treated with a SJMB 3/97 protocol. Median age at the time of diagnosis was 9.6 years (range 5.0 and 17.7). Diagnoses included medulloblastoma (7), anaplastic astrocytoma (5), high grade ependymoma (3) and PNET (3). Primary tumor was localized in the fourth ventricle (10), supratentorial area (5), thalamus (2) and hemisphere of the cerebellum (1). One child had dissemination at the time of diagnosis. Complete was monitored in 8 pts, biopsy or subtotal removal of the tumor (residual tumor greater than 1,5 cm2) was performed in 10 pts.

Amplication of c-myc was detected in 13 pts., amplification of N-myc in 4 pts. DNA aneuploidy was found in 6 pts.

Results: After surgery all children were irradiated on the craniospinal axis with boost on the primary tumor with concomitant vincristine. After radiotherapy 9 patient were in complete remission, 6 pts in partial remission, 3 pts. experienced disease progression.

After chemotherapy were 66% (12/18) pts. in CR, 22% (4/18) pts in PR, 1 pt. in SD, 1 pt. in PD and 1 pt. died of progression.

The hemat- and gastrointestinal toxicity were in all patients WHO grade III-IV, neurotoxicity gr. II-III, 1 pt. finished the treatment after 3 cycles of chemotherapy due to neurotoxicity, 1 pt. showed ototoxicity gr. III, 1 pneumotoxicity gr. IV.

Conclusions: The crucial prognostic factors in children with embryonal brain tumors are complete resection of the tumor, no evidence of leptomeningeal disease and no evidence of amplification of c-myc oncogene. Initial evaluation show feasibility of this regimen in the treatment of high risk brain tumors with promising results, despite high but manageable toxicity. Further evaluation is necessary.

P712
Pre-emptive oral vs. I.V. ganciclovir therapy against cytomegalovirus disease after allogeneic hematopoietic stem cell transplantation (HSCT) in patients with solid tumor
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Objective: Does the pre-emptive oral ganciclovir (GCV) therapy show comparable toxicity and efficacy as the intravenous therapy against CMV disease after allogeneic HSCT? Additional 3 patients (2 with initially oral and 1 with I.V. therapy) received another and successful oral course due to asymptomatic reinfection within 3 months. The peak CMV DNA load was 570 copies (median 35). The median GCV through levels were 2.6 mikromol/L (range 1.0-4.6) and 1.8 mikromol/L (range 0.7-7.5) in oral and I.V. groups, respectively. The median peak I.V. GCV level was 16.4 mikromol/L (range 8.0-23.6). Dose reduction was performed in 3 patients, 2 with oral and 1 with I.V. GCV, due to nephrotoxicity. In addition, 1 patient needed hemodialysis for acute renal failure and confusion with a GCV concentration of 50.9 mikromol/L.

Conclusion: CMV DNAemia with moderate CMV load was common in spite of the nonmyeloablative conditioning prior to HSCT. No tissue invasive CMV disease developed. Two of 9 patients with oral pre-emptive GCV were converted to I.V. therapy. GCV concentrations were comparable in oral and I.V. treated patients.

Additional abstracts to this topic

Allogeneic hematopoietic stem cell transplantation after dose-reduced conditioning induces remissions in recurrent ovarian cancer - a report of two cases.

Stem cell supported high-dose chemotherapy (HDT) seems as a promising approach in patients with advanced or recurrent epithelial ovarian cancer. Nevertheless relapses occur after autografting in a substantial number of patients. Further treatment has palliative intention in most cases. Although the effect in ovarian cancer is controversial, allogeneic hematopoietic stem cell transplantation (HSCT) is offered to selected patients with suitable HLA-matched donors. We report two patients who underwent allogeneic HSCT for recurrent epithelial ovarian cancer after sequential HDT.

Patient 1 (56 years) was diagnosed FIGO stage III in 1997. Surgical debulking was followed by adjuvant chemotherapy (taxol/carboplatin). In 1999 a first peritoneal relapse could be treated successfully with taxol/cyclophosphamid, whereas two retroperitoneal bulk tumors (about 5 cm in diameter each) detected in 2000 were refractory against sequential HDT (carboplatin/taxol/melphalan) as well as alternative salvage treatment with topotecan.

Patient 2 (32 years) underwent surgery and adjuvant chemotherapy for FIGO stage III disease in 1991. Consecutive metastases at vaginal stump, chest wall and retroperitoneal lymph nodes were treated by surgical extirpation, radiation and chemotherapy including taxanes, platin and topotecan. Further disease progression with left infraclavicular soft tissue metastases, inguinal and iliacal lymph node involvement had to be detected in 2000. Sequential HDT (carboplatin/ topotecan/tresulan) induced only short tumor regression.

Dose-reduced preparative regimen included busulfan 4 mg/kg orally d – 6 and – 5, and intravenous fludarabin 30 mg/m² dx2 for 2 weeks plus 5mg/kg/d 5 days/week for 3 weeks. GCV concentrations were monitored weekly.

Results: 10/17 (59%) patients developed CMV DNAemia. 2 patients with CMV syndrome, within the first month after HSCT. Five more patients developed CMV DNAemia between months 2 and 5. Nine of the 15 CMV DNA+ patients were administrated oral and 5 patients I.V. GCV. Two oral GCV cases were converted to I.V. therapy due to increasing CMV DNA load and/or clinical CMV syndrome. Both of them died with CMV DNAemia in tumor progression. Additional 3 patients (2 with initially oral and 1 with I.V. therapy) received another and successful oral course due to asymptomatic reinfection within 3 months. The peak CMV DNA load was 570 copies (median 35). The median GCV through levels were 2.6 mikromol/L (range 1.0-4.6) and 1.8 mikromol/L (range 0.7-7.5) in oral and I.V. groups, respectively. The median peak I.V. GCV level was 16.4 mikromol/L (range 8.0-23.6). Dose reduction was performed in 3 patients, 2 with oral and 1 with I.V. GCV, due to nephrotoxicity. In addition, 1 patient needed hemodialysis for acute renal failure and confusion with a GCV concentration of 50.9 mikromol/L.

Conclusion: CMV DNAemia with moderate CMV load was common in spite of the nonmyeloablative conditioning prior to HSCT. No tissue invasive CMV disease developed. Two of 9 patients with oral pre-emptive GCV were converted to I.V. therapy. GCV concentrations were comparable in oral and I.V. treated patients.

S192
Significance of P-glycoprotein expression in childhood malignant tumors
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P-glycoprotein (PGP) is a protein involved in efflux of various compounds. Its overexpression in cancer cells decreases intracellular drug concentrations, and thus, confers a multidrug resistance phenotype. Quantitative and functional analysis of PGP is important in determining resistance and therapeutic response. The pretreatment expression of PGP is a prognostic sign of the failure of therapy in many different cancers. We present the results of PGP expression examination in 91 tumor tissue samples obtained from children treated for different malignant tumors. The correlation between the level of PGP expression and tumor histology, clinical outcome, use of therapy, relapse rate and metastatic disease was made. Patient ages ranged from 2 months to 22 years. Samples were analyzed by flow cytometry and Khi2 test was applied. The highest levels of PGP expression were frequently found in the group of soft tissue sarcomas, neuroblastomas, and Ewing family of tumors (p<0.05).

Soft tissue sarcomas, neuroblastomas, and hepatoblastomas cells have shown significantly more frequent PGP expression (p<0.05). Brain tumors and nephroblastomas have shown significantly less frequent expression of PGP (p<0.05). In soft tissue sarcomas, we found a nonsignificant increased PGP expression in patients with relapse after or during chemotherapy (N.S., p<0.05). In patients with metastatic disease PGP overexpression was observed in soft tissue sarcomas and neuroblastomas (p<0.05).

We found a significant PGP overexpression in soft tissue sarcomas, neuroblastomas and hepatoblastomas. A significant increased PGP expression we proved in patients with soft tissue sarcomas and neuroblastomas with metastatic disease. In brain tumors and nephroblastomas we found significantly less frequent expression of PGP. PGP positive tumor cells were detected more frequently in disseminated disease. There was no significant difference between the tumors examined before the chemotherapy administration and the tumors influenced by the chemotherapy. Our results show that PGP positive phenotype is associated with a metastatic disease especially in soft tissue sarcomas and neuroblastomas. In those tumors PGP expression is frequently seen in recurrence and PGP presence in patients before chemotherapy may be a sign of low response to chemotherapy. We verify that flow cytometry is useful method for PGP detection not only in leukemias but also in solid tumors including brain and bone tumors.

17. Cytokines and Gene Therapy

P713

Mobilization of peripheral blood stem cells (PBSCs) with chemotherapy and granulocyte-colony stimulating factor (G-CSF): A randomized evaluation of different doses of G-CSF

Objective: To date, there is no randomized study comparing different doses of G-CSF following mobilization chemotherapy (MC). Therefore, in this study the effect of different doses of G-CSF following MC on the yields of CD34+ PBSCs were evaluated in patients with hematologic malignancies and solid tumors.

Methods: Fifty patients were randomized to receive G-CSF 8 (n = 25) versus 16 (n = 25) µg/kg/day following one of these mobilization regimens: cyclophosphamide (CY) and etopoide; CY and epirubicine; or CY and paclitaxel.

Results: The median number of CD34+ cells collected after 8 µg/kg/day of G-CSF was 2.36x10^6/kg (range 0.21-7.80) as compared to 7.99 (range 2.76-14.89) after 16 µg/kg/day (P=0.001). Among patients randomized to 8 v 16 µg/kg, all (100%) achieved > 4.0 x 10^6 CD34+ cells/kg and less aphereses were required to achieve > 2.5x10^6 CD34+ cells/kg after the higher dose (P=0.001). Twenty of 25 (80%) patients in the low dose and 23 of 25 (92%) in the high dose G-CSF arm underwent high dose chemotherapy and autologous stem cell transplantation. Median days of WBC engraftment in patients mobilized with 8 µg/kg and 16 µg/kg of G-CSF were 12 and 9, respectively (P<0.001). But there was no difference between 2 groups regarding the other parameters of peritransplant morbidity such as days of platelet engraftment (P=0.10), RBC (P = 0.56) and platelet transfusions (P=0.22), days of TPN requirement (P=0.84), days of fever (P=0.93), and days of antibiotics (P=0.77).

Conclusion: These data showed that higher doses of G-CSF following MC were associated with a clear dose response effect based on the collected cell yields. Based on these results 8 µg/kg/day is as effective as 16 µg/kg/day except for a rapid neutrophil engraftment in the high dose arm. Therefore, in the routine clinical practice, despite some advantage in the use of higher doses of G-CSF, lower doses may be used for PBSC collections following chemotherapy based mobilization regimens in this cost-conscious era.

P714

Stem cell mobilization by G-CSF in solid and hematological malignancies - Single daily dose better than split dose in obese patients

In the past, different results were reported for single daily and two divided daily G-CSF doses in stem cell collection where no study exists examining the effect of body mass index (BMI) on mobilization. Collected CD34(+) cells numbers were compared in 86 patients with solid and hematological malignancies receiving either single daily 14 mcg/kg/day G-CSF (filgrastim) as group I (n=36) or divided daily two G-CSF doses 2X7 mcg/kg/day as group II (n=50). Both groups were subdivided into a and b groups according to their BMI as group a (BMI < 25 kg/m2) and group b (BMI >25 kg/m2). Two groups were similar in terms of age, gender and disease characteristics. All the patients have received G-CSF as a single or two divided doses subcutaneously and apheresis has been done on the 5th day, 4 hours after the last dose. No significant CD34(+) cell number difference between groups Ia and Ib, groups IIa and Iib, groups Ia and Ila were found. On the other hand, the median ratio and the number of CD34(+) cells in group
P715

G-CSF and GM-CSF administration following PBPC transplantation in women with cancer - impact on transplantation outcomes in a randomized comparison


PBPC transplantation (PBPTC) combined with post-PBPC administration of myelopoeitic growth factors is a valid therapeutic intervention to rapidly restore haematopoiesis after the delivery of intensive, myeloablative anti-cancer chemotherapy. On the other hand, the best growth factor regimen to potentiate PBPC-mediated immuno-haematopoietic recovery has yet to be determined. We compared in a randomized evaluation the effects produced by post-PBPTC G-CSF and GM-CSF on myeloid/lymphoid recovery and transplantation outcome in women with chemosensitive cancer. Thirty-seven ovarian cancer and thirty-four breast cancer patients ranging in age from 24 to 60 years were treated with carboplatin, etoposide and melphalan (CEM) high-dose chemotherapy and subsequently randomized to receive G-CSF (5 ug/kg subcutaneously) or GM-CSF (5 ug/kg subcutaneously) until day +13 post-PBPTC. Patients were compared for their haematopoietic recovery, post-transplantation clinical management and immune recovery. Finally, clinical outcome was estimated as time to progression (TTP) and overall survival (OS). Haematopoietic recovery and post-transplantation clinical management were comparable in both the G-CSF and GM-CSF series. Conversely, significantly higher T lymphocyte counts were observed in G-CSF patients during the early and late post-transplantation follow-up. Patients who received G-CSF showed a significantly longer median TTP. A parallel analysis revealed that patients who recovered a higher CD3+ count had a significantly longer OS and TTP. The enhancement in post-PBPTC T cell recovery observed in G-CSF patients encourages the use of G-CSF to ameliorate immune recovery which seems to play a role in post-PBPTC disease control of cancer patients. GM-CSF might be administered to prolong immunosuppression after auto-PBPTC for autoimmune diseases or allogeneic PBPTC.

P716

Low-dose interleukin-2 plus G-CSF/EPO administration early after autologous PBSC transplantation - Results of a prospective study in women with breast and ovarian cancer


This study evaluated the effects of low-dose interleukin-2 (IL-2) plus G-CSF/EPO on post-PBSC transplantation (PBSTC) immune-haematopoietic reconstitution and NK activity in patients with breast (BrCa) and ovarian cancer (OvCa). To this end, two consecutive series of patients were prospectively assigned to distinct post-PBSC cytokine regimens (from day +1 to day +12) which consisted of G-CSF (5 ug/kg/d) plus EPO (150 UI/kg/every other day) in 17 patients (13 BrCa and 4 OvCa) or G-CSF/EPO plus IL-2 (2x10^5 U/ml/d) in 15 patients (10 BrCa and 5 OvCa). Haematopoietic recovery and post-transplantation clinical cares were manageable and comparable in G-CSF/EPO- and in G-CSF/EPO plus IL-2 treated patients. No significant differences were found between the two groups of patients in the kinetics of most lymphocyte subsets except naive CD45RA+ T cells which had a delayed recovery in G-CSF/EPO plus IL-2 patients (p=0.021). No significant difference was observed between NK activity in the two different groups, albeit a significantly higher NK activity was observed in G-CSF/EPO plus IL-2 series on day +20 (p=0.020). These results demonstrate that low-dose IL-2 can be safely administered in combination with G-CSF/EPO early after PBSCCT and that it exerts some positive effects on post-PBSCCT myeloid reconstitution but not on immune recovery.

P717

Delayed dose of glycosylated-G-CSF post autologous peripheral blood stem cell transplant: preliminary results of a randomized trial


Although G-CSF utilization after PBSC transplantation determines a faster granulocytes recovery, not all studies have shown clear advantages associated with its usage and hence the necessity to conduct sufficiently extensive randomized trials in order to clarify this subject. Since a delayed start of G-CSF has been shown to have a comparable efficacy in respect to G-CSF started at day +1, we choose to study this schedule. We have initiated a prospective randomized trial in 201 patients with solid tumors. Patients affected by acute leukaemia were excluded from the study. The treatment was stopped for neutrophils count > 500 for at least 2 days. Until now 31 patients have been enrolled in the study. 18 Lymphoma, 11 MM and 2 solid tumors; 15 patients were randomized to receive G-CSF from day +5 and 16 patients were randomized in no treatment arm. There were no significant differences in the two groups of patients regarding the characteristics of PBSC infused, age, state of diseases and type of the conditioning regimens used (HD-PAM, BEAM, TT-BUS-PAM). In the treatment group G-CSF was administered for an average of 7 days. Compared to control group, patients treated with G-CSF showed shorter neutropenia (N< 500) (Logrank p=0.0004) and a reduction in the need of parenteral nutrition (12 days in the G-CSF group vs 14 days in the control group). No statistical differences were noticed for other clinical parameters, such as platelet recovery, platelets transfusion requirement, duration of hospital stay and fever episodes. The preliminary analysis of our data shows that the use of a low dose of G-CSF with a delayed beginning (from +5) is effective in the reduction of neutropenia post PBSCCT transplantation. The number of patients required in order to evaluate whether administration of G-CSF in associated with other clinical and economic advantages is about 40 patients per arm. The study is still ongoing.
High-dose chemotherapy followed by peripheral stem cell transplantation is widely used in lymphoma patients. Growth factor has been used after peripheral stem cell reinfusion with the aim to further reduce the duration of neutropenia. We retrospectively studied 63 consecutive lymphoma patients (NHL 37, HD 27) treated with high dose chemotherapy and peripheral stem cell transplantation, subdividing them in 3 groups on the basis of administration of G-CSF. The first group received G-CSF starting 1 day after reinfusion independently from CD34+ number. In the second group, G-CSF was administered 5 days after reinfusion because CD34+ cells were less than 5 x 10^6/kg. Patients in the third group, did not received G-CSF because CD34+ cells were more than 5 x 10^6/kg.

The number of CD34+ cells reinfused was significantly different between the study groups. However, we did not found relevant differences between group 1 and 2 in term of hematopoietic recovery. Only the number of days to obtain an ANC more than 1 x 10^9/L was significantly shorter in group 1 compared to group 3 (p = 0.05). On the other hand, the number of days with an ANC less than 0.5 x 10^9/L, and to obtain an ANC more than 0.5 x 10^9/L and 1 x 10^9/L were significantly shorter in group 2 compared to group 3 (p = 0.00, 0.0009, and 0.00001, respectively). Patients in group 3 experienced a shorter days with platelets less than 20 x 10^9/L (p = 0.05).

Patients in group 2 showed a higher incidence of infections (FUO + documented infections) even if without consequence on number of days with antibiotics, antimycotics or time to hospital discharge. In conclusion, the routinely administration of G-CSF after peripheral stem cell is not recommended if an adequate (more than 5 x 10^6/kg) of CD34+ cells is reinfused. When this target is not obtained, G-CSF starting 5 days after reinfusion can reduce the aplasia period with an economical advantage compared to early administration (day +1).

**Effects of epoietin beta on RBC transfusion in inflammatory breast cancer patients receiving sequential high dose chemotherapy: Pegase 05, a randomized FNCLCC trial**

T. Palangie for the Working Party Sessions

Purpose: to assess in a randomised trial the effects of Epoietin Beta on transfusion requirements in patients with inflammatory breast cancer treated with sequential high dose Doxorubicine (D) + Cyclophosphamide (C) and Taxotere (TXT). Patients: 54 entered into the trial . Treatment consisted in 7 courses of D 75mg/m2 + C 6g/m2 in cycle1 (C1) and C2 every 3 weeks ; TXT 100mg/m2 in C3, C4 and C5 every 2 weeks; D 75mg/m2 + CPM 3g/m2 in C6 and C7 . PBSC were collected after C2 + G-CSF, and infused after C6 and C7 . G-CSF was administered on day 5 post transplant or following chemotherapy. Only 40 patients with HB level < 14 g/dl at baseline were randomised to Epoietin Beta 150 UI/kg 3 times per week, versus control. The primary end point was the reduction of RBC units transfused in treatment group (n = 21) / control (n = 19).

Results: 15 out of 21 patients in the treatment arm and 13 / 19 in the control, completed the study successfully; reasons for withdrawal were infectious toxicity and patient’s decision . Epoietin Beta therapy resulted in significant decrease in the rate of transfusion per chemotherapy cycle as reported in the table:

<table>
<thead>
<tr>
<th>N transfusion / cycle</th>
<th>control</th>
<th>epo</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>57.4%</td>
<td>72.2%</td>
<td>0.03</td>
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<tr>
<td>1-2</td>
<td>32.2%</td>
<td>26.2%</td>
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<tr>
<td>&gt;=3</td>
<td>10.4%</td>
<td>1.6%</td>
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Peripheral-blood progenitor-cell collection was increased in patients treated with G-CSF and EPO as compared to patients with G-CSF alone, but the difference was not significant. Conclusion: The result from this randomised trial suggest that Epoietin Beta decrease transfusion requirements in patients treated with sequential high dose chemotherapy.

**Persistence of anemia due to inadequate erythropoietin production after allogeneic stem cell transplantation**

M. Gregor, J. Passweg, A. Tichelli, A. Gratwohl (Basel, CH)

Background : Anemia due to inadequate erythropoietin (EPO) production is well known in the first weeks after allogeneic stem cell transplantation (SCT). Persistence of posttransplant anemia and delayed red cell reconstitution are associated with infections, graft-versus-host-disease (GVHD), renal insufficiency, or hemolysis. The role of EPO in these conditions is unclear. We report 3 patients with persisting anemia and inadequate low serum EPO responsive to therapy with recombinant human (rhu) EPO. Patients: Patient 1 : 38 year old female with Multiple Myeloma IgG kappa had a low haemoglobin (Hb) of 87g/l eight months after SCT. EPO was low with 12.7 IU/l (normal 12-23). RhuEPO at a dose of 3 x 10000 IU was started and later tapered to 4000 IU weekly. Hb remained stable around 135g/l on continuous rhuEPO therapy for one year.

Patient 2 : 36 year old male with AML M4 eo (inv 16) in 1st relapse developed anemia with Hb 90g/l 10 weeks after transplant. EPO was low with 12.0 IU/l. RhuEPO at a dose of 2 x 10000 IU weekly raised the Hb up to 150g/l. RhuEPO was tapered and finally discontinued. His Hb remained stable at 120g/l three months after stopping rhuEPO.

Patient 3 : 25 year old female with CML in chronic phase had stable anemia with Hb 93g/l ten months after SCT. EPO was inadequately low with 25.6 IU. Therapy with rhuEPO at a dose of 2 x 10000 weekly raised Hb to 110 g/l after 4 weeks of therapy. All patients received a peripheral blood precursor SCT either from an HLA-identical sibling (patients 1 and 2) or an HLA-identical unrelated donor (patient 3). They are in continuous complete remission for 7, 12 and 20 months. All patients developed steroid responsive acute GVHD grade II, which was inactive at the time of anemia and rhuEPO therapy. No patient had active infection or hemolysis. Renal function was slightly impaired in all patients (Creatinine clearance 50, 64 and 80 ml/min).

Conclusion : Inadequate EPO production can persist for months after allogeneic SCT. It might result from clinically inapparent GVHD or even minimal renal insufficiency. Patients with low EPO seem to profit from therapy with rhuEPO.

**Alpha interferon as treatment for leukemia/lymphoma relapse after nonmyeloablative transplantation (NST)**

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Relapse after NST is an important cause of treatment failure. The post-transplant chimeric state provides an opportunity to evaluate biologic therapies designed to enhance the immune mediated graft-vs-leukemia (GVL) effect by increasing the activity of residual donor lymphocytes. Alpha Interferon (IFN-a) enhances MHC antigens expression, which can increase occurrence of graft-vs-host disease (GVHD). It also has direct anti-proliferative activity against various malignancies. Induction of GVL effect by IFN-a...
was studied in 14 patients with leukemia/lymphoma relapsing following NST. IFN-a (Referon-A) 3 MU IU/day s.c was given to patients with early relapse following transplantation from mismatched or matched unrelated donors (MUD) whenever donor lymphocyte infusion (DLI) was not possible in 6 patients. In 13 patients with overt relapse, IFN-A was combined with DLI. Patients were off treatment if grade 3 toxicity and or >grade 2 GVHD occurred. Fourteen patients (9 females; 5 males), median age 27 years (range 7-57), 5 AML, 5 ALL, 2 CML, 1 Richter's syndrome and 1 NHL, were included. Following NST (fludarabine 30mg/m2x6; busulfan 4mg/Kgx2; 8 with ATG 10mg/Kgx4; 3 without ATG), with addition of TBI, 1 with additional Campath-1H in vivo; 11 received peripheral blood stem cells (PBSC) from HMC identical siblings, 2 from MUD and 1 from haploidentical sibling (with additional Cytoxan and CD34+ CD4- CD8- PBSC). Treatment was started in median time of 3 mo. post NST (range 1-30). Out of 14 patients, 6 patients developed AGVHD (5 patients grade II-III and 1 patient grade IV). Two out of 6 patients with acute GVHD received DLI in addition to IFN-a. Out of the 6 patients who developed GVHD, 4 had cutaneous skin lesions of disease that disappeared within 7 (range 7-30) days. Three of them maintain complete response for 16-24 mo. (median of 23). Overall, 6 patients, 3 treated with IFN-a alone and 3 with DLI plus IFN-a, are alive without evidence of disease, for 6-42 mo. (median 22). Two patients are alive with disease, 2 patients died due to sepsis and 5 due to disease progression. We conclude that IFN-a therapy alone and mostly with DLI can induce GVL effects and long-term remission following NST, but further investigations are needed.

P722
Flow cytometric determination of intracellular cytokines in monocytes and association with the development of acute graft-versus-host disease (aGVHD) and hepatic veno-occlusive disease (VOD) in allogeneic stem cell transplant (allo-SCT) recipients
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Inflammatory cytokines (TNF-a, IL-1, IL-6) play a crucial role in the development of aGVHD, and have also been implicated in the pathogenesis of VOD. In a prospective study, we employed flow cytometric (FC) detection of intracellular cytokines (ICs) to assess the production of cytokines by monocytes and investigate its association with the development of aGVHD and VOD in allo-SCT recipients. Sequential determinations of TNF-a and IL-1b in peripheral monocytes were performed on days 4 and 5. Of 6 patients, 4/6 had elevated TNF-a and IL-1b levels on days 4 or 5. Of the 2 patients without aGVHD, 1 had elevated TNF-a and IL-1b at days +4 and +5. Of the 3 patients with grade II-III aGVHD, 2 had elevated TNF-a and IL-1b at days +4 and +5. Of the 1 patient with grade IV aGVHD, 1 had elevated TNF-a and IL-1b at days +4 and +5. Similar results were found in the donors. Smaller proportion of patients with VOD had low producers of TNF-a and IL-1b compared to patients without VOD (15% vs. 30%) p=0.025, OR-8. In conclusion: It is an intriguing possibility that low production of TNF-alpha, TGF-beta, IL-10 and IL-6 has a protective role against development of HVOD, whereas the profile of the other cytokine production in our series did not seem to be associated with HVOD. Larger number of patients should be evaluated to confirm these results.

P724
Polyclonal expansion of primary human T-lymphocytes for retrovirolytically mediated gene transfer can lead to functional impairment and skewed distribution of memory and effector T-cell subsets

Retrovirolytically mediated gene transfer into primary human T-lymphocytes usually involves strong polyclonal stimulation before transduction. Although little is known about the functional profile of these cells after expansion, this is a critical aspect for all applications involving adoptive transfer of transduced T-cells into patients. Actually, several clinical trials with transduced T-cells have reported defective function in vivo. To explore the impact of polyclonal stimulation in T-cell immunocompetence, PBLs from donors were expanded in parallel with various doses of either PHA or immobilized anti-CD3 and anti-CD28 (3/28). The responses of 3/28-stimulated cells to third party stimulators of TNF-alpha, TGF-beta, IL-10 and IL-6. The majority of patients typed as homozygous low producers of TNF-alpha, (100% in both groups), high producers of TGF-beta both homozygous and heterozygous (67% vs. 77% in patients with VOD and without VOD, respectively), heterozygous intermediate producers of IL-10 (71% vs. 60%, respectively) and high producers of IL-6 both homozygous and heterozygous, (92% vs. 87%, respectively). Similar results were found in the donors. Smaller proportion of patients with HVOD were low producers of INF-gamma as compared to patients without HVOD (15% vs. 30%) p=0.025, OR-8. In conclusion: It is an intriguing possibility that low production of INF-gamma has a protective role against development of HVOD, whereas the profile of the other cytokine production in our series did not seem to be associated with HVOD. Larger number of patients should be evaluated to confirm these results.
(CCR7+/CD45RA+), central memory (CCR7+/CD45RA-), effector memory (CCR7-/CD45RA-) and effectors (CCR7-/CD45RA+). CCR7- subsets secrete IFNγ and express intracellular perforin. PHA-expansion leads to a rapidly skewed distribution of these T-cell subsets, with an average 6-fold increase of the central memory cells and a marked decrease of all the others. Conversely, cells from the same donors expanded with comparable levels of 3/28-stimulus retained their physiological profile to a larger extent. When stimulated with autologous dendritic cells pulsed with CMV or EBV-derived HLA-A2 peptides, PHA and 3/28-cells from HLA-A2 donors gave rise to comparable percentages of HLA-tetramer positive cells. However, tetramer-positive PHA-cells expressed lower levels of perforin, half the amount of IFNγ upon peptide-specific stimulation, and a lower percentage of effector cells when compared with their 3/28 counterparts. In conclusion, polyclonal expansion of T-cells with PHA skews their functional profile and impairs their immunocompetence in vitro compared to 3/28. This might contribute to a defective function of the transduced T-cells after their adoptive transfer in vivo.

P725

Bcl-2 antisense in the treatment of large cell anaplastic lymphoma with relapse after autologous hematopoietic cell transplantation – a case report

A. Chybicka, R. Chaber, J. Toporski (Wrocław, PL)

Mitochondrial protein bcl-2 is an important inhibitor of apoptosis. Blocking expression of bcl-2 gene by antisense oligonucleotides (AS-ODN) administration may result with lower production of bcl-2 protein what makes neoplastic cells more sensitive to chemotherapy. We used bcl-2-AS-ODN in the treatment of 11-years old boy with Anaplastic Large Cell Lymphoma, which underwent two autologous PBSCT. The diagnosis was made in March 1999. The patient was initially treated with LCAL BFM 93 protocol and due to partial response autologous PBSCT was performed (BEAM conditioning). The patient was in complete remission for 18 months and relapsed in May 2001. First relapse was treated according NHL BFM 95 for relapsed LCAL and was followed by the second auto-PBSCT in September 2000 with conditioning consisted of Busulfan, Vepesid, Cyclophosphamid. No remission was achieved. Palliative therapy was introduced in October 2000, (Vinblastin) and resulted with transient and short remissions. In the June and September 2001 bcl-2 antisense infusion was given and was combined with chemotherapy based on Topotecan and Vinblastin with good result - stable remission is observed for 20 weeks. We used bcl-2-AS-ODN in the treatment of 11-years old boy with Anaplastic Large Cell Lymphoma, which underwent two autologous PBSCT. The diagnosis was made in March 1999. The patient was initially treated with LCAL BFM 93 protocol and due to partial response autologous PBSCT was performed (BEAM conditioning). The patient was in complete remission for 18 months and relapsed in May 2001. First relapse was treated according NHL BFM 95 for relapsed LCAL and was followed by the second auto-PBSCT in September 2000 with conditioning consisted of Busulfan, Vepesid, Cyclophosphamid. No remission was achieved. Palliative therapy was introduced in October 2000, (Vinblastin) and resulted with transient and short remissions. In the June and September 2001 bcl-2 antisense infusion was given and was combined with chemotherapy based on Topotecan and Vinblastin with good result - stable remission is observed for 20 weeks. In conclusion, bcl-2 AS-ODN may be useful in the treatment of relapsed lymphomas after conventional chemotherapy and BMT.

P726

Efficient and durable gene marking of cord blood stem cells: comparison of two cytokine cocktails

S. Lucchi, L. Lazzari, L. Porrettì, R. Lopa, R. Ferone, P. Rebulla (Milan, it)

Gene marking of cord blood (CB) hematopoietic stem cells (HSCs) is crucial to investigate the role of HSCs in hematopoietic reconstitution after transplantation. We evaluated the efficiency of the infection of CB CD34+ cells by Gibbon Ape Leukemia Virus pseudotyped retroviral supernatant, containing the low affinity nerve growth factor receptor (LNGFR) marker gene, under the control of Moloney murine leukemia virus long terminal repeat (study 1; n=7). Moreover, we determined the maintenance of the gene marker in long term culture initiating cells (LTC-IC) present in the suspension of the infected cells (study 2). Isolated CD34+ cells from CB were prestimulated for 24 hours in a serum-free medium containing cocktail A (used in our previous studies to expand HSCs compartment): IL-6 (10 ng/ml), IL-11 (10 ng/ml), FL (50 ng/ml) and TPO (10 ng/ml) versus cocktail B (used by Thrasher et al, 1998): SCF (100 ng/ml), FL (100 ng/ml), IL-6 (20 ng/ml) and IL-3 (20 ng/ml). The cells were then infected on a retronectone coated plate previously loaded with retroviral supernatant. The infection was carried out in serum-free medium by exposing cells to retroviral supernatant containing the above cocktails for 3 times every 12 hours. Results of study 1: the median and range fold expansions of nucleated cells (NC) were 2.8 (2.1-8.8) and 6.8 (3.9-17.7) with cocktails A and B respectively. The median fold expansions of CD34+ cells were 2.6 (1.6-6.7) and 5.1 (3.5-12.4) with cocktails A and B respectively. The percentages of LNGFR positive NC were 59% (21-79) (A) and 77% (31-84.5) (B). The percentages of LNGFR positive CD34+ cells were 67% (23-86) (A) and 86% (34-97) (B). Differences were statistically significant by the Wilcoxon signed-rank sum test (p<0.05). Results of study 2 were as follows. By using a flow cytometry gate on live cells recovered after 4 weeks of the LTC-IC culture, we found 3.7% (0.7-14) CD34+ cells, of which 35.5% (25-95) were LNGFR+ with cocktail A; and 4.8% (1.1-11) CD34+ cells, of which 85.5% (37-98) were LNGFR+ with cocktail B. Differences did not reach statistical significance. Our data suggest that, probably due to IL-3, cocktail B shows about 10% higher efficiency in transducing HSCs.

Additional abstracts to this topic

Influence of post-transplant granulocyte-colony stimulating factor (G-CSF) administration on peritransplant morbidity in patients undergoing autologous stem cell transplantation (Asct)


Objective: To evaluate the effect of post-transplant G-CSF on the parameters of peritransplant morbidity.

Methods: Three sequential and consecutive cohorts of 20 patients each with hematologic malignancies and solid tumors received either post-transplant G-CSF at a dose of 5 μg/kg/day IV in the morning started on day 0, day 5, or no G-CSF. G-CSF was given in the morning started on day 0, day 5, or no G-CSF. G-CSF was given until the absolute neutrophil count was greater than 5 x 10⁹/L. Engraftment kinetics and other parameters of peritransplant morbidity such as transfusion and TPN requirements, fever, antibiotic administration as well as hospital stay were evaluated with univariate and multivariate analysis.

Results: Patients receiving post-transplant G-CSF starting on day 0 and 5 recovered granulocytes more rapidly than those not receiving post-transplant G-CSF (P=0.000 for ANC>0.5 and 1x10⁹/L). Post-transplant G-CSF was not significantly associated with more rapid platelet engraftment in the univariate and multivariate analysis. Post-transplant G-CSF starting on day 0 and 5 were significantly associated with a decreased duration of fever (P=0.002 and 0.001, respectively) and antibiotic administration (P=0.000 and 0.006,respectively) compared to reference group of without G-CSF. Post-transplant G-CSF were also significantly associated with a short hospital stay compared to reference group of without G-CSF (P=0.002 and 0.001, respectively) and antibiotic administration as well as hospital stay were evaluated with univariate and multivariate analysis.

Conclusion: Post-transplant G-CSF is associated with a faster granulocyte recovery and shortened duration of hospitalization, fever as well as nonprophylactic antibiotic administration in patients who receive ASCT. This study also showed that post-transplant G-CSF on day 5 may be as effective as day 0 administration on the clinical outcome. Day 5 administration instead of day 0 may be an economical approach in the routine clinical practice in this cost-conscious era.
Serum soluble interleukin-2 receptor levels as predictive parameter of acute graft-versus-host disease
C. De Souza, J. Visentainer, S. Lieber, M. Favarelli, A. Vigonito, F. Aranha, K. Eid, G. Oliveira, E. Miranda (Campinas, Paraná, BR)

Serial concentrations of soluble interleukin-2 receptor (sIL-2R) were analysed in 172 sera of 13 patients that had received full match allogeneic bone marrow transplantation from sibling donors. The aim of this study was to evaluate the usefulness of sIL-2R as a predictive parameter for acute GVHD. Serum sIL-2R concentrations were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) prior to BMT, and weekly after transplantation. Preconditioning regimens did not appear to affect the sIL-2R levels. A significant correlation between sIL-2R and the occurrence of acute GVHD may be shown. The sIL-2R levels markedly increased at the engraftment period in 5 patients who developed aGVHD. Only one patient presented aGVHD without increasing of sIL-2R level. The mean serum sIL-2R concentration began to increase in the second week following transplantation and reached a peak concentration of 1357.83 ± 887.09 pg/ml (Mean ± SD) on day +18. These data were confirmed using independent sample T-test (P=0.008). This change was not seen in the 7 patients without aGVHD. Therefore, the sIL-2R levels at the engraftment period appear to be useful in order to predict the development of aGVHD. Further controlled studies using sIL-2R between the 2nd to 4th weeks could help in monitoring of aGVHD.

The value of TNF-alpha and IL-10 gene polymorphisms in the incidence and severity of graft-versus-host disease

Various mechanisms have been proposed to explain the complication of stem cell transplantation (SCT) but these are diverse and complex. The relationship between cytokine concentrations and SCT related complications have been studied in SCT patients.

Vascular endothelial damage and hyper cytokine involved in some ARDS like respiratory disorders.

We investigated whether different cytokine gene polymorphism is associated with severe acute GVHD and early mortality in HLA matched related HSCT. The subjects were 163 consecutive pts who underwent allo-SCT between 1995-2000, patients [adult: 128 age range 18-42 years, child: age, range, 1-17 years] and their donor. Samples were the pre transplant stored DNA.TNF alpha (-308 g/a) and IL-10 (-1082 g/c) polymorphism was detected. We also have failed to demonstrate any association of either TNF-alpha or IL-10 polymorphisms with these genotypes like other published report elsewhere. Until more extensive studies disprove these findings, it seems premature to use cytokine genotyping to predict transplant outcome.

18. Graft Engineering

P727
Relation between CFU-GM and CD34+ doses and engraftment
N. Stecová, E. Tóthová, J. Rosocha, A. Elbertová, M. Frièová, T. Guman, Š. Raffaič (Kosice, SK)

Autologous Peripheral Stem Cells Transplantation (AP SCT) is standard therapeutic approach in the treatment of many haematological malignancies. It’s success depends on many factors, one of the most important is quality of autologous graft. The quality of graft is most frequent -ly evaluated by CD34+ cells count determination and by CFU-GM progenitor cells cultivation in ap-heresis product. Engraftment time, haematologic reconstitution and frequency of infective compliations depends on CD34+ and CFU-GM reinfused cells count. Minimal recommended doses in auto-graft are 2.0 - 2.5.10/ 6/kg body weight of CD34+ cells and 2 - 4.10/4/kg of CFU-GM colonies . We have studied 30 patients with different haematological malignanies, who received autologous peripheral stem cell transplantation since November 1999 till December 2001: 8 AL (CR1),12 MM(CR1, PR1),8 NHL (CR1,PR1,2HD (CR2,PR2). Ratio M/F was 13/17, patient age ranged from 19-65 years (median 42). Transplant procedures were per-formed at mean time of 12 months after achieving CR or PR. PBSCs was obtained after chemotherapy with HD- CY (22) or HAM (8) followed by G-CSF. Patient was conditioned with BuCy2 (7), BuMel(1), HD-Melphalan (12) or BEAM (10) according to diagnosis. Median CFU-GM dose was 114,1.10/4/kg (range 6,90-275,40) and 3,8.10/6/kg CD 34 positive cells (range 1,22-10,5).

No significant correlation was found between doses of progenitor cells and hemopoietic recovery time or infectious coplications in our patients. Engraftment depended on diagnosis and conditioning regimen.

P728
CD 34+ enriched - CD 19 depleted autologous peripheral blood stem cell transplantation for chronic lymphoproliferative disorders: High purging efficiency but increased risk of severe infections

Introduction: Purging procedures have been developed to decrease the incidence of relapse after autologous stem cell transplantation. We used a ‘positive’ (CD34) and ‘negative’ (CD19) double selection procedure to improve the efficacy of ‘single purging’ of hematopoietic harvests in poor prognosis lymphoproliferative disorders.

Material and methods: The inclusion criteria were patients under 65 years old with a diagnosis of B-CLL in stage C, or B with poor prognosis factors; follicular lymphoma after a non-localized relapse (or in first response with a 4-5 IPI score) and mantle cell lymphoma, diffuse or blastic variants, in first or subsequent response. Patients with chemoresistant disease, or in partial remission with a bone marrow neoplastic infiltration >50% were excluded. Mobilization regimens were ifosfamide-VP16 or CY 3g/m2, both adding G-CSF. Patients were conditioned with CY-TBI or BEAC. All patients included in the study had a positive molecular marker of their disease. Minimal residual disease (MRD) was studied by flow cytometry and PCR techniques during the purging procedure and after transplantation.

Results: Twenty-six patients fulfilled entry criteria. Median age of patients was 50 years (range: 33-66), 17 were male and 9 female. Thirteen (50%) of the patients mobilized an adequate number of CD34+ cells (>3X10E6/kg) to proceed with the double selection protocol. Twelve of the 13 harvests became PCR negative after purging. Ten patients were grafted with the selected products and all but one engrafted without delay. After a median follow-up of 30 months, 2 of 10 patients suffered a molecular relapse at 7 and 19 months, respectively. The earlier relapse was observed in the patient who received a MRD+ product. Only one patient has experienced a clinical relapse. Three patients died due to obliterans bronchiolitis, pneumococcal sepsis and septic shock of unknown origin, respectively, and three others presented life-threatening infections.

Conclusions: Therefore, CD34+/CD19 positive/negative selection is an effective purging approach in patients with chronic lymphoproliferative disorders. This favorable effect is, however, counterbalanced by the high frequency of life-threatening infections.
Persistence of ATG levels in patients after stem cell transplantation


Rabbit anti-thymocyte globulin (rATG) is currently used as an immunosuppressive agent in allotransplants as a graft-versus-host disease prophylaxis. We wanted to analyse how long ATG levels are detectable in patients after a stem cell transplantation. In ex vivo experiments rATG binds 100 percent of the leukocytes up to a concentration of 0.8µg/ml. Erythrocytes were not bound over 80 percent by rATG even at higher concentrations (up to 200µg/ml) of ATG and at a concentration of 0.8µg/ml showed almost no binding of rATG. We measured the concentration of rabbit IgG, the development of anti-ATG-antibodies by ELISA, and the amount of rATG by flow cytometry analysis in the serum of 4 Patients who received 3 times 20mg per Kg body weight (3 Patients) or 3 times 30mg per Kg body weight prior to stem cell transplantation. We used rATG from Fresenius, Germany which uses Jurkat T cells as immunogens for the preparation of ATG. Serum level of rabbit IgG began to decrease between day 21 and 48 after transplantation while antibodies against ATG were found to raise between day 12 and 22 after transplantation. rATG in serum was measured by FACS-analysis after blocking of the Fc receptor. Levels of rATG in serum were distinguishable down to 0.8 µg/ml.

Blood taken from a patient who had a take of the donor bone marrow 16 days after transplantation showed still an average binding of 70 percent of the lymphocytes. Approximately 90 percent of the CD3+, CD4+, CD6+, CD8+ had detectable rATG on their surface while only 82 % of the NK cells had a positive signal for rATG.

Collection efficiencies of MNC subpopulations during autologous CD34+ peripheral blood progenitor cell (PBPC) harvests in small children and adolescents

V. Witt, G. Fischmeister, D. Schärer, D. Printz, U. Poetschger, G. Fritsch, H. Gadner (Vienna, A)

Purpose: There is increasing demand for mononuclear cell (MNC) harvests not only for PBPC but also for immune therapies using dendritic cells and donor lymphocytes. We determined the collection efficiencies (CE) of various MNC sub-populations during stem cell harvests using a Fenwal CS3000 Plus Omnix system in small children and adolescents.

Patients and methods: The cell content of 151 leukapheresis products (LP) was prospectively evaluated in 48 pretreated patients with solid tumors and hematological malignancies. The median age was 13 years (range 0.8-28) and the median bodyweight (BW) 44 kg (range 9 – 92). Depending on the BW of the patients, the media used for priming were saline (SP) in 97, human albumin (HA) in 10, and packed red blood cells (RBC) in 44 apheresis procedures (BP). Results: The major nucleated cell (NC) fractions collected were monocytes (52% of NC) and CD3+ T cells (26%). The median cell yield for monocytes was 322 x 106/kg (range 89-874) representing a CE of 53%. The median number of CD3+ T cells was 84 x 106/kg (range 5.6-380; CE = 74%). CD34+ cells represented a very small cell fraction of the LP (1.4% of NC), with a median yield of 4.3 106/kg (range 0.2–87) and a CE of 62%. The cell yield of various MNCs was significantly correlated with the cell count in the peripheral blood (PB) and with the blood volume processed (ANOVA, p.<0.0001). No influence on the CE was observed for the priming procedure, the patients’ age or sex, or the other adaptations used in the harvesting protocol. Conclusion: The Fenwal CS3000 Plus OmnIX system with the stem cell program and the described adaptations is also predictably useful for harvest of monocytes or lymphocytes of pediatric patients. We present regression equations that predict the cell yield of various MNC subpopulations in apheresis products.
Superior in patients receiving mobilized peripheral blood compared undergoing transplantation for hematologic malignancies. Recent mobilized peripheral blood (PB) is being used with increasing microparticles transplantation (PBSCT) using antibody coated high density CD8+ T cell depleted allogeneic peripheral stem cell.

P733 Positive enrichment of CD34+ cells with the ClinMACS device: Effect of column sizes on separation results
M. Schimm, P. Lang, S. Kucj, J. Greil, D. Niethammer (Tuebingen, D)
The ClinMACS device has been proven for enrichment of CD34+ cells from apheresis products with both high recovery of cells and profound depletion of T-cells. We investigated the performance of the large tubing set and column versus the small tubing set and column in regard on purity, depletion of T-cells and recovery of cells.
A total of 114 separations were performed between May 2000 and November 2001; 62 separation were performed with the larger set for 52 separations, the smaller set was used. The median number of cells prior to separation was 2.88 x 10^10 (range 0.20- 6.90) for the smaller set and 7.02 x 10^10 (range 1.15- 2055) for the larger set containing 7.8% (range 0.25 - 6.40) and 0.65% (range 0.11 - 10.10) of CD34+ cells. Separations were performed immediately after collection or, more frequently, after overnight storage. After separation, a median cell number of 151 x 10^6 (range 23 - 960) and the median purity was 97.7% (range5.9 - 98.2) mononuclear cells compared with 47.7% (range 5.9 - 98.2) mononuclear cells compared with 47.7% (range 5.9 - 98.2) mononuclear cells compared with 47.7% (range 5.9 - 98.2) mononuclear cells compared with 47.7% (range 5.9 - 98.2) mononuclear cells compared with 47.7% (range 5.9 - 98.2) mononuclear cells.

In conclusion, this stem cell processing method provides a very good stem cell recovery and purity, extensive T cell depletion and sufficient B cell depletion to ensure sustained engraftment, no GvHD and no EBV-LPD. Finally our study shows the CD34+ cell dose plays a crucial role in platelet recovery.

P734 CD8+ T cell depleted allogeneic peripheral stem cell transplantation (PBSCT) using antibody coated high density microparticles
R. Sollifer, E. Alyea, B. Pankh, S. Lee, J. Antin (Boston, USA)
Mobilized peripheral blood (PB) is being used with increasing frequency as a source for allogeneic stem cells for patients undergoing transplantation for hematologic malignancies. Recent studies suggest that engraftment and perhaps overall survival are superior in patients receiving mobilized peripheral blood compared with bone marrow. However, graft-versus-host disease (GVHD), both acute and chronic, continue to be major problems. We have recently demonstrated that depletion of CD8+ T cells from donor lymphocyte infusions (DLI) can reduce GVHD without compromising anti-leukemic efficacy or conversion to complete donor hematopoietic chimerism. Based in part on these results, we have begun to evaluate CD8+ T cell depletion as primary GVHD prophylaxis in patients undergoing allogeneic PBSCT. CD8 depletion is being performed with anti-CD8 IgG monoclonal antibody high density microparticles (Eligix CD8-HDM). Nine patients (7M/2F, median age 49 years) with AML (3), MDS (2), NHL (2), ALL (1), and CLL (1) have thus far been treated. All patients received primed HLA identity high dose CD8 depletion of allogeneic PBSG. Conditioning consists of cyclophosphamide (60 mg/kg) and fractionated TBI (1400 cGy Total). All patients received tacrolimus as the sole pharmacologic agent for GVHD prophylaxis. G-CSF was initiated on Day +1 and continued until engraftment. Patients received a median of 5.9 x 10^6 CD34+ cells/kg. The CD8-HDM depletion process resulted in a 77% recovery of CD34+ cells. The graft manipulation process took approximately 2 hours to complete. Patients received a median of 8.0 x 10^6 CD3+ cells/kg. The CD8-HDM depletion process resulted in a > 0.5% CD8+ cells by FACS analysis. Engraftment has been rapid. The median time to reach 500 neutrophils/ml was 9 (range, 9-11) days. Platelet transfusion independence with a count > 20,000/ml occurred at 10 (9-28) days. There have been 2 patients with grade 2 and one patient with grade 1 acute GVHD. There have been no relapses or deaths with short follow-up. CD8 depletion of allogeneic PBSG can be performed simply and effectively with the CD8-HDM system without impairing engraftment. Additional follow-up is needed to assess the effect of such graft manipulation on GVHD and relapse.

P735 Comparing two different programms for collecting peripheral blood stem cells in healthy donors with a continuous flow cell separator
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Granulocyte-Colony Stimulating Factor (rhG-CSF) mobilised haematopoietic stem cells in peripheral blood (PBSC) are increasingly being used as a source of marrow repopulating cells in allogeneic stem cell transplantation. The number of collected PBSC may be linked with the type of cell separator and the harvesting program. A non randomised study compared PBSG harvests from the COBE Spectra version 6.1 AutoPBSC and the version 5.1 LRS MNC (mononuclear cell). 526 standard leukaphereses were performed in 135 related donors, primed daily with 12 or 16 micrograms/kg subcutaneously rhG-CSF (Lenograstim; rhG-CSF). Leukaphereses started after 4 days rhG-CSF administration. All donors underwent 3 or 4 collections on consecutive days. We performed 323 leukapheresis with MNC and 203 with AutoPBSC. The median processed blood volume was 9028ml (range 4525-14290) for MNC procedure and 8668 ml (range 6000-14,000) for the Auto. This slight difference was not statistically significant. Blood flow rate was 60-70ml/min. Median duration time was significantly longer with the AutoPBSC: 184± 22.3 min vs 162± 26.7 min. with the MNC procedure (p< 0.001). The median yield of nucleated cells was 45.50 x 10^9 (range 8.85-84.00) with the MNC and 33.80x10^9 (range 14.19-66.20) with the AutoPBSC (p<0.000). The components collected with the MNC contained 47.7% (range 5.9-98.2) mononuclear cells compared with the 52.5% (range 1.6-91.1) with AutoPBSC (p=ns). The number of CD34+ cells in the product was 366.87 x 10^6 (range 37.7- 897.1) for MNC and 286.73x10^6 (range 42.9-724.9) for AutoPBSC (p<0.001). The collection efficiency was greater for MNC, being 50.8% compared with the 44.6% for AutoPBSC (p<0.014). The number of CD34+ cells//litre of whole blood processed was 36.8±106/L (range 3.84-104.08) with MNC and 29.2±106/L (range 4.12-76.76) with Auto PBSG (p<0.004). Contaminating fractions in the product were: Ht 1.2% (range 0.4-2.4) for MNC, Ht 1.1% (range 0.3-2.1) for AutoPBSG (p<0.006).
platelets 121.9/mm (range 1.3-334) for MNC vs 95.6/mm range (19-327) with Auto (p<0.034). Granulocyte contamination was not significant in either: 50.9% (range 23.5-89.8) with the MNC and 48.2% (range 20.4-91.5) with Auto (p 0.35).

In conclusion, the AutoPBSC procedure is less efficient and more time consuming than the MNC but simplifies PBSC collection; the product is less contaminated by platelets and erythrocytes.

P736
Ex vivo manipulation of hematopoietic stem cells - purging with mafosfamide and amifostine protection of progenitor cells
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Various cytotoxic agents are used to eliminate possible contaminating tumour cells in autologous grafts in pharmacological purging studies. However, these agents are toxic to haematopoietic progenitors too, and ex vivo purging can diminish the quality of graft. From that reason, attempts are made to selectively protect the haematopoietic progenitors.

Objectives: We have evaluated the effects of amifostine (Ethyol) pretreatment on human haematopoietic progenitors during ex-vivo purging with mafosfamide and cytotoxic effects of mafosfamide on selected neuroblastoma and Ewing’s sarcoma tumour cell lines. Mafosfamide in vitro cytotoxic experiments, samples of bone marrow and peripheral blood progenitor cells at a final concentration of 2x107 MNC/ml were treated with amifostine alone (4 mg/ml, at 37°C for 15 minutes), mafosfamide alone (100 µg/ml, at 37°C for 30 minutes ) and mafosfamide with amifostine pretreatment. Changes in nucleated cell count, CD 34+ cell count and CFU-GM and BFU-E recovery were studied. Tumor cell lines (NB, ES) were treated under the same conditions described and neoplastic cell viability was than tested by MTT test.

Results: Mafosfamide treatment led to significant reduction of CFU-GM and BFU-E recovery in haematopoietic progenitor cells, while changes in NC and CD 34+ cell counts were minimal. We were unable to demonstrate significant protective action of amifostine pretreatment haematopoietic progenitors (non significant trend for BFU-E and CFU-GM protection p<0.1 NS, t-test, was found). Amifostine did not interfere with mafosfamide antitumour activity and tumour cell viability was decreased to 20-30% of controls after mafosfamide treatment.

Conclusion: Amifostine do not interfere with mafosfamide antitumour activity, but only non-significant protective action of amifostine on haematopoietic progenitors could be demonstrated during ex vivo treatment at a concentration of 100 µg/ml of mafosfamide. Supported by grant IGA MZ CR No: NC 5329-3 and Ministry of Education grant No. 111300005

P737
Positive selection of CD34+ progenitor cells from allogeneic peripheral blood by the use of a clinical-scale magnetic-activated cell separation device
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The aim of the present study was to evaluate the performance of a magnetic-activated cell separation device (CliniMACS, Miltenyi Biotec) in peripheral blood progenitor cell (PBPC) grafts from HLA-identical normal donors for alloengraftment transplantation and to analyze the variables influencing CD34+ cell enrichment and recovery and the efficacy of non-target cell depletion as well as the capacity of allogeneic CD34+ selected cells for supporting hematological reconstitution.

Thirty PBPC from twenty-one healthy donors were positively selected for CD34+ cells by the use of a clinical, normal-scale ClinMACS device. Leukapheresis products (LKS) had a median value (range) of total nuclear cells (TNCs) and percentage of CD34+ cells of 6.16x10E10 (2.27-9.86) and 0.75% (0.1-2.1), respectively. After selection, median percentage of CD34+ cells in the CD34+ cell-enriched component (purity) was 96% (58-99.5). A median number of 2.5 x 10E8 total CD34+ cells were recovered after selection (range: 0.11-10), which represents a recovery of 64% (35-109) of CD34+ cells. Multiple regression analysis showed that the ratio of magnetic particles used per CD34+ cell processed had the highest predictive impact on the CD34+ cell yield (R² = 0.27; p = 0.003) and total amount of CD34+ cell in the starting component on the purity (R² = 0.79; p < 0.005). Logarithms of T and B-cell depletion had median values of 5 and 3.2, respectively. The median number of CD34+ cells/kg, CFU-GM/kg and CD3+ cells/kg infused to the patients was 5.9x10E6/kg (1.5-13.9), 1.5x10E5/kg (0.11-17) and 0.04x10E5/kg (0.02-2.2), respectively. All patients engrafted with median times for recovery >0.5x10E9/L of granulocytes and > 20x10E9/L untransfused platelet count of 14 days (10-28) and 14 days (8-83), respectively. One patient developed secondary graft failure and was successfully rescued by an unmanipulated PBPC graft from the same donor. These data indicate that normal scale ClinMACS kit can be used in a large range of TNC and CD34+ cell load and achieves high degree of purity, yield and T cell depletion from allogeneic PBPC components.

P738
Depletion of CD8+ T-cell and recovery of CD34+ cells from mobilized stem cell apheresis after processing with CD8-HDM in conjunction with the ELIGIX(tm) cell separation system
Q. Chang, R. Schmittling, H. Houde, H. Zhu, D. Cook, R. Monroy (Medford, USA)

The depletion of CD8+ T cells from mobilized stem cell (SC) apheresis collections was evaluated pre-clinically using CD8-HDM Cell Depletion System, as a potential means to reduce GVHD in matched-related alloengraftment transplantation. The depletion of CD8+ T cells and recovery of CD34+ cells was assessed after 2 and 3 depletion cycles. CD8-HDM consists of an anti-CD8 monoclonal antibody conjugated to high density microparticles (HDM). SC collections obtained after overnight shipment at 2-8°C were washed and re-suspended in 1% HSA-saline, combined with CD8-HDM in a Depletion Chamber, and rotated end over end for 10 minutes. HDM and captured CD8+ cells were allowed to settle by gravity for 5 minutes. The cells remaining in suspension were again processed in a similar fashion for a total of 3 depletion cycles. SC collections (N=7) contained an average of 3.6 x 10E10 cells (range: 2.0 to 5.5 x 10E10). Aliquots removed after 2 and 3 depletion cycles were assessed for cell counts, viability, cell phenotype and CFU-GM. CD8+ T cell depletion, as assessed by flow cytometry, showed a median 1.7 logs (range: 1.1 to >2.7 logs) and >2.2 logs depletion (range: 1.6 to >2.7 logs) after 2 and 3 cycles, respectively. Median CD34+ cell recovery was 83.7% (range: 79% to 93%) and 73.3% (range: 65% to 91%) for 2 and 3 cycles, respectively. CD4+ T cell recovery was >90% after each cycle and CFU-GM recovery measured after 3 cycles was 92.5%. These studies demonstrated that in order to achieve the most stringent depletion of CD8+ cells a third cycle was required but higher CD34+ cell recoveries were achieved after 2 cycles. Additional lab scale studies have shown comparable CD8+ cell depletions and CD34+ cell recoveries over an equivalent total nucleated cell range of 2 - 10 x 10E10 cells using CD8-HDM. Thus, CD8-HDM used in conjunction with the Eligix(superscript:TM) Cell Separation System is suitable for purging CD8+ T cells from mobilized SC collections up to 1 x 10E11. The efficacy of this system for reducing GVHD in allogeneic SC transplantation is being tested.
The performance of the ELIGIX(tm) T-cell-DLI cell separation system in a simulated clinical setting for processing CD8 depleted DLI

H. Houde, H. Zhu, R. Schmittling, Q. Chang, D. Cook, R. Monroy (Medford, USA)

Donor leukocyte infusions (DLI) depleted of CD8+ T cells have been shown to mediate a graft-versus-leukemia effect with a reduced incidence of graft-versus-host disease relative to unmanipulated DLI (Aylea et al, Blood 91: 3671). We have previously demonstrated a prototype method to deplete CD8+ T cells from apheresis collections using CD8-HDM, which consists of an anti-CD8 monoclonal antibody conjugated to high density microparticles. The CD8-HDM method was developed to replace cumbersome methods of CD8+ T cell depletion using antibody and complement. An Eligix TCell-DLI Cell Separation System incorporating the CD8-HDM has been developed which contains a disposables kit, two accessory bench-top devices for clinical implementation of the HDM depletion procedure and a process optimized to purify >95% CD8+ cells in only two depletion cycles. To assess the performance of this System, apheresis products were obtained from 22 normal volunteers and some products with high cell numbers were split into two parts, such that 34 depletion processes were performed in a simulated clinical setting. The initial number of processed nucleated cells ranged from 0.2 to 2.7 x 10^10 cells, and all processes were performed aseptically in a biological safety cabinet. The median depletion of total CD8+ T cells from these products as measured by flow cytometry was 1.7 logs (range: 1.4 - 3.5 logs). The depletion of 2 subpopulations of CD8+ T cells, CD8+ bright and CD8+ dim cells, was also documented. The CD8+bright T cells, representing ~95% of the CD8+ cells, were depleted by a median of 2.2 logs (range: 1.7 ? 3.4 logs) where depletions were frequently below the limits of the assay. In contrast, the CD8+dim T cells were only reduced by a median of 65%. Thus, the residual CD8+ T cells remaining after processing were CD8+dim cells. CD8+CD56+ cells were also depleted to a median of 11.5%. The CD4+ T cell recovery was highly reproducible with a mean of 88% (SD 6%) and a range of 76% to 99.8%. The percent recovery of CD4+ cells was consistent over the entire range of cells entered into the process. Process times were less than 2 hours. The Eligix (superscript: TM) TCell-DLI Cell Separation System represents an improved method both in processing time and purging efficacy and is appropriate for use in clinical trials for patients requiring DLI therapy.

P741
Preserved colony formation capacity of hematopoietic progenitor cells after repeated freezing and thawing

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Stem cell harvests are routinely frozen in DMSO and rapidly thawed and infused in order to avoid toxic effects of DMSO on stem cells after thawing. We have been unable to find information about the effect of thawing and refreezing the cells. Such information is vital for proper management of accidents resulting in thawing of the cells. Methods: We tested ten peripheral stem cell harvests that were frozen in 10% DMSO at a programmed rate (CRYO MED) and stored at -150° C until thawing in a water bath at 37° C. The number of cells were counted in a Bürker chamber after Türk staining, and viability was assessed after trypan blue staining. Cells were seeded on plates with methylcellulose containing growth factors (GF H4434, StemCell Technologies). Colonies were counted after incubation in humidified atmosphere at 37° C for 14 days. The effect of temperature after thawing was tested by estimation of cell number, viability, and colony forming capacity 10, 30, 60, and 120 minutes after thawing. The cells were kept in 10% of DMSO at 0°, 25°, or 37° C. The effect of temperature after thawing was tested by diluting the cell suspension with phys. saline to 2% DMSO immediately after thawing or washing with phys. saline, and resuspension in phys. saline to 0% DMSO. The procedures were performed on ice at 0° C. Tests were done 10, 30, 60, and 120 minutes after thawing. The effect of repeated freezing. The stem cell harvest was thawed and immediately placed on ice. After 30 minutes on ice the harvest was frozen again and stored at -150° C until the test was repeated. Three freezing and thawing procedures were done and tests repeated after each thawing. Results: The number of viable cells decreased significantly with each freezing procedure. After thawing, 10% DMSO had a negative effect on CFC at 20° C and 37° C, but not at 0° C. However, CFC was only modestly reduced even after three freezing procedures in samples stored at 0°C after each thawing. Conclusion: The colony forming capacity of hematopoietic progenitor cells is preserved even after repeated freezing procedures. Hematopoietic progenitor cells are vulnerable to the toxic effects of DMSO at room temperature, and should be stored on ice. Thawed cells may be refrozen.

P740
Effect of red blood cell content on progenitor function after cryopreservation of cord blood buffy-coat products

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Introduction: CB volume reduction is necessary in order to reduce storage space and to avoid the use of high cryoprotectant volumes (DMSO). Different strategies have been proposed in CB banking, as RBC sedimentation (HES) and automatic buffy-coat collection (by means of different available devices: Optipress(R), etc).

Objective: To analyse the cryopreservation of products after thawing and refreezing the cells. Such information is vital for proper management of accidents resulting in thawing of the cells. Methods: We tested ten peripheral stem cell harvests that were frozen in 10% DMSO and rapidly thawed and infused in order to avoid toxic effects of DMSO on stem cells after thawing. We have been unable to find information about the effect of thawing and refreezing the cells. Such information is vital for proper management of accidents resulting in thawing of the cells. Methods: We tested ten peripheral stem cell harvests that were frozen in 10% DMSO at a programmed rate (CRYO MED) and stored at -150° C until thawing in a water bath at 37° C. The number of cells were counted in a Bürker chamber after Türk staining, and viability was assessed after trypan blue staining. Cells were seeded on plates with methylcellulose containing growth factors (GF H4434, StemCell Technologies). Colonies were counted after incubation in humidified atmosphere at 37° C for 14 days. The effect of temperature after thawing was tested by estimation of cell number, viability, and colony forming capacity 10, 30, 60, and 120 minutes after thawing. The cells were kept in 10% of DMSO at 0°, 25°, or 37° C. The effect of temperature after thawing was tested by diluting the cell suspension with phys. saline to 2% DMSO immediately after thawing or washing with phys. saline, and resuspension in phys. saline to 0% DMSO. The procedures were performed on ice at 0° C. Tests were done 10, 30, 60, and 120 minutes after thawing. The effect of repeated freezing. The stem cell harvest was thawed and immediately placed on ice. After 30 minutes on ice the harvest was frozen again and stored at -150° C until the test was repeated. Three freezing and thawing procedures were done and tests repeated after each thawing. Results: The number of viable cells decreased significantly with each freezing procedure. After thawing, 10% DMSO had a negative effect on CFC at 20° C and 37° C, but not at 0° C. However, CFC was only modestly reduced even after three freezing procedures in samples stored at 0°C after each thawing. Conclusion: The colony forming capacity of hematopoietic progenitor cells is preserved even after repeated freezing procedures. Hematopoietic progenitor cells are vulnerable to the toxic effects of DMSO at room temperature, and should be stored on ice. Thawed cells may be refrozen.
Clots in cryopreserved autologous peripheral blood stem cell concentrates (PBsc) at the time of thawing - Possible causes and prophylactic strategies

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Objectives: The formation of clots is a rare but serious problem at the time of thawing of PBSC and may have negative influence on the affected patients. Our effort was to find out influences on the formation of clots and to implement prophylactic strategies.

Methods: In a retrospective study we first evaluated the risk rate of clotting in a two year observation period. We looked at correlation with leucocyte concentration (cNC), leucapheresis conditions, diagnosis of the patients and duration of transfusion. The clinical reactions and hematological engraftment of the concerned patients were recorded.

Results: In 195 bags retransfused we observed six clotting incidents (3.1%). The diagnoses of the patients were NHL (3), myeloma (2) and AML (1). Side-effects during and after the transfusion were neurological signs and dyspnea (1), temporary renal insufficiency (1), none (4). Leucocyte engraftment was not affected (median on day 10 (9-12)) but platelet engraftment showed a tendency of delay (median day 15 (11-50) as compared to day 11 (9-17), control group). The median cNC of the PBSC was 331 (114-661) x 10^9/l (control group: 215 (123-601)), not significant. Two cell separators and three software programs were involved with no risk difference. The ACD-A (acid-citrate-dextrose)-ratio ranged from 1:11 to 1:22 (with addition of heparin for the latter). In cases the duration of transfusion was more than 10 minutes, in the other three less than ten minutes. After addition of 10% ACD-A to each thawed PBSC bag we did not see any clot formation in 50 transplantations of 71 bags retransfused independently of all factors mentioned above.

Conclusions: The causes of clotting are in most cases unclear. Activated monocytes, disintegrated granulocytes, cNC, lack of ACD-A at the time of collection as well as DMSO have been accused for this phenomenon. We neither could find one singular cause for clotting, so our strategy is wide-ranging: transfusion time less than ten minutes, limitation of cNC below 300 x 10^9/l, substitution of ACD-A in case of reducing the ACD-A-ratio at the end of apheresis and addition of 10% of volume ACD-A to the bags immediately after thawing. In this way we could improve the safety of retransfusion of cryopreserved and thawed PBSC.

P743

Ex vivo expansion of primitive cord blood cells in the presence of thrombopoietin (TPO) and erythropoietin (EPO)

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Cord blood is considered to be an easily accessible source of primitive and committed haemopoietic cells and represents an alternative to PBSC and bone marrow cell source for transplantation. Clinical experience has shown that haemopoietic recovery after cord blood transplantation is delayed compared to peripheral blood and bone marrow transplants. In addition the small volume of cord blood grafts limits their use in young children and low body weight adults. The aim of the study was the evaluation of the capability of CD34+ cord blood cells for ex vivo proliferation. The material consisted of mononuclear cells (MNCs) from 25 cord blood samples from normal full term deliveries isolated on Ficoll_Hypaque and CD34+ cells collected following immunomagnetic separation. Ex vivo expansion of MNCs, CD34+, CD34+/338- and CFU-GM were studied after a 7- day liquid culture in the presence of SCF (50 ng/ml), IL-3 (20 ng/ml), IL-6 (20 ng/ml), and either EPO (4IU/ml) or TPO (100 ng/ml). Mean CD34+ cell yield was 61.9 % and the mean CD34+ population purity was 83.2%. The results showed 64, 48.5, 8-fold expansion with TPO and 78, 37, 3.5-fold expansion with EPO for MNCs, CD34+ cells and CFU-GM respectively. Finally, 16-fold expansion was observed in the CD34+38- fraction. A positive linear correlation was found between the expanded CD34+ cells and the number of CFU-GM colonies.

These data suggest that EPO is more efficient for the ex vivo expansion of cells capable for short-term haemopoiesis although the presence of TPO in the growth factors' combination is also adequate for the expansion of hematopoietic progenitors. The combination of the expanded hematopoietic cord blood cells with the unmanipulated ones could probably overcome the difficulties of late engraftment and problems of quantitative insufficiency of cord blood grafts.

P744

Purification of leukocyte subsets for quantification of mixed chimerism: comparison of two different selection techniques

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The degree of mixed chimerism (MC) is important for an early diagnosis of graft rejection or disease relapse in patients after allogeneic stem cell transplantation (allo-SCT). Besides monitoring bone marrow (BM) and peripheral blood (PB) samples it seems necessary to detect the engraftment of specific cell populations, e.g. T-cells (CD3+), T-helper cells (CD3+/CD4+), cytotoxic-T cells (CD3+/CD8+), monocytes (CD14+), NK-cells (CD3-/CD56+) and granulocytes. After stem cell transplantation, however the purity of subset isolations is lower than in blood samples from healthy donors.

We compared the immunomagnetical cell isolation system MACS (Miltenyi Biotec) with a new lymphoid cell enrichment kit, RosetteSep (StemCell Technologies). This new kit crosslinks the unwanted cells with antibodies to form rosettes with red blood cells. The different leucocyte subsets can be separated from the rosettes by density gradient centrifugation.

The subsets were isolated according to both manufacturer instructions with a starting blood volume of 5 ml for the MACS and 2 ml for the RosetteSep technology. Leucocyte subsets from BM and PB were enriched from patients post allogeneic transplantation with haematological and oncological diseases. The samples were taken between day 28 and 295 post transplantation. The mean value of the CD3+ cell purity from BM (n=7) was 81.1% +/-4.6, and from PB (n=9) 80.9% +/-7.0 using immunomagnetical antibodies, while selections with RosetteSep kit in PB (n=4) led to a purity of 68.8% +/-4.5. Evaluation of the applicability of the RosetteSep kit in healthy donors resulted in a purity of 92.3% in CD3+ selected cells.

The purity with RosetteSep kit in PB for CD4+ cells (n=2) was 66.5% +/-4.6, for CD8+ cells (n=3) 72.9 +/-5.7 and for CD56+ cells (n=5) 70.8% +/-4.9.

Both systems are important technologies to isolate leucocyte subsets. In summary, while the MACS system led to a higher purity, the RosetteSep kit seems to be the less time consuming method.

P745

Correlation of flow cytometric CD34 counts and HPC (human progenitor cells) determined by the Sysmex XE-2100 hematology analyzer

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Patients pretreated with multiple cycles of chemotherapy often need prolonged periods to mobilize an appropriate number of CD34 positive stem cells for successful apheresis. Therefore, multiple cost and time consuming flow cytometric CD34 measurements are required. The aim of this study was to evaluate the reliability of the parameter HPC as a marker for immature cells and its usefulness for prediction of CD34 counts. This parameter is provided by the Sysmex XE-2100 hematology analyzer during processing of blood cell differential.

After G-CSF stimulation 240 peripheral blood samples (136 from patients with malignancies and 104 from healthy donors) were
investigated in parallel considering CD34 and HPC counts. CD34 and HPC measurements resulted in ranges of 0.448/µl and 0.2670/µl, respectively. Thus, given an amount of < 10/µl for HPC there were 60 of 73 samples (82%; 26 pts. and 10 healthy donors) displaying CD34 counts < 10/µl, our threshold for performing aphereses. HPC counts < 5/µl were present in 56 samples (24 pts. and 4 healthy donors). All but three of these samples, from healthy donors, contained < 10/µl of CD34 positive cells. Multiple analyses at up to 7 consecutive days were approached in 20 of the patients. Thus, almost all patients (18/20) showed multiple low values for HPC as well as for CD34 positive cells.

In summary, these data show that HPC measurements with the Sysmex XE-2100 can be used to predict the optimal time point for apheresis. HPC counts < 5/µl in peripheral blood of patients who are scheduled for autologous transplantation predict a low probability of a sufficient CD34 yield in an apheresis on the same day. Confirmation by FACS is not required in these cases. On the other hand, healthy stem cell donors should be monitored with both methods to exclude false negative HPC measurement, since apheresis will be started regardless of the results in most cases. The reasons for false negative HPC counts in healthy donors have to be explored.

P746

Short- and long-term hematopoietic reconstitution after 1,019 courses of high-dose chemotherapy and peripheral blood progenitor cell support in a single institution adopting the 2x10^6 CD34+ cells/Kg threshold


Controversies still exist about the number of peripheral blood progenitor cells (PBPC) able to support prompt and stable hematopoietic rescue after high-dose chemotherapy (HDCT). According to most reviews, the recommended dose for autologous transplantation is 5-8x10^6 CD34+ cells/kg. This recommendation was based upon faster platelet recovery with increasing CD34+ cell dose, and ascertainment that reconstitution of durable hematopoiesis capable of coping with further chemotherapy and/or radiotherapy occurred in a single 1994 study on 34 cancer patients who received 8x10^6 CD34+ cells/kg. To study the safety of the 2x10^6/CD34+ cells/kg threshold, we retrospectively evaluated 433 patients [281 with breast cancer (BC), 98 with lymphoma, 54 with other malignancies] who underwent 1,019 HDCT courses followed by reinfusion of PBPC collections containing a median of 2.4x10^6/CD34+ cells/kg. BC patients received 3 cycles of HD-EC, T-EC, ICE or T-ICE; lymphoma patients received HD sequential therapy. Median follow-up was 33 months (range 8-56). CD34+ cells were enumerated using a FDA-approved, single platform kit. Results of follow-up was 33 months (range 8-56). CD34+ cells were always found within appropriate limits. Prompt hematopoietic recovery (<16d to 1,000 neutrophils and 10,000 platelets/µl) was observed in 99% of patients mobilized with G-CSF alone (n=281) and in 92% of patients mobilized with chemotherapy plus G-CSF (Ch-G, n=204). In the latter group (more frequently pretreated), recovery was delayed of 1-2 and 3-4 weeks in the remaining 6 and 1%, respectively. In multivariate analysis, >2 previous chemotherapy courses (and not other variables) were significantly associated with delayed engraftment. CD34+ cells and/or CFU-GM collection below median values or lower quartiles were not associated with disease progression or inferior survival. Eighteen percent of patients mobilized by G-CSF alone failed to collect >6x10^6/CD34+ cells/kg as planned to support 3 HDCT courses. In 43/52 (82%) of these patients, a crossover Ch-G mobilization was effective. In patients who failed to mobilize after Ch-G, the crossover to G-CSF alone mobilization was significantly less effective. Despite frequent secondary radiotherapy and/or chemotherapy, all patients had long-term engraftment. MDS did not occur. The frequency of secondary leukemia (0.4%) was better or similar when compared to studies where the PBPC threshold was 2-4 fold higher.

Additional abstracts to this topic

Influence of diagnosis on peripheral blood stem cell mobilization
M.C. Fernandez-Jimenez, R. Arrieta, F. Hernandez-Navarro (Madrid, E)

Background and objective: Peripheral blood stem cell (PBSC) transplantation has become a widely accepted therapeutic option for patients with several diseases. However, factors involved in PBSC mobilization are still poorly understood. The purpose of this study was to evaluate the impact of diagnosis on PBSC mobilization.

Patients and methods: We analyzed data from 148 patients (75 with solid tumors and 73 with hematologic malignancies) and 43 donors who had undergone mobilization with G-CSF at a dose of 10 mcg/kg/day for four days through leukapheresis. Leukapheresis procedures were performed according to two harvest schedules: standard leukapheresis (n=114) and large-volume leukapheresis (LVL) (n=77). The relationship between the number of blood CD34+ cells previous to harvest (pre-harvest CD34), the leukapheresis size, the diagnosis and the CD34+ yield was studied.

Results: In patients with solid tumors and donors the median amount of CD34+cells/kg harvested was significantly higher in LVL than in standard apheresis, whereas in patients with hematologic malignancies, there was no significant difference for the two procedures. In addition, patients with solid tumors and donors had significantly higher number of pre-harvest CD34 than patients with hematologic malignancies had. Differences were also noted between diagnoses on the median number of pre-harvest CD34 to reach >= 2.5 x 10^6 CD34+ cells/kg in the first collection (see table).

Conclusion: Patients with hematologic malignancies exhibit lower amount of pre-harvest CD34 and lower PBSC recruitment during the apheresis than patients with solid tumors and donors. The number of pre-harvest CD34+ cells in each group of patients taken together with the leukapheresis size is a useful tool to predict de CD34+ yield.

<table>
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<th>Diagnosis</th>
<th>Donor</th>
<th>Breast cancer</th>
<th>Non-Hodgkin's Lymphoma</th>
<th>Hodgkin's disease</th>
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<td>Pre-harvest CD34+/µl</td>
<td>to collect</td>
<td>2.5 x 10^6</td>
<td>CD34+ cells/kg</td>
<td>85</td>
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II2 activated graft for autologous transplantation in hematologic malignancies
M.J. Baptista, I.L. Barbosa, S. Roncon, A. Avila, P. Torres, A. Campos, F. Campilho, C.P. Vaz, P. Pimentel, A. Carvalhais (Porto, P)

Several groups have shown that it is possible to use IL2 for culturing peripheral blood progenitor cells (PBPC) “in vitro” 24 hours before graft infusion increasing their cytotoxic capacity. In this study we report our results on the use of IL2 activated graft, their cellular content and patient hematologic engraftment. Five patients with poor prognosis hematologic malignancies (3 Non Hodgkin Lymphoma; 1 Hodgkin Disease, 1 Acute Myeloid Leukemia) were conditioned with BEAM (n=4) or busulfan+melfalan (n=1).

Toxicity related with infusion was mild and IL2 administration in the early post-transplant period was well tolerated. Table 1 shows the median number of leucocytes, CD34+ cells, NK cells and their bright and dim subpopulations infused to the patients.
All patients engrafted regardless CD34+ cell dose, which was less than 1x10^6/kg b.w. in two patients. The number of days required for neutrophil engraftment varied between 11 and 22 days (median 12 days) and for platelet engraftment between 13 and 26 days (median 19 days). These results are in agreement with those reported by other groups.

In summary, based on our preliminary experience with these 5 patients, IL-2 activation of the graft did not impair hematopoietic reconstitution.

<table>
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<th>Recovery of NK cell subsets from PBPC collections following short in vitro IL2 incubation</th>
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<td>M.J Baptista, I Barbosa, S. Roncon, A. Avila, P. Torres, A. Campos, A. Campos, F. Campilho, P Vaz, P. Pimentel, A. Carvalhais (Porto, P)</td>
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</table>

IL-2 activated PBPC have been used for autologous transplantation of patients with hematologic malignancies. These stimulated grafts seem to have increased cytokotoxic activity that may be assigned to NK cell population CD3-CD56+. Two subpopulations of NK cells can be distinguished, based on the intensity of CD56 antigen expression: CD56bright and CD56dim. The aim of this work was to evaluate the effect of 24 hours IL-2 incubation on NK cell subset present on PBPC from healthy donors (n=7) and patients with haematologic malignancies (n=7). The parameters evaluated were CD56 dim /CD56bright ratio the total NK cell population recovery after culture.

Thawed PBPC were incubated for 24 hours with 1000 U/ml IL-2 and cell populations pre and post incubation were evaluated by flow cytometry.

In frozen PBPC collections, median percentage of NK cells was similar in donors and in patients. 2.8% and 2.5% respectively.

In the donors, the NK population was > 89% CD56dim, and <12% CD56bright. Amongst patients there was wider variation in the percentage of CD56dim (76–95%) and in the CD56bright (5–24%).

Before culture, the CD56dim/bright ratio for donors was 13.5 and 5.9 for patients, however no statistical difference was found (Table 1). Under this cultured condition, the recovery of the NK cells subpopulations was variable. In some cases there was losses of 50%, in others there was an expansion of NK cell numbers (Table 2).

Following IL-2 incubation no significant changes in the parameters evaluated were noticed, both for donors and for patients (Table 1 and 2). Although, these preliminary results suggest a higher percentage of recovery of CD56bright in donors, they required further studies to confirm these data.

**Table 1:** Number of IL2 treated cells infused per Kg patient body weight.

<table>
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<tr>
<th>Median Range</th>
<th>Less x 10^6/Kg</th>
<th>CD34x10^6/Kg</th>
<th>NKx10^6/Kg</th>
<th>NKdim x10^6/Kg</th>
<th>NKbright x10^6/Kg</th>
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<td>3.04 – 8.57</td>
<td>1.43 – 8.57</td>
<td>1.33</td>
<td>2.36 – 7.88</td>
<td>0.39</td>
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</table>

**Table 2:** NK cell recovery following IL-2 culture (median/Kg min-max).

<table>
<thead>
<tr>
<th>% NK (total PBPC population)</th>
<th>CD56+/CD56- ratio</th>
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<tr>
<td>Donors Pre</td>
<td>1.6 (0.6 – 4.4)</td>
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<td>8.5 – 52.9</td>
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<tr>
<td>Post</td>
<td>2.5 (1.6 – 7.7)</td>
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<td>3.2 – 26.7</td>
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<tr>
<td>Patients Pre</td>
<td>2.0 (0.9 – 4.3)</td>
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<td>3.6 – 17.2</td>
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</table>

**Table 1:** Comparison of NK cell population pre and post IL-2 incubation.

**19. Pediatric**

**P747**

**Bone marrow transplantation (BMT) in thalassemia: a modified condition protocol with fludarabine and reduced cyclophosphamide**

S. Eber, M. Fasnacht, R. Seger, S. Corbacioglu, H. Ubierto, T. Gündör (Zurich, St Gallen, CH)

Bone marrow transplantation (BMT) related mortality is increased in thalassemic patients with cardiomyopathy due to advanced hemosiderosis: Several cases of fatal acute cardiac tamponade were observed in early posttransplant period. A less cardiotoxic regimen was described by Lucarelli et al (BMT 2001;28:11-13) containing busulfan 14 mg/m2 as well as a reduced cyclophosphamide dose of 90 mg/kg and fludarabine (100 mg/m2). In addition this protocol comprises a 34 day long prephase with hydroxurea and azathioprine.

We have transplanted two 18-year old thalassemic females (both with mild to moderate liver fibrosis; class 2-3 according to Lucarelli et al). Patient 1 (ferritin level 1600 ng/ml) had impaired diastolic function. Patient 2 (ferritin level 2300 ng/ml) had systolic dysfunction and was treated with enalapril (10 mg/kg); this therapy was stopped before the conditioning phase. Both patients were put on continuous i.v. desferrioxamine (60 mg/kg) for 6 weeks before transplantation. We used the above protocol omitting the prephase with hydroxurea and azathioprine. GVHD prophylaxis consisted of ATG (Thymoglobuline; 5 mg/kg x 4), cyclosporin A and methotrexate. Both patients had HLA identical bone marrow donors (a 3 - 4 year old brother) and no GvHD occurred in either patient. Patient 1 (2 x 10^6/kg CD 34) had a neutrophil take at day +18 and platelet take at day +30. FISH analysis using the X-Y difference proved >97% donor chimerism at day +180. Diastolic dysfunction persisted but remained asymptomatic. In patient 2 due to blood donor-recipient weight discrepancy only a low stem cell dose could be substituted (0.7 x 10^6/kg). She had a sluggish neutrophil recovery at day +37 (absolute neutrophil count remaining low at about 500/mm3) and maintains platelet counts between 20.000 to 30.000/mm3 without substitution since day +62 post transplant. Chimerism remained >97% after day +60. This patient presented with signs of cardiac failure when 1200 ml of bone marrow was infused at a hemoglobin level of 112g/l. Symptoms resolved with diuretic therapy. On day +60 persisting systolic dysfunction was demonstrated via echocardiography and therapy with ACE-inhibitor was restarted.

Conclusion: Our results show that cyclophosphamide can be partially substituted for fludarabine without compromising full bone marrow ablation and engraftment. The regimen was well tolerated and further cardiac damage could be avoided. The addition of hydroxurea and azathioprine might be unnecessary.
variable (3-loci; 2, 2-loci; 7, 1-locus; 10, phenotypically identical; 7). They suffered from hematological malignancies (n=23) or non-
malignant diseases (3). The disease status at transplant was PR or refractory in 14 out of the 23 patients. T cells were depleted in two of the grafts by using E-rosetting method plus anti T cell monoclonal antibodies. A median of 4.1x10^8/kg of marrow nucleated cells was harvested and infused into the patients. GvHD prophylaxis regimen included CyA or FK506 + sMTX in 23 cases, CyA alone in 2, and MTX alone in one case. After transplant, 3 grafts including 2 after T cell depletion were rejected. Neutrophil (>500/ul) and platelet (50,000/ul) engraftment days were 20 and 25 days, respectively. Fatal GvHD was compared to only one patient. Seven patients died of TRM (VOD; 3, Pulmonary infiltrates; 2, bronchiolitis obliterans; 1, LPD; 1) and 5 died of disease recurrence. Twelve cases are still alive. One of 12 is surviving on relapse.

Our results support that HLA phenotypically identical or partially matched parent could become immediately available and effective alternative donors for HSCT in pediatric population in certain area where distribution of HLA is more homogenous.

P749
T cell reconstitution in children with hematological malignancies during the first 6 months post bone marrow transplantation
D. Barge, A. Gennery, G. Spickett, A. Cant, M. Abinun, T. Flood, R. Skinner (Newcastle, UK)
Aims: 14 patients with haematological malignancies had T cell subsets and function measured during the first 6 months post allogeneic BMT to establish normal reconstitution patterns and to determine which measurements of immunological function are useful in management of these patients.

Methods: T cell subset markers to CD3, CD4, CD45RA, CD45RO, HLA-DR, and CD7 were measured by two-colour flow cytometry. Function was measured using the standard tritiated thymidine uptake assay against a mitogen panel, reported as % of normal control.

Results: 5 T cell depleted haploidentical (TCD) marrow recipients had 1000 CD3+ cells/ul by day +122 to +175. Increases in PHA responses >50% were present by 115-180 days. By day+180, HLA-DR expression, initially 70 - 100% was < 30% and all had >500 CD4+ cells/ul. 3 patients receiving HLA-identical sibling who marrow had CD8+ cell increases before 90 days not seen in 2 umbilical stem cell recipients. 2/5 non-TCD patients had >500 CD4+ cells/ul at +90 and +170 days; the remaining 3 had >250 CD4+ cells/ul. Patients who cleared virus post BMT had variable T cell numbers and PHA responses; all responded to anti-CD3 & IL-2 >40%. <10% PHA responses correlated with <50 CD3+ cells/ul, >90% T cells HLA-DR+ or CD7+.

Conclusion: Normalisation of T cell numbers and PHA responses by 100-180 days in TCD recipients was not seen in whole marrow recipients. The correlation seen between PHA responses, CD3, and CD45RA cell numbers in TCD and whole marrow was absent in cord blood recipients. Poor proliferation responses to anti-CD3 and IL-2 may indicate increased susceptibility to viral infection.

P751
Haploidentical transplantation - speed of immune reconstitution and post transplant infections
J. Motwani, S. Lawson, P Darbyshire (Birmingham, UK)
23 paediatric patients (11 males; 12 females) received a CD34 selected partially matched related donor transplant for malignant (13) and non-malignant (10) disease over the last 3 years. The median age of recipients was 7 years 9 months (range 3 months - 14 years). There were 6 deaths (26%) – relapse (3), TBI toxicity (1), disease progression (1) and parainfluenzae III infection (1). The median cell dose was 9.77 x 10^6/Kg (range 2.42 – 34.2). Three patients received a second stem cell transplant for poor engraftment/mixed chimerism (2) and rejection (1). Five patients received additional CD34 infusions for poor immune reconstitution based on T cell recovery at 3 months. Two patients received T cell infusions, one for parainfluenzae type III infection, and the other for relapse. All patients received regular immunoglobulin infusions for a median of 10 months (range 3.5 – 15 months). The median times to recovery for lymphocyte subsets were: CD3 - 9 months (range 4 – 20 months); CD4 – 11 months (range 4 – 20 months); CD8 - 8 months (range 3 – 16 months); CD19 - 3 months (range 2 – 13 months); NK cells - 4 weeks (range 3 weeks – 8 months). The median times to immunoglobulin subset recovery were: IgG - 13.5 months (range 6 - 20 months); IgA - 7 months (range 4 weeks - 32 months) and IgM - 7 months (range 4 weeks - 22 months). Ten patients developed acute infection which resolved after treatment in nine (EBV (1); Mycobacterium kansasii (1); HZV (2); HSV (1); CMV (3); Pseudomonas (1) and Parainfluenzae III (1) ). The patient with Parainfluenzae III infection died. Median follow up is 507 days (range > 1018 days). 16 of 23 patients are alive and well in remission with complete donor chimerism. One patient has relapsed disease, but remains stable on oral chemotherapy. In conclusion, despite delayed immune reconstitution following haploidentical haemopoietic progenitor cell transplant, our observed infection rate is very low. This may be due to strict isolation policy and regular support with immunoglobulins practised in our centre.

P752
Quantitative analysis of whole blood chimerism in children after sex-mismatched allogeneic bone marrow transplantation (BMT) - High correlation between FISH and STR-PCR method
Quantitative chimerism in patients after sex-mismatched allogeneic BMT can be monitored successfully using polymerase chain reaction technique (PCR, microsatellite markers) and molecular cytogenetic analysis - fluorescent in situ hybridisation (FISH). We report application of automated DNA sizing technology (ALF Express Sequencer) and FISH for analysis of post-BMT
chimerism. Blood samples obtained from the donor and the recipient prior to BMT and the recipient at short time intervals were analysed. Genomic DNA was isolated from frozen whole peripheral blood by salting-out method, amplified with fluorescent polymerase chain reaction primers (specific for STR marker loci: FGA, VWA, TH01, F13A1, D21S11) and analysed using quantitative automated DNA sizing technology. FISH was carried out on interphase nuclei with the use of probes specific for X and Y-chromosomes (DXZ 1 and DYZ 1 loci) Chimerism status was assessed in 10 patients (Table 1.)

Monitoring of chimerism after allo-BMT during medical treatment has an important role. It is possible to assess dynamics of chimerism status using quantitative method, which is necessary for assessment effectiveness of patient’s treatment. Two methods used in our molecular studies of chimerism complement one another. Correlation between FISH and STR-PCR results obtained in this study was very high (r=0.97) and statistically significant (P<0.0001). Therefore FISH and STR-PCR can be used interchangeably.

This work was supported by The State Committee for Scientific Research, Projects No. 4 PO5E 07419, No. 6 PO5E 035 20 and No. 4 PO5E 108 18.

### Table 1

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### Table 2

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P753

Quantitative assessment of whole blood chimerism in children conditioned for allogenic HSCT with 30 g/m2 treosulfan-based preparative regimen


Treosulfan (TRS) demonstrates pronounced effect against hematopoetic cells (Westerhof et al., 2000), but reduced organ toxicity (Casper et al., 2001). Therefore, TRS seems to be especially useful in adults (Caspers et al., 2000) and children (Wachowiak et al., 2001) with pre-transplant factors of increased risk of severe regimen related toxicity (RRT). However, it is not yet established in children, whether preparative regimens (pre-reg) based on TRS at a dose of 3 x 10 g/m2/day gives myeloablative effect sufficient to achieve complete post-transplant hematopoetic chimerism. Therefore, quantitative assessment of whole blood chimerism has been performed in 5 children (Table 1) conditioned with TRS (total dose of 30 g/m2) and transplanted from MSD.

For monitoring of hematopoetic chimerism an automated DNA sizing technology and FISH have been applied. Genomic DNA was isolated from frozen whole peripheral blood by salting-out method and amplified with fluorescent PCR primers (specific for STR marker loci: FGA, VWA, TH01, F13A1, D21S11) and analysed using quantitative automated DNA sizing technology. FISH was carried out on interphase nuclei with the use of probes specific for X and Y-chromosomes (DXZ 1 and DYZ 1 loci) in a case of sex-mismatched bone HSCT.

Conclusion: 30 g/m2 TRS-based pre-reg assures myeloablative effect sufficient for engraftment, however in some children mixed post-transplant chimerism is observed. Therefore, there is a need of pharmacokinetic studies of TRS for its individualized dose adjustment and/or for further dose escalation to assure optimal myeloablative and/or anti-leukemic effect.

This work was supported by The State Committee for Scientific Research, Projects No. 4 PO5E 07419, No. 6 PO5E 035 20 and No. 4 PO5E 108 18.

### Table 1

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P754

Serial analysis of hematopoetic chimerism in children with severe aplastic anemia

W. Hoelle, H. Kreyenberg, P. Lang, D. Niethammer, T. Klingebiel, J. Beck, P. Bader (Tuebingen, Frankfurt, Greifswald, D)

Between December 1993 and January 2000 19 transplantations have been performed in 17 children with severe aplastic anemia (SAA) at the University Children’s Hospital in Tübingen, Germany. The median follow-up is 45 months (23 - 74 months). Posttransplant engraftment has been monitored by sequential analysis of chimerism using quantitative PCR based amplification of short tandem repeat (STR) markers. During the first 100 days analysis were performed weekly, thereafter once a month until 18 months posttransplant. During the course of follow up five children developed increasing autologous cells (in-MC), one patient failed to achieved sustained engraftment and 11 patients became complete chimeras (CC) at all subsequent analyses. The first 2 patients with in-MC (2/5) developed an rapid increase of autologous cells and finally rejected their graft. A second transplant has been performed, the patients then became CC and remained in remission. In the following two patients with in-MC immunomodulation have been performed by repetitive transfusion of low dose DLI (2.5 x 104/kg) when the amount of autologous cells increased over 30%. Both patients responded to the treatment, the amount of autologous cells decreased after DLI below 10% and the patients remained disease free. None of them developed GVHD. In another patient with in-MC no DLI was given because the level of autologous DNA did not exceed more than 30%. This patient developed a stable mixed chimerism and is doing well 63 months after transplant. These results gave evidence that low dose DLI in children with SAA and in-MC posttransplant is feasible and may help to prevent graft rejection without inducing GVHD.

### Table 2

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S207
P755
Risk factors for moderate-to-severe chronic graft-versus-host disease in children
J. Svennisson, M. Remberger, B. Gustafsson, A. Gustafsson
Jernberg, J. Winiarski, O. Ringdén (Huddinge, S)

Objectives: Graft-versus-host disease (GVHD) is one of the main complications limiting the outcome after allogeneic haematopoietic stem cell transplantation (HSCT). Underlying mechanisms that lead to moderate-to-severe GVHD might not be the same in adults as in children, and need to be identified in order to reduce mortality and morbidity. Here, we have studied moderate-to-severe chronic GVHD in children following allogeneic HSCT.

Methods: 253 children, aged 0-18 years and transplanted with sibling or unrelated donors at Huddinge University Hospital between 1975 and 2001, were retrospectively studied. Thirty-nine potential risk factors were suggested. Variables that were significant or near significant (P < 0.10) in Cox regression univariate analysis were further tested in multivariate analysis.

Results: Fifty-one of 253 patients (20 %) developed mild chronic GVHD. They were not analysed in this study. 14 of 253 patients (5.5 %) developed moderate-to-severe chronic GVHD. Two risk factors were identified: 1) CML and 2) previous acute GVHD grade I-II IV.

1) CML patients ran a 6-fold risk of developing moderate-to-severe chronic GVHD (P=0.03). The probability was 33% with CML, versus 7% for non-CML diagnoses.

2) Patients with previous grade II-IV acute GVHD had a 3-fold risk (P=0.03). The probability of moderate-to-severe chronic GVHD was 6% without and 16% with preceding grade II-IV acute GVHD.

Factors that were not significant in this study included unrelated donor, HLA one antigen mismatch, type of conditioning regimen, stem cell source, cell dose, etcetera.

Conclusion: CML and grade II-IV acute GVHD are strong risk factors predisposing for moderate-to-severe chronic GVHD in children. This is in line with previously published data from adult patients. CML patients might therefore need additional GVHD prophylaxis. Children with preceding grade II-IV acute GVHD are also at risk for moderate-to-severe chronic GVHD and should be carefully monitored.

P756
High-dose chemotherapy in children with advanced malignant germ cell tumors - Single center experience
E. Kabickova, D. Sumerauer, P. Kobylka, R. Kodet, E. Cumlivska, J. Valkova, J. Koutecky, J. Mracek, B. Malinova (Prague, CZ)

Purpose: To evaluate toxicity and effectiveness of consolidation high-dose chemotherapy with autografting in children with incompletely responding or relapsing malignant germ cell tumors.

Methods: Twenty patients with poor-risk germ cell tumors, of whom 14 were boys, underwent megatherapy between January 1993 to May 1999. The median age was 16.2 years (1.8 to 19). Reasons for high-dose chemotherapy were: poor response to first-line chemotherapy (16x) or relapse (4x). Primary sites at diagnosis were testis (9), ovary (3), embryonal carcinoma (2) and mixed germ cell tumor (13). All but two patients had initially elevated serum alphafetoprotein and/or human chorionic gonadotrophin. Seven patients had initial stage III, thirteen stage IV disease. The conditioning regimen contained carboplatin, etoposide and melphalan or cyclophosphamide. Bone marrow was used as the source of hematopoietic stem cells in six patients, peripheral blood in fourteen.

Results: After a median follow-up of 3.5 years (1.5 to 7) the disease-free and overall survival were 75% and 80%. Two boys relapsed, median time to relapse was 4.5 months. One patient died from disease progression, 3 secondary to infectious complications, Grades III-IV (WHO) renal toxicity were observed in five patients, hepatic toxicity in eleven patients.

Conclusions: High-dose chemotherapy with autografting is effective strategy for children who relapsed and may be included as consolidation of first-line treatment for poor-risk patients.

P757
Dose-intensive therapy and myeloablative chemotherapy with hematopoietic stem cell rescue in childhood poor prognosis Ewing’s sarcoma (HR-ES)
A. Prete, R. Rondelli, P. Rosito, A. Pession (Bologna, I)

Objectives: To improve the prognosis of paediatric patients (pts) with HR-ES. Methods: Previously untreated pts, aged less than 18 years at diagnosis, with newly diagnosed HR-ES of bone because metastatic or localised but with tumour volume more than 100 cm3. Treatment consisted of: cytoreduce-reactivating induction therapy with two courses of Vincristine (Vcr) 2 mg/m2, Cyclophosphamide (C) 2200 mg/m2 and Adriamycin (Adr) 90 mg/m2 in two days (Hiper-VAdC), alternated to two courses of Etoposide (VP16) 600 mg/m2 in three days plus C 4000 mg/m2 (G-CSF). G-CSF supports each cycle of chemotherapy in order to improve dose intensity and enhance peripheral blood stem cell mobilisation after CE; Surgery and/or Radiotherapy for local control of primary and/or metastatic sites of disease; Maintenance chemotherapy consisting of two courses of Vcr 1.5 mg/m2, C 1200 mg/m2 and Adr 80 mg/m2 in two days (VAdC) alternated with two courses of VP16 500 mg/m2 plus Ifosfamide 9000 mg/m2 in five days (IE). At the end of this phase pts who were not in progression of disease were eligible for consolidation therapy and received Busulfan (Bu) 4 mg/kg/die for 4 days, VP16 800 mg/m2/die for 3 days and Thiotepa (TT) 300 mg/m2 followed by peripheral blood stem cell rescue.

Results: From April 1993 to May 1999, 43 pts 10 with localised and 33 with metastatic disease were enrolled in this protocol. Four pts progressed during the maintenance phase and 34/39 pts eligible were grafted. At time of graft 12 pts were in CR. The median number of CD34+ infused was 6.9 (2.5-40.1) x10^6/kg. Despite 10 patients received both Bu and total lung irradiation, nor pulmonary toxicity and toxic death related to consolidation procedure were registered. After a median follow up from the diagnosis of 47 (23-89) months, 20/43 patients are in CR, and 2 are alive with disease. The 6 years OS (SE) and PFS (SE) were 48.6% (16.3) and 42.3% (8.3) respectively. Patients without metastasis at diagnosis fared substantially worse than pts with localised disease (6 years PFS 35.8% vs 64.0%, p=0.06), moreover pts with bone metastasis (PFS = 14.4%) have a poorer outcome.

Conclusion: The results if compared with other national and international experience, demonstrate an increment in terms of 6 years OS. Only extension of disease at diagnosis, site of metastasis and surgery on primary site of disease seems to influence the outcome in terms of PFS.

(Supported by National Research Council, Project # 9302236PS39)

P758
Megatherapy and hematopoietic stem cell rescue in the primary bone high-risk and inoperable Pnet/Ewing’s sarcoma. Ten Years experience
J. Malis, E. Kabickova, J. Valkova, J. Koutecky, J. Mracek, B. Malinova (Prague, CZ)

Between 1990 and 1999 thirty six primary bone high risk and inoperable PNET/Ewing’s sarcoma pts (22 boys, 14 girls) were enrolled in the transplantation program. The median age of pts was 15.6 yrs (2.1 - 19.2). Primary tumor sites were pelvic in 18, spinal column 7, femur 4, humerus 2, tibia 2, metatarsus 1, clavicle
P759

Autologous stem cell transplantation in childhood lymphomas


Between 1995-2001 a total of 49 hematopoietic stem cells transplantations (HSCT) were performed in 47 children with non- Hodgkin (NHL) and Hodgkin (HD) lymphoma. Two children received double transplantations. The age of patients ranged from 2.8/12 to 8.1/12 years (median 6.8/12 years).

NHL LCAL was diagnosed in 14 children, NHL-T cell in 6 children and B cell NHL – in 20 children. Hodgkin disease was diagnosed in 9 patients. In 17 children transplant was performed while in CR1 (all belonged to high risk groups), in 1 – in CR2, in 3 – in CR3. However, 12 transplanted children did not achieve complete remission (PR). HSCT was also performed in 4 relapsed children. As conditioning regimen the BEAM protocol was introduced in most cases (42 pts). The source of stem cells was peripheral blood – in 42 cases, bone marrow – in 2, bone marrow plus peripheral blood – in 5 cases.

33 out of 47 pts are still alive with the observation time ranging from 1 month to 3.5 years (median 2 years). The probability of survival for the whole group of transplant patients with lymphoma is 0.7 and EFS – 0.65.

P760

Collection of peripheral blood stem cells (PBSC) in children with solid tumors: experience from 1991 to 2000 in a French oncology institute

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PBSC transplantation is now routinely used as a rescue after myeloablative treatment. We present here the experience of PBSC collection in a large series of children. From January 1991 to December 2000, 784 cytaphereses were performed in 334 consecutive children with solid tumors in the pediatric transplantation unit of IGR. The median weight and age of the patients were respectively 22 kg (8-95), and 84 months (8-319). Patients had previously received 6 courses (1-31) of chemotherapy (CT). A median of 1 (1-5) high dose chemotherapy (HDC) course followed by PBSC transplantation was planned. The goal of the collection was to harvest 3x106 CD34+/kg graft. An additional central line was inserted for collection in 75% of the patients. Femoral site (71%) was the most frequently used. Mobilization was performed in steady state in 61% of the cases and with CT + haematopoietic growth factor in 39%. A median number of 4.6x106 CD34/kg (0-90) was harvested with a median number of 2 (1-6) cytapheresis. The targeted number of CD34+ cells was collected after a first cycle of cytapheresis in 66% of children. Compared to mobilization in steady state, mobilization with CT and cytokine allowed to harvest more CD34+ cells (4.7 vs 4.2 p<0.106), with less cytapheresis (1 vs 3 p<0.007). A highly significant correlation between circulating CD34+ cells and CD34+ cells collected (r=0.769 p<0.0001) was observed. Transfusions were necessary in 76% of the patients. Clinical tolerance was excellent in 80% of the cases and improved with time.

PBSC collection was achievable even in very young children with a very good tolerance. Monitoring of peripheral CD34+ cells allows to better chose the time to start cytapheresis. Mobilization with CT and cytokine despite increased organization problems is more efficient than mobilization in steady state.

P761

Results of stem cell mobilization and subsequent engraftment after G-CSF + GM-CSF in children with leukemia and solid tumors


Background: Mobilized blood cells produce faster hematopoietic and immune reconstitution than BM, becoming the main cell source for autologous rescue and making high - dose therapy safer and more cost effective and may even replace BM in transplants. G-CSF alone (autologous and allogeneic) or a combination of hematopoietic growth factors such as G-CSF and GM-CSF are the most commonly used mobilization protocols in autologous transplantations.

Material and Methods: In a 5 – year - period, 32 patients (15 AML, 4 ALL, 4 NHL, 2 Hodgkin’s Disease, 2 Neuroblastoma, 2 RMS, 1 CML, 1 Astrocytoma, 1 Adrenocortical CA) underwent autologous stem cell apheresis after mobilisation with G-CSF (10 mcg/Kg/day) + GM-CSF (5 mcg/Kg/day). Apheresis, performed on the COBE Spectra separator, was started when the WBC count was > 2.0 x 106/ml. Results: The patients were 17 boys and 15 girls with a average age of 10.8 + 4.1 y (range: 3 to 18). All patients mobilized and an average number of 5.25 + 8.52 CD34+ cells x 106/kg (range 0.9 - 256.2) were collected for each patient. Median 2 leukapheresis/patient (n: 28/32) (Range: 1 to 4). No major side effect were observed and all pts. completed the mobilization procedure. 25 / 32 pts. were treated with high dose chemotherapy followed by infusion of PBSC. The median time to PMNs > 500/µl and PLT > 30000/µl were 17.0 (range : 9.0 – 67.0) and 26 (range : 14 - 160) days, respectively. 17/25 transplanted patients were alive & well for 10 - 66 months. DFS was 69% (5 years). There was no correlation between CD34 yield (< 2.0, 2.0 - 5.0, >5.0 x 106/kg) and number of 2 (1 -6) cytapheresis. The targeted number of CD34+ cells was collected after a first cycle of cytapheresis in 66% of children. Compared to mobilization in steady state, mobilization with CT and cytokine allowed to harvest more CD34+ cells (4.7 vs 4.2 p<0.106), with less cytapheresis (1 vs 3 p<0.007). A highly significant correlation between circulating CD34+ cells and CD34+ cells collected (r=0.769 p<0.0001) was observed. Transfusions were necessary in 76% of the patients. Clinical tolerance was excellent in 80% of the cases and improved with time.

PBSC collection was achievable even in very young children with a very good tolerance. Monitoring of peripheral CD34+ cells allows to better chose the time to start cytapheresis. Mobilization with CT and cytokine despite increased organization problems is more efficient than mobilization in steady state.
Hypo, without relevant side-effects, during peri-PBSC transplantation period. Hypo, though severe, was manageable with intravenous phosphate substitution. It was greatly correlated to neutrophil process and time of recovery. Hypo, in fact, lasted a little more than ANC < 500/ml and covered the same period. Thus, we concluded that a prophylactic intravenous phosphate substitution has to be taken into account during PBSC transplantation in children.

Conclusions: Almost all our patients (92%) experienced severe Hypo during peri-PBSC transplantation. We didn’t observe any side-effects of Hypo. Intravenous phosphate substitution was given when necessary in every case of Hypo and was administered every 24 hours. We didn’t observe any side-effects of Hypo.

Oral infection rates were similar both in melphalan and non-melphalan treated groups.

Conclusions: Although melphalan as part of the conditioning regimen, in our experience improves outcome:
1. melphalan induced mucositis is not related to the presence of GVH or vice-versa.
2. considerable morbidity results from oral mucositis.
3. opiate analgesia and parenteral nutrition made this acceptable to care givers.
4. further information on children’s perception of acceptability is required.
5. cytoprotection, e.g. by amifostine, if possible would have considerable positive impact on the quality of life, warranting further investigation.

P763
Hyposphosphatemia (Hypo) in children undergoing peripheral blood stem cells (PBSC) transplantation
D. Russo, C. Cappelli, F. Libera, C. Ossella, M. Gonfiantini, M. De Pasquale, A. Sordi, L. Amoroso, A. Clerico (Rome, I)

Severe Hypo can cause several complications which include: myopathy, cardiomyopathy, paraparesis, seizures, thrombocytopenia and osteomalacia. Some studies about bone-marrow transplantation (BMT) in adults showed a severe Hypo during peri-BMT period in a high percentage of patients. Our study aims to evaluate the degree of Hypo in pediatric patients affected by solid tumors undergoing PBSC transplantation.

Patients and Methods: From October 97 to October 2001, 51 patients underwent high-dose chemotherapy (HD-CT) supported by PBSC transplantation. They were 35 males and 16 females and the median age was 9 years (range 1-18). HD-CT regimens included Thiotepa, Melphalan, Carboplatin, Etoposide and Busulfan in different associations. Phosphatemia was evaluated from the day of transplantation to time of hematological recovery (ANC >500/ml; Hb >9 g/dl; Plts >50,000/ml). Intravenous phosphate substitution was given when necessary.

Results: For their transplantation our patients received a median number of 2x106 CD 34+ cells (range 1.2 - 4.5). Forty-nine out of 51 patients (92%) presented severe Hypo during peri-BMT period. Hypo nadir median value was 1.6 mg/dl (range 1.0 - 1.8) and median day of Hypo nadir was 8 days post-BMT (range 6-9). Median time of ANC <500/ml was 6 days (range 3-12), median first and last day of ANC < 500/ml were +4 (range 2-5) and +10 (range 9-13). In all 49 patients Hypo was noted from 24 hours before the first day of ANC <500/ml up to 48 hours after the first day of ANC >500/ml. Intravenous phosphate substitution was necessary in every case of Hypo and was administered every 24 hours. We didn’t observe any side-effects of Hypo.

Conclusions: Almost all our patients (92%) experienced severe Hypo, without relevant side-effects, during peri-PBSC transplantation period. Hypo, though severe, was manageable with intravenous phosphate substitution. It was greatly correlated to neutrophil process and time of recovery. Hypo, in fact, lasted a little more than ANC < 500/ml and covered the same period. Thus, we concluded that a prophylactic intravenous phosphate substitution has to be taken into account during PBSC transplantation in children.

P764
Acute renal failure (ARF) in children treated with hemopoietic stem cell transplantation (HSCT) - A prospective study
A. Munoz, M. Maldonado, R. Estepa, N. Gallego (Madrid, E)

All children who underwent a HSCT from January 1985 to December 2000 in our centre, were prospectively studied to determine the incidence of ARF in this population. ARF was defined as a sudden increase of serum creatinine concentration up to twice the normal range for age and sex and at least > 1 mg/dl for more than 48 hours. The following parameters were investigated: age, sex, underlying disease, type of HSCT, conditioning regimen and interval between HSCT and ARF. There were 234 HSCT(146 boys and 88 girls, median age 8.5 years). One hundred and twelve were allogeneic HSCT and the rest autologous. Hematologic malignancies (53%), solid tumors (33%) and genetic diseases were the underlying pathologies. There were 56 episodes of ARF were observed in 21 patients and the median interval between transplant and ARF was 36 days. ARF was significantly higher in boys (p=0.005), in allogeneic HSCT (p=0.001) and in children <12 years old (p=0.003). Most important pathogenic factors of ARF were nephrotoxic agents, multorgan failure and infections. There were 13 early transplant related deaths. Higher serum levels of creatinine and requirement of dialysis were significantly associated with mortality (p=0.003). No relationship was found between underlying disease and ARF.

Conclusions. The incidence of ARF after HSCT is mainly related with older age and allogeneic transplant. Mortality in patients with established ARF is high.

P765
Monitoring of etoposide in children with ALL undergoing bone marrow transplantation
L. Skibińska, J. Wachowiak, M. Kaczmarek, D. Boruczkowski (Poznan, PL)

In order to improve therapeutic effects of allogeneic bone marrow transplantation(allo-BMT), the pharmacokinetics of etoposide was determined and correlated with clinical response and toxicity in pediatric patients. Additionally, because simultaneous administration of etoposide and cyclosporine is considered during therapy, the influence of cyclosporine on free etoposide level was examined in vitro study.

Eleven patients (age 3 to 16 years) with ALL were studied. They received etoposide (dose: 60 mg/kg) during 4 h continuous infusion, 3-4 days before (allo-BMT). Cyclosporine as an immunosuppressive agent was given 24 h before (allo-BMT).

Blood samples were collected in heparinized tubes before and after the end of infusion (from 0.25 to 96 h). Plasma etoposide concentrations (total and free fraction of the drug) were determined by HPLC method using teniposide as an internal standard. A two compartment model was fit to measured drug concentrations for each subject and pharmacokinetic parameters were calculated. Laboratory parameters such as albumin and total plasma protein, bilirubin, hepatic enzyme levels, count of granulocytes and leucocytes were determined. The relationship between pharmacokinetic parameters, clinical response and toxicity was examined.

Etoposide is extensively (approximately 97%) bound to plasma proteins. There was positive correlation between non-bound etoposide and albumin level in patients. We observed correlation between free fraction of etoposide and count of leucocytes. Higher etoposide concentrations were significantly
associated with smaller count of leucocytes. There were not any correlation between total drug concentrations and leucocyte level. Higher hepatic enzyme levels (AspAT) in 2 patients was accompanied with significantly higher free fraction of etoposide concentration. Enhanced mucositis was observed in patient with delayed drug elimination (t0.5, beta = 27.2 h). Moreover, relapse of leukemia was observed in patient with very low free fraction of etoposide (1.4%). In vitro study have demonstrated the increasing free etoposide concentration in the presence of cyclosporine.

Conclusions:
- The free fraction of etoposide has a closer correlation with toxicity and clinical response than total drug concentration.
- Cyclosporine co-administered with etoposide may enhance its unwanted side-effects.

This work was supported by The State Committee for Scientific Research - Project No. 4 POSE 108 18.

P766

Cryopreservation at high cellular concentration with 5 % DMSO for PBSC transplantation in children
A. Curcoy, I. Alcorta, J. Estella, S. Rives, T. Toli, E. Tuset (Esplugues (Barcelona), E)

Background: Infusion of cryopreserved stem cells carries a risk of serious adverse effects to the use of cryoprotectant agent dimethylsulfoxide. It is desirable to reduce the amount of DMSO in cryopreserved products to minimize its toxicity. Our approach consists in using high cellular and low DMSO concentration for cryopreservation. We evaluate the hematological recovery of patients transplanted with this approach.

Methods: The apheresis products of 13 consecutive patients submitted to our BMT unit for autologous PBSC transplant were cryopreserved with 5 % DMSO in autologous plasma at a final cellular concentration equal or greater than 200 x 10^6/mL. The product was frozen and preserved at -80 °C in a mechanical freezer. The patients had been mobilized with G-CSF 24 mcg/Kg for 3-5 days (n=11) or a combination of chemotherapy and G-CSF (n=2). All patients were submitted to large volume leukapheresis processing 6 blood volumes with the Cobe Spectra AutoPBSC procedure. Short term hematological recovery was analysed by the number of days required to achieve an ANC >500/mm3 and a platelet count >20000/mm3 without transfusion support. Long term hematological recovery was assessed by analysing blood counts at 3 and 6 months post-transplant, considering as adequate an Hb level > 10 g/dL, PLT > 100000/mm3 and ANC > 200 x 10^6/mL in 5 % DMSO is accompanied by a rapid and sustained engraftment and permits the infusion of a reduced amount of DMSO.

P767

Relapsing large cell anaplastic lymphoma: from local to disseminated disease
C. Pizzagalli, P. Avoledo, A. Gratwohl, T. Kühne (Basel, CH)

The prognosis of large cell anaplastic lymphoma has improved with recent protocols. We report about a young patient, now 16 years old with relapsing lymphoma. At the age of 9 years the patient was referred because of swelling of the upper lip and of the right cheek lasting for weeks, and swelling of the angular lymphnodes at the right side. Large cell anaplastic Non Hodgkin Lymphom of T-cell type stage II was diagnosed. At the age of 9 years chemotherapy according to protocol NHL-BFM90 risk group K3 was initiated. In June 1995 two months after finishing chemotherapy the first local relapse appeared. Reinduction chemotherapy according to protocol NHL-BFM95 risk group K3 was started. The therapy was carried out between July 1995 and December 1996. At the age of 11 years the second local relapse appeared. The tumor was surgically removed and again reinduction chemotherapy according to protocol NHL-BFM95 risk group K3 was started followed by conditioning (VP16, Busulfan, Cyclophosphamid) and autologous stemcell trans-plantation. At the age of 15 years a new relapse with disseminated metastatic spread in the skin, subcutis, muscles, bones and internal organs was found. A reinduction chemotherapy was applied followed by allogenic stemcell transplantation from an HLA-identical brother. 12 months after bone marrow transplantation the patient is still in complete remission. In conclusion this case illustrated that a patient with LCAL, progressing from local to disseminated disease and relapsing after autologous stemcell transplantation can be rescued with allogenic stemcell transplantation.

Additional abstracts to this topic

Importance of committed progenitor cells number infused during an autologous PBSCT for rapid hematological reconstitution in children
J. Isaikina, Y. Strongin, M. Potapnev, O. Aleinkova (Minsk, BLR)

The aim of study was to evaluate progenitor cell parameters for hematological reconstitution in patients after auto-PBSC transfusion. 28 children with oncohematological diseases were underwent PBSC mobilization with G-CSF before auto-PBSC. The conditioning regimen was myeloablative. G-CSF was appointed since day +5 after PBSCT. Two groups of pts were identified: group 1 "rapid neutrophil recovery" (n=20) and group 2 "slow neutrophil recovery" (n=8) with neutrophil engraftment (>500/ul) on day 11.3 (range 9-14) and 19.3 (range 15-41) respectively. Four transplant parameters, including number of mobilized MNC/kg, CD34+ cells/kg, CFU-GM/kg, CD34+: CFU-GM ratio and time of the platelet (PLT) and red blood cells (RBC) recovery were measured. The CFU-GM were assayed in methycellosiuse (Gibco BRL). CD34+ cells were determined by flow cytometry. Mann- Whitney U-test was applied for statistical analysis.

Results: In the first group mean number of MNC/kg, CD34+cells/kg, CFU-GM/kg and CD34+:CFU-GM ratio in the infused PBSC were 6.39x10^8 (range 2.42-14.9), 5.40x10^6 (range 1.3-28.95), 11.36x10^5 (range 2.24-27.5), 4.78 : 1 respectively. The mean recovery time for PLT>=20000/ul and to RBC >=1% of reticulocytes was 15.06 day (range 11-37), 12.84 day (range 11-27) respectively. In the second group mean number of MNC/kg, CD34+ cells / kg., CFU-GM/kg and CD34+ : CFU-GM ratio in the infused PBSC were 6.85x10^8 (range 1.82 -16.1), 3.73x10^6 (range 1.02-13), 3.64x10^5 (range 0.1-12) and 14.59 : 1 respectively. The mean recovery time was 39.4 (range 12-80) for PLT and 23.3 day (range 15-39) for RBC. There were no significant difference in number of infused MNC/kg and CD34+cells/kg between groups. But in the second group of pts we observed statistically significant reduction the number of CFU-GM/kg (p=0.009)infused and higher ratio of CD34+ : CFU-GM (p=0.01), which indicate that infused
stem cells were less mature. The PLT (p=0.01) and RBC (p=0.001) recovery time was also slower in this group. 

Conclusion: Yield of CFU-GM/kg (> 2x 105/kg) and CD34+: CFU-GM ratio less then 5 : 1 rather then number of MNC/kg and CD34+cells/ kg infused during an auto-PBSCT predict rapid hematological reconstitution in children.

Correlation between peripheral blood stem cells (PBSC) oxidative stress and time of hematological recovery


Aim of the study was to correlate the oxidative stress level of transplanted PBSC and the time of hematological recovery (THR) in children affected by solid tumors and treated with repeated cycles of high-dose chemotherapy.

Patients and methods: The oxidative stress was evaluated taking account both the 8-hydroxydeoxyguanosine (8-OHdG) present in the DNA and the glutathione content. Between February 1998 and August 2001, 13 PBSC harvests and 26 reinfusions were performed. From each harvest and reinfusion product, a PBSC sample was collected and processed to evaluate the 8-OHdG in the DNA and the GSH content. The time of hematological recovery was evaluated after reinfusion and was considered when achieved when: Hb 9 g/dL, ANC >500/ml, PLT 100.000/ml. We graded 8-OHdG and GSH content from 0 to 10 to better correlate them to THR.

Results: We discovered a direct correlation between the oxidative stress values at the time of harvest and the values at the time of transfusion. In fact, the lowest oxidative stress value was found in the same bag at the time of harvest and transfusion. A correlation was also found between oxidative stress and ANC, but not with THR. In fact, the higher the oxidative stress is the longer ANC becomes, but there is no change in THR. Our median THR was 42 days (range 20-67), median ANC <500/ml was 6 days (range 0-12); 8-OHdG and GSH both had a median value of 7 (range 1-10 and range 0-10, respectively).

Conclusion: The correlation between oxidative stress at the time of harvest and at the time of transfusion allows us to assume that PBSC preservation doesn’t affect the oxidative stress status. The oxidative stress status of PBSC at the time of reinfusion could exert a significant effect on the ANC <500/ml period. Maybe the chemotherapy treatment administered before the harvest could negatively influence the PBSC efficiency. If our results will be further confirmed, the oxidative stress status of PBSC has to be taken into consideration to improve the efficiency of PBSC transplantation.

Thrombotic thrombocytopenic purpura after BMT due to severe deficiency of von Willebrand Factor cleaving protease - A case report

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Thrombotic thrombocytopenic purpura (TTP) in the setting of BMT is a complication of growing interest. The classic diagnostic pentad: fever, haemolysis, thrombocytopenia, renal failure and neurologic symptoms may be incomplete or match with other complications of posttransplant phase. The fatality rate is up to 90%.

Hereditary TTP was found to be due to the absence of a protease capable of cleaving vWF multimers to normal size (vWF-CP). Here we present the case of a boy resembling the features of TTP after PBSC with low activity of vWF-CP. PBSC was performed in a ten year old boy for myelodysplastic syndrome (RAEB). After conditioning with busulfan, thiotepa, cyclophosphamide and OKT3 20.5 x 10 6 CD 34 + cells per kg BW from a HLA-identical female donor were transfused and he engrafted rapidly. Complete chimerism was established after he was transfused twice CD 3+ cells on day 28 and 57. The patient developed headache, nausea and had a tonic-clonic seizure on day 64. AVHHD manifested at the intestine and immunosuppression was started at day 77 with mycophenolodisone and cycloporine(CyA). Consequently platelet count fell from 195 x 10 9 to 15 x 10 9. CyA was stopped for renal insufficiency and mycophenolic acid was added. On day 138 with aggravation of AVHHD CyA was started again. The following days platelet count gradually declined. CyA was changed to FK 506. On day 155 there were overt signs of haemolytic anaemia, with 3% fragmentocytes in the bloodsmear. FK 506 was stopped and plasma was infused, followed by a plasma exchange, both without any effect on haemolysis. Later in the course the boy delivered pulmonary and renal failure, requiring mechanical ventilation and dialysis. The boy died 6 days later. Activity of vWF-CP on day 155 was 10% of normal plasma activity.

Retrospectively determined plasma samples showed 7% vWF-CP activity before BMT within normal range (57%), at day 77 vWF-CP activity was 21%. After the plasma exchange vWF-CP activity was 19%.

Although vWF-CP activity in this patient was always in a range that does not cause haemolysis in patients with idiopathic TTP, his microangiopathic signs correlated well with low activity of vWF-CP. We conclude that vWF-CP plays a role in pathogenesis of post BMT-microangiopathy.

I.v. busulfan to replace oral busulfan in children: initial pharmacokinetic results

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High-dose busulfan (bu) is an important part of many pre-transplant conditioning regimens in children with a standard dose of 1 mg/kg every 6 h x 16 doses. Problems arise with oral bu due to the high intra- and interpatient variability of systemic exposure measured as the area under curve (AUC). Furthermore, bu is only available as a 2 mg/tablet (Myleran R) - that means a 30 kg-child has to swallow 240 bu tablets over the four days of treatment. Therefore, we started a study to replace oral bu by the new i.v. formulation of bu (Busulfex TM) at 0.8 mg every 6 h (Exception: First dose = double dose of 1.6 mg/kg).

Objective: Our purpose was to produce a dose intensity comparable to that achieved by oral bu at comparable toxicity and lower interindividual variability of AUC. The target AUC was 1600 ± 600 µM x min.

Methods: So far, 5 children were included into the study (Median age: 3.9 years, range: 1.4-16.1) receiving 15 doses i.v. busulfan based on the actual body weight. Plasma levels of bu for PK analysis were measured using a new LC-MS-method requiring only 200 µl of plasma at the following time points: After the 1st dose (3, 4, 4.5, 5, 6, 8 and 12 h), trough values prior to 3rd, 7th and last dose at 6 h after the last dose. Results: The AUC after the first dose in 3 of 5 patients was in the target range. The geometric mean of the AUC of all patients was 1128 µM x min (range: 968-1494). No case of severe hepatic venoocclusive disease (VOD) was observed.

Conclusion: The first results suggest that a busulfan i.v. dose of 0.8 mg/kg produces a dose intensity (measured as AUC) comparable to that achieved by oral administration. The study is ongoing without modifications to enroll at least 10 patients.

Mixed chimerism in allotransplanted children with immunodeficiencies

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The group of 11 children presently analysed composed of: five, three, and two patient suffering from SCID, WAS, Omen syndrome and Hyper IgM syndrome, respectively. Eight patients received graft from alternative (6 parental haploidentical, 2 unrelated) donors and three from matched siblings. Haploidential grafts were T cell depleted with the use of elutriation (5 cases) or magnetic beats (1 pt). Sibling matched transplanted children did not receive any conditioning (SCID and Omen Syndrome) or Bu Cy (Hyper IgM Syndrome). All alternative donor transplanted cases were on ATG given in addition to Cy (1 SCID pt) or BuCy (5 patients) based regimen. In two haploidential and one unrelated donor transplants there was a graft failure which prompted us to
Bone marrow purging. In the second attempts the extent of T cell depletion (haploidentical transplant) was lowered and in VUD transplantation setting another donor was used. One child with Omen syndrome died 23 days post transplant. All other children are alive.

Chimerism was followed with the use of short tandem repeats technique twice weekly during first month post transplant and then usually in one month intervals. During the observation period (from 0.5 to 4 yr.) we found stable full chimerism in 6 children and in 5 mixed chimerism was seen.

Among children with mixed chimerism two received matched sib (SCID and Omen Syndrome) and they had above 90% of T cells of donor origin and 3 haploidentical transplants (Hyper IgM, WAS and SCID cases) and they had from 40% to 90% of T cells of donor origin. Chimerism post transplantation in presented patients might be affected by a lack of conditioning (sib matched transplants) or T cell depletion (haploidentical transplants). VUD fully conditioned transplanted cases showed full chimerism soon after transplantation. In spite of mixed chimerism the patients improved clinically.

Cytoprotection of normal bone marrow and progenitor cells by amifostine, during MC540 phototreatment in pediatric malignancies

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Bone marrow purging has reduced the incidence of relapse in many hematological malignancies during autologous transplantation. However it is responsible for delay of hemopoietic reconstitution of the graft and serious complications. For this reason it is necessary to find cytoprotective substances, which improve the survival of normal progenitor and stem cells. Amifostine (WR-2721) is a thiol that is dephosphorylated in the active metabolite WR-1065 and selectively protects normal tissues from chemotherapy and radiotherapy. The aim of this study was to evaluate the effect of WR-2721 and WR-1065 on the survival of normal progenitors from children with malignancies and normal controls after MC 540 phototherapy. Bone marrow cells from 11 children with acute leukemias in remission (AL-r), 5 children with neuroblastoma and 7 normal controls were studied. Cell suspensions at 10 7 cells/ml were incubated with 1.5 mg/ml WR-2721 or 0.2 mg/ml WR-1065 for 15 min, washed twice and then incubated with MC 540 (20 mg/ml) for 1 hour. Afterwards, they were exposed to different Argon Laser 514 nm doses. Cell survival was estimated by trypan blue supravital staining following 24 hour incubation and the survival of normal progenitors has been estimated by colony formation assay. Our results showed that Amifostine improves the survival of bone marrow cells from children with acute leukemia in remission (20±2.6 % vs. 54.5±13.2%, p=0.02) after MC540 phototherapy while it does not influence the survival from normal controls (36.3±2.9 % vs. 43.87±7.5%, p=0.4). No statistical differences have been observed between AL-r and controls, either in the presence or not of Amifostine. WR-2721 and WR-1065 significantly protect BFU-E and CFU-GM from the phototoxic effect of MC 540 at the acute leukemia group. BFU-E and CFU-GM from children with acute leukemia in remission are more photosensitive than the normal controls (p=0.02). This difference is not observed after Amifostine pretreatment (p=0.1). In children with neuroblastoma, Amifostine seems to improve the survival of progenitors as follows: BFU-E (89.1±12.67% vs. 45.47±10%, p=0.01), CFU-GEMM (80.41±13.3% vs. 37.14±10.09%, p=0.02) and CFU-GM (79.6±5.1% vs. 58.2±7.9%, p=0.016). In conclusion, Amifostine selectively protects normal bone marrow and progenitor cells from children with acute leukemia in remission and neuroblastoma during MC 540 phototherapy and it is a promising agent in ex vivo bone marrow purging.


FTBI- and Busulfan-based preparative regimens (prep-reg) for HSCT may lead to severe early and late RRT, while NST increase the risk of graft rejection, GVHD and leukemia relapse. Therefore, there is still a need of prep-reg which demonstrates sufficient myeloablative, immunosuppressive and anti-leukemic effects, but low toxicity. For this reason we report results concerning 5 adults undergoing 2nd HSCT - such criteria seemed to be fulfilled by prep-reg with treosulfan (TRS) and fludarabine. Therefore, from July 2000 the TRS-based regimen has been applied in 8 children demonstrating before HSCT high risk of RRT. Median age was 10 years (range 4-13). Two patients (pts) were transplanted for inherited disorders (WAS, ALD), 6 for malignant diseases (NR-AML-MS, HR-AML in I CR, MDS in post-transplant relapse, ALL in III CR, B-NHL in II CR, LCH in PR) – for 2 pts it was 2nd HSCT. In all pts various pretransplant risk factors of severe RRT were identified. Seven pts received HSC from MSD and one from MUD. Prep-reg consisted of TRS 3 x 10 g/m2/day i.v. (total dose 30 g/m2) given in combination with other drugs acc. to diagnosis, identified risk factors of RRT and/or prep-reg used before 1st HSCT. Early non-hematological RRT was graded acc. to criteria of Bearman et al. (1988). Engraftment was achieved in all pts. (ANC >0.5 G/1 within 10-22 days, med. 19 days; platelets >20 G/1 within 10-63 days, med. 19 days). AcuteGVHD III occurred in 4 pts. with subsequent limited cGVHD in one of them. In 5 pts no features of early RRT have been observed, but in child undergoing MUD-HSCT (2nd transplant) for B-NHL in II CR conditioned with TRS, VP-16, CY and ATG a severe hemorrhagic cystitis occurred on day +20. Beside, in 2 pts early RRT I0 and/or II0 mucosa was observed. To date (30.11.2001) there was no early or late deaths related to HSCT complications. Relapse occurred in one of 6 pts transplanted for malignant disease (HR-AML) (afterward he underwent 2nd HSCT, achieved CR, but died because of subsequent relapse). Seven children are alive with median follow-up of 4 mths (range 2-16 mths).

Conclusion: Prep-reg based on TRS at a total dose of 30 g/m2 demonstrates in children myeloablative effect sufficient to assure engraftment and ussually low early RRT. To estimate late RRT and anti-leukemic effect further observation is necessary.

20. Late Effects and Quality of Life

P768

Long-term follow-up of pulmonary function after allogeneic hematopoietic transplantation

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Background: Pulmonary toxicity is a frequent event in patients undergoing haemopoietic transplantation (HT), basically due to conditioning regimes and infections. Patients and methods: we reviewed serial pulmonary function tests (PFT) in 41 patients who underwent allogeneic HT in our Centre between January’96 and June’01, with a median follow-up of 38 months. Twenty-five patients (61%) were men and sixteen were women (39%). The patient’s ages ranged from 13 to 62 years old (median 45). The underlying diseases were: 12 CML, 12 AML, 5 ALL, 4 aplastic anaemia, 4 NHL, 2 MDS, 1 m. myeloma, and 1 CLL. The donor was a sibling in 39 cases (95%) and unrelated in 2 (5%). Stem cell source was PB in 38 cases (93%) and BM in 3 (7%). Twenty-seven patients (66%) were conditioned with fractionated total body irradiation (FTBI) and busulfan based on weight without further adjustments based on plasma levels), ten patients (24%) with TBI-based regimes (with lung protection) and four patients (10%) received
cyclophosphamide-ATG. Thirty-two cases (78%) had clinical chronic graft versus host disease (GVHD) requiring immunosuppressive therapy some time after transplant. The PFT were performed pre-transplant, at days +90, +180, +360, and then yearly after-transplant. FEV1 (forced expiratory volume in 1 second), FVC (forced vital capacity), FEV1/FVC, TLC (total lung capacity), DLCO and DLCO/VA (diffusing capacity) were evaluated.

Results: 1) Considering the average as well as the median values of the series, a significant fall (around 25%) in the diffusing capacity was observed at day +90. The diffusion capacity improved at day +180 (15% of reduction) and became normal after day +360. We did not identify any factors influencing on this complication. 2) Three patients (7%) developed moderate to severe obstructive pulmonary disease at one, two and three years after transplant, respectively. The three of them had chronic GVHD and had received conditioning with busulfan-based regimes.

Conclusions: Hematopoietic transplant was associated with minor and major impairments of PFT in our series: 1) A transient fall in the diffusing capacity was observed in most patients at day +90. 2) A considerable proportion of patients developed moderate-severe obstructive pulmonary disease late after transplant. Chronic GVHD and busulfan-containing regimes might be influencing this complication.

P769
Thyrotoxicosis after allogeneic bone marrow transplantation
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Thyroid dysfunction is an important problem in patients receiving bone marrow transplantation. However, hyperthyroidism is rare and only a few cases have been reported. We describe the case of a 48 year-old woman with CML who underwent allogeneic bone marrow transplantation and who developed an hyperthyroidism. She was first treated with hydroxyurea, interferon alpha and cytarabine for one year. Because of the lack of cytogenic response she received an allogeneic bone marrow transplantation from her HLA identical sister. Conditioning regimen consisted of busulfan 16mg/kg and cyclophosphamide 120 mg/kg. Cyclosporine A and methotrexate were used for GVHD prophylaxis. Cyclosporine A was stopped 1 year after transplantation and no GVHD appeared. PCR analysis of peripheral blood cells confirmed a complete chimerism of donor origin. Fifteen months after transplantation, fatigue, tremor and weight loss leaded to the diagnosis of Grave's disease confirmed by a high level of thyroid stimulating antibodies. She was treated with carbimazole and achieved an euthyroid state. The donor, aged 47 year developed thyrotoxicosis, 3 months after transplantation. She complained of tachycardia, diarrhea, weight loss and tremor for several months.

Thyroid stimulating antibodies were elevated leading to the diagnosis of Grave's disease. She was treated with carbimazole and radioiodine. An hypothyroidism appeared rapidly and she was then treated with thyroxine.

Adoptive auto-immunity seems to be the main cause of hyperthyroidism appearing after allogeneic transplant but other causes can be discussed. Indeed this patient bore HLA DR3/B8 antigens, she had a family history of thyroid dysfunction and she was treated with interferon alpha before bone marrow transplantation.

P770
Retrospective study of long-term morbidity of an anthracycline based adjuvant treatment followed by one cycle of high-dose chemotherapy (HDC) with autologous blood cell transplantation in high-risk breast cancer patients
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To study the late morbidity in our HDC program in adjuvant breast cancer, we made a retrospective review of the 80 surgically resected high risk (based on pT, N+ number, histology and age) stage II (37p) and III (43p) breast cancer patients treated in our Center between 02/94 and 10/01 with HDC consisting of Mitoxantrone 25mg/m2, Carboplatin 800mg/m2 & Thiotepa 10mg/m2 (5p treated with STAMP-V) and PBPC support, following 4-6 cycles of standard adjuvant AC chemotherapy. Locoregional radiotherapy was added in most p. and Tamoxifen for all ER+ cases.

The peri-transplant morbidity (before day 30) has been previously reported and to summarize only one case of treatment related death (S. mitis bacteremia) occurred. With a median follow-up of 4.5 y. 32p relapsed and 60% are alive and disease free. After an extensive review of organ functions (thyroid, liver, lung, cardiac, blood and hormonal status) we have found induced menopause in 70 (in 2p reverted) and only elevated TSH in 14% and decreased cardiac LEVF >15% in 2p without clinical relevance as a significant findings. Neither graft failure nor myelodysplastic syndroms were observed.

In conclusion: Very limited morbidity was found with one cycle of HDC and PBPC support after conventional adjuvant treatment for breast cancer in the long term.

P771
Does donor recipient ABO-incompatibility have any effect on the course and outcome of allogeneic hematopoietic cell transplantation?

There are some reports showing the impact of ABO incompatibility on disease free survival, overall survival, and relapse and transplant related mortality after allogeneic bone marrow and peripheral blood stem cell transplantation. In order to reevaluate this finding we retrospectively analyzed 269 patients who underwent allogeneic hematopoietic cell transplantation (AHCT) from their HLA identical sibling donors in Ibi2 Sina Hospital Stem Cell Transplantation Unit. The median age of the patients was 30 (range: 14-48), and there were 120 CML, 92 AML, 23 ALL, 18 SAA, 6 MDS and 10 with miscellaneous diagnosis patients. The majority of the patients were standard risk patients and had received a Cy-based conditioning regimen (Cy-TBI or Bu-Cy). We used CSA and short-term methotrexate for GVHD prophylaxis. All patients received unmanipulated stem cells except red cell depletion of the BM harvest for major ABO mismatched and plasma depletion of the donor for minor ABO mismatched transplants if the serum antibody titers were high. We compared the ABO incompatible group (n=106) with ABO identical group (n=150) for the incidence of acute graft versus host disease (A-GVHD), chronic graft versus host disease (C-GVHD) and relapse, and percentage of five year estimated overall survival. The results are summarized in the table below. We did not find any significant difference both for the incidence acute-, chronic GVHD and relapse, and duration of overall survival, either. In spite of significant difference in the incidence of A-GVHD and C-GVHD between peripheral blood - and bone marrow stem cell use (46% vs 36%, respectively) our inter-group analysis within ABO identical and incompatible transplants according to the stem cell source did not reveal any significant difference, either. We concluded that 1- ABO incompatibility did not have any significant impact on the
occurrence of A-GVHD, C-GVHD and relapse, and duration of overall survival, 2. The type of stem cell source, bone marrow versus peripheral blood stem cells, did not affect these statistical results, either.

Most common symptoms were dyspnea on exertion and non-productive cough; median time between BMT and the onset of symptoms was 6 months and all the patients developed chronic GVHD. Pulmonary function tests showed both obstructive-pattern and diffusion defect. In addition bronchial dilatation and air trapping were detected by thoracic high-resolution computed tomography. Recurrent lower airway infections were frequently seen among this population and contributed to the progressive loss of diffusion defect and increased rate of rehospitalization in BO. In the majority of the patients a partial response was achieved using systemic corticosteroids, inhaled steroids and bronchodilator therapy. Three patients died at median +30 months while in complete remission because of progressive respiratory failure. Patients in BO and non-BO group showed similar pretransplant status, stem cell source and incidence of chronic and acute GVHD. We did not observe any relapse on BO group and the median duration of overall survival for BO and non-BO group were 16.5 and 33.5 months, respectively. But we did not find any significant difference on survival curves between BO and non-BO patients (p=0.29). BO is an important cause of non-relapse late mortality and mortality; its both diagnosis and treatment are difficult and heterogeneous. Patients with respiratory symptoms should be evaluated functionally and randomized prospective controlled studies particularly investigating the effects of inhaled steroids for prevention and treatment of BO are strongly recommended because of its unpredictable development and fatal course.

**P772**

**Thromboembolic complications after allogeneic hematopoietic cell transplantation: the Ibni Sina experience**

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Thrombotic complications and especially hepatic veno-occlusive disease (VOD) are the third leading cause of morbidity and mortality after stem cell transplantation, arising mostly due to tissue damage and cytokine release. Risk factors for VOD are pretransplant liver injury or hepatitis, TBI, preparative regimens and the use of hepatotoxic drugs. We have analyzed 308 allogeneic hematopoietic cell transplant recipients retrospectively for the occurrence of thrombo-embolic complications like VOD, DVT, PTE and TTP within our 13 years' transplant experience. The majority of the patients were at standard risk and they received stem cells from their HLA identical siblings. The results are summarized below. We observed an increased frequency of thrombotic complications in patients who received allogeneic peripheral blood stem cells in comparison to bone marrow (p=0.008). VOD incidence observed is lower than reported in the literature (7.4%) and p<0.05. This issue may be related to the absence of TBI containing conditioning to others, and also to the careful selection of transplant recipients (standard risk patients). In two patients with hereditary thrombophilia we observed both VOD and late PTE in the former, and catheter related thrombosis in the latter. The increased incidence of thrombotic complications in peripheral blood transplant recipients needs further evaluation.

**P774**

**Dental development after conditioned bone marrow transplantation for primary immunodeficiency disorders in early infancy**


There are few published data on the development of the permanent dentition in children undergoing conditioned bone marrow transplantation (BMT) in early infancy.

Aims: The purpose of this work was to investigate developmental delay in the permanent dentition of children who had undergone bone marrow transplantation for primary immunodeficiency disorders.

Patients and methods: Children aged over 6 years were recruited from the BMT (Immunodeficiency) follow-up clinic at Great Ormond Street Hospital. All subjects had undergone BMT before the age of 1 year and were conditioned with busulphan and cyclophosphamide. An oral examination was completed. Radiographs included a dental panoramic tomograph and a lateral skull view.

Results: Twelve children have been recruited so far. The diagnoses were: severe combined immunodeficiency (n = 10), Wiskott Aldrich Syndrome (n = 1) and Omenn syndrome (n = 1). The mean age at transplantation was 8.3 months (sd 8.8). Alterations in dental development affected all subjects. Some (n = 6) were relatively minor including bulbous crowns, narrow roots, small teeth and chronological hyperplasia. More serious disturbances were present in 6 children resulting in between 2 and 18 missing permanent teeth.

Conclusions: Children undergoing conditioned bone marrow transplantation in early infancy have alterations in dental development. The exact pathophysiology of this has not yet been determined. There are significant dental and social implications for the most seriously affected subjects as well as considerable financial implications for the necessary remedial work.

**P773**

**Late non-relapse mortality after allogeneic hematopoietic cell transplantation: the impact of Bronchiolitis obliterans**


Respiratory complications affect 40-60% of the patients who underwent allogeneic hematopoietic cell transplantation (AHCT) and are one of the major causes of morbidity and mortality. Obstructive airway disease as a result of bronchiolitis obliterans (BO) occurs at 2-13% of transplanted patients. Post transplant 6-12 months usually is the time for BO and mostly associated with chronic GVHD. Between 1995-2001, 251 patients with various hematological malignancies underwent AHCT from their HLA identical siblings. The conditioning regimens were mainly Bu-Cy and GVHD prophylaxis was short-term methotrexate and cyclosporine. Twelve patients (8 F/4 M) diagnosed as having bronchiolitis obliterans (BO) after AHCT (incidence 4.7%) were evaluated retrospectively. Median age at transplantation was 32.5.

Late non-relapse mortality after allogeneic hematopoietic cell transplantation: the impact of Bronchiolitis obliterans


Respiratory complications affect 40-60% of the patients who underwent allogeneic hematopoietic cell transplantation (AHCT) and are one of the major causes of morbidity and mortality. Obstructive airway disease as a result of bronchiolitis obliterans (BO) occurs at 2-13% of transplanted patients. Post transplant 6-12 months usually is the time for BO and mostly associated with chronic GVHD. Between 1995-2001, 251 patients with various hematological malignancies underwent AHCT from their HLA identical siblings. The conditioning regimens were mainly Bu-Cy and GVHD prophylaxis was short-term methotrexate and cyclosporine. Twelve patients (8 F/4 M) diagnosed as having bronchiolitis obliterans (BO) after AHCT (incidence 4.7%) were evaluated retrospectively. Median age at transplantation was 32.5.
Quality of life of thalassemia transplanted patients

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During the last twenty five years the improvement in the treatment of thalassemia major has determined a significant increase of survival and a better surveillance of complication. Bone Marrow Transplantation (BMT) has improved the life of the patients affected by thalassemia. Many of them have obtained recovery from a disease considered not curable. The quality of life in terms of psychological attitude towards the disease, has changed as well. From a series of 100 consecutive patients with transfusion dependent homozygous beta thalassemia, we considered ten patients who had been successfully transplanted between 1990 and 2000. All donors were HLA identical siblings. Conditioning regimen included busulfan (14-16 mg/kg) and cyclophosphamide (200 mg/kg). GVHD prophylaxis was dose with ciclosporine A. Two years after BMT the patients have been enrolled in a program of regular phlebotomy for the treatment of iron overload (serum Fe levels more than 2.00 microgr/l and moderate or severe iron overload at liver biopsy). Percutaneous biopsy was performed under ultrasound guidance before and after completion of phlebotomy program to calculate the reduction in liver iron concentration.

The aim of our study was to evaluate the quality of life in thirty thalassemia major conventionally treated subjects, matched for age with transplanted ones. The median age of the two groups was 16.6 years. We have used questionnaires with the purpose of investigating on education, employment, emotional status, hobbies, denial isolation and social life. We did not find significant differences between the answers to the questionnaires of the two groups of patients regarding hobbies, education and employment, while thalassemia patients think negatively about physical ability (80% vs 20%), emotional life (67% vs 33%), denial and isolation (85% vs 15%). Thalassemia transplanted subjects are more optimistic than conventionally treated patients. They have positive expectation about their future, have a positive life orientation, internal health locus and are not hopeless in their attribution about life. At present only meloablative regimen performed by marrow transplantation can eradicate thalassemia. A longer follow-up after BMT is needed to monitor and treat related complications and quality of life.

How to assess quality of life in multiple sclerosis patients after autologous peripheral stem cell transplantation

A. Novik, T. Ionova, L. Chelombit, P. Denisov (St. Petersburg, RUS)

Multiple sclerosis (MS) is one of the most dramatic neurologic diseases that a greatly impacts a patient's quality of life (QoL). QoL is the most important outcome of MS treatment. Correct choice of a QoL questionnaire is one of the key-issues in QoL assessment. As methodology of QoL assessment in MS patients after autologous peripheral stem cell transplantation (AP SCT) is lacking the choice of relevant QoL measures is necessary. The aim of this study was to compare the performance of the BMT questionnaire – FACT-BMT and the disease-specific questionnaire - FAMS in MS patients after APSCT.

Materials and methodology: five patients with MS who underwent APSCT (EBMT protocols) were studied. QoL was assessed by FACT-BMT and FAMS at baseline, at discharge and at 3, 6, 12, 18, 24 and 30 months after APSCT.

Results: All five transplantations were clinically successful. QoL improvement was observed for all the patients at the end of follow-up. Positive changes in QoL parameters were noted by both questionnaires. Strong correlation between the physical well-being scale of FACT-BMT and the symptom scales of FAMS was found. However FAMS appeared to be a much more sensitive measure in MS patients after APSCT than FACT-BMT. In particular, for patient P. (female; 48 years old; spinal primary progradent subtype; EDSS 7.5) 2-3 fold increase in QoL parameters of FAMS scales 3 months after APSCT was shown whereas there was only slight improvement of QoL values by FACT-BMT at this time-point. Higher sensitivity of FAMS as compared with FACT-BMT was demonstrated for patient Sh. (21 years old; cerebral-spinal primary progradent remitting subtype; EDSS 4.0). FACT-BMT domains remained stable for 3 months after transplantation in doing so there was an increase only in special BMT-scale scores. At the same time all QoL parameters of FAMS showed measurable improvement.

On the other hand FACT-BMT provided general information on the major QoL domains – physical, psychological, social and functional well-being as well as general QoL and its use was convenient in QoL monitoring at follow-up.

Conclusion: Comparison of the two questionnaires – FACT-BMT and FAMS in MS patients after APSCT showed that the disease-specific questionnaire FAMS is more sensitive in this patient population than FACT-BMT. FACT-BMT is useful in QoL monitoring after APSCT. Simultaneous use of two questionnaires can provide comprehensive information on QoL changes in MS patients after APSCT.

Quality of life as a measurable outcome of autologous peripheral stem cell transplantation in patients with multiple sclerosis

A. Novik, T. Ionova, G. Bisaga, V. Melnichenko, L. Chelombit, P. Denisov (St. Petersburg, RUS)

Quality of life (QoL) is increasingly used as a treatment outcome along with traditional clinical outcomes in multiple sclerosis (MS) patients. Autologous peripheral stem cell transplantation (AP SCT) is a new and promising treatment strategy for patients with MS. Monitoring of clinical and QoL outcomes of APSCT in MS patients is worthwhile. The aim of the study was to provide evaluation and comparison of EDSS level as clinical outcomes and QoL parameters in MS patients before and at different time-points after APSCT.

Materials and methods: Four patients with MS who underwent APSCT (EBMT protocols) were studied. EDSS and QoL evaluation (FACT-BMT - specific for patients with BMT, FAMS - specific for MS patients) was provided at baseline, at discharge and at 3, 6, 8, 12, 18, 24 and 30 months after APSCT.

Results: Stabilization of the disease was achieved in all cases. Follow-up period varied from 8 to 30 months. Comparison of EDSS before APSCT and at follow-up revealed its slight decrease in 3 patients: from 7.5 to 6.5 (Patient P.; female; 48 years old; spinal primary progradient subtype; EDSS 6.0; APSCT 20.02.01. Fluctuation of EDSS level were observed during this follow-up period.

Conclusion: QoL is a measurable outcome of APSCT in patients with MS. APSCT results in distinct improvement of QoL parameters after APSCT whereas there is only a slight decrease of EDSS level. QoL appears to be a more sensitive indicator of outcome than EDSS level in MS patients after APSCT. Further research is to be done to identify values of EDSS level and QoL parameters as the criteria of clinical outcome in MS patients after APSCT.
**P778**

**Emotional and physical intimacy after stem cell transplantation**

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Objectives: The use of allogeneic hematopoetic stem cell transplantation (HSCT) as a treatment for blood-related diseases has increased in the past several years. The increasing number of surviving HSCT recipients has fostered a large body of research examining post-transplant quality of life (QOL) in this group. Based on our recent review of the literature (Dew, Switzer et al., 1997), it is clear that stem cell recipients experience some immediate QOL gains post-transplant, and that these improvements are generally sustained post-transplant. However, difficulties with intimate relationships may be particularly prevalent among HSCT recipients. Research, to date, has neither described the full range of intimacy deficits that HSCT recipients experience, nor investigated the full range of factors that might be related to better or poorer intimate relationship function. Our goals were to describe HSCT recipients' experiences in intimate relationships, and to begin to identify the characteristics of individuals who are at high risk for experiencing problems in intimate relationships.

Methods and Results: Potential subjects included surviving adult HSCT recipients who were transplanted at domestic National Marrow Donor Program (NMDP) transplant centers between 01/01/93 and 01/01/01. Transplant recipients >18 years old and in an intimate relationship (> 6 months duration) at the time of transplant were included. Thirty-two transplant recipients who met eligibility requirements were randomly selected to participate in a 45 minute in-depth telephone interview. The interviews are designed to gather information on general physical and psychological health, and key issues related to intimate relationships. Data will be analyzed using standard content analysis techniques to identify themes in HSCT recipients' responses and to discover less commonly mentioned, but important factors related to intimacy post-transplant.

Conclusion: Although multiple studies have examined global QOL in HSCT recipients, no studies have focused specifically on intimate partnerships post-transplant. The evidence that HSCT recipients may experience deficits in sexual functioning and that such deficits could lead to decreased satisfaction with recipients' intimate relationships makes it critical to examine the effects of the transplant on physical and psychosocial issues as they relate to general intimacy and sexuality in particular.

**P779**

**A study of the effects of religious faith and family support on the quality of life in survivors undergoing allogeneic stem cell transplantation**

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Objective: A number of studies have found that for cancer patients, religious, spiritual concerns and family support are paramount. The complex cultural and religious beliefs and values in Jordan present unique patient needs which must be addressed. The medical literature revealed very little research related specifically to the Arab culture. We report on the effects of religion, spirituality and family support on the physical, emotional health and psychosocial adjustment of patients undergoing allogeneic Stem Cell Transplantation (alloSCT).

Methods: A cohort of 40 patient survivors (29 females, 31 males), who underwent alloSCT between November 1995 and November 2001, for a variety of benign and malignant haematological conditions were interviewed using questionnaire-based study. All patients were over eighteen-years of age. The age range and median were (18-48, 29 years). Eighty percent of patients identified Islam as their religion; the other 20% were Christians.

Results: Older patients (over 20 years) in both religions consistently reported higher degrees of spiritual well being and identified as being religious than did younger patients. Also, married patients reported higher degrees of spiritual well being and identified as being religious than did single patients. Major religious changes occurred in Moslems and Christians patients (M&C) (40/48 vs. 10/12, p = 0.001 respectively). Visits on a daily basis by a family member in M&C (87% vs. 92%, p<0.05 respectively). Blood product donation by a family member in M&C (72% vs. 78%, p<0.05 respectively). All our stem cells donors were siblings and sense of obligation and satisfaction at the same time were there in M&C (90% vs. 99% respectively). "My suffering is God's will" was observed in M&C in (100%).

Conclusions: Religious, spiritual faith and family support provides patients who underwent alloSCT with important tools for coping with their illness and should be recognized by treating physicians. It may be important to encourage patients seek religious support and to reconnect with their religious community. Cultural issues pertaining to family practices played an important role in the care giving process. Finally, the role of religion and strong family ties to maximize the patient's ability to use religious resources and family support as a source of coping with pain and suffering can be a deeply rewarding area of medical practice.
P781

Severe central nervous system complications after allogeneic stem cell transplantation (SCT)

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In three male patients (pts), 38 to 46 years old, severe central nervous system complications developed after allogeneic SCT. Diagnoses at transplantation were AML in 1, CR in two pts and refractory ALL in 1 pt. Two to five chemotherapy courses including high-dose Ara-C were given before SCT. The pt with ALL additionally received intrathekal chemotherapy and high-dose MTX, but no cerebral irradiation. The donors were HLA-identical sibling, HLA-identical and HLA-mismatched unrelated donors. The conditioning regimen consisted of BuCyVP16, TBI (12Gy)/Cy and TBI (12Gy)/Cy/VP16. For GVHD prophylaxis CSA/MMF was used, with ATG in the two pts with unrelated donors additionally. Maximal acute GVHD was grade II. 1 pt suffered from limited chronic GVHD. In all of them symptoms developed slowly and fluctuating, starting between day 50 and 300 after SCT. All pts became dependent on 24 hours daily care finally. In one pt symptoms started with orthostatic hypotension and a loss of short-term memory, progressing to dementia in about 40 days. He died because of central nervous system failure 9 months after SCT, autopsy was unspecific. In the other two pts severe symptoms of parkinsonism developed relatively fast, unresponsive to antiparkinsonian drugs. One pt died after a septic complication, the other is alive in a disable state. In all pts cerebral CT and NMR showed only slight atrophy and EEG was unsuspicious. In cerebrospinal fluid only slight elevation of protein was seen, no evidence for a central nervous system infection could be demonstrated at any time. We could not find a conclusive explanation for these fatal complications.

P782

Incidence and risk factors for hepatic veno-occlusive disease (VOD) after allogeneic hematopoietic stem cell transplantation in 251 consecutive adult patients: a single center study


Two-hundred fifty one patients who received allogeneic hematopoietic stem cell transplant in our Institution were evaluated for occurrence of VOD. The median age was 36 years (15-55), 141 were males and 110 females; they were affected with CML (77 cases), idiopathic myelofibrosis (2 cases), acute leukaemia (139 cases) or advanced myelodysplastic syndrome (33 cases). The donor was a HLA identical sibling for 195 recipients and a well-matched unrelated donor in 56 cases. Sixty patients received peripheral blood stem cells and 191 bone marrow. Conditioning regimen was a TBI based combination in 100 cases and busulfan-cyclophosphamide or other chemotherapeutic associations in 151 cases. GVHD prophylaxis was performed with Cyclosporine and short course methotrexate in 156 cases and with Cyclosporine A and methylprednisolone in 70 cases. VOD was defined according to the following criteria occurring before day +30: hyperbilirubinemia >2 mg/dl, ascites or weight gain >5% of baseline body weight and painful hepatomegaly. VOD was classified according to the described criteria as mild, moderate or severe. The influence of previously described risk factors were analysed using logistic regression models. VOD developed in 12 cases (4.7%). It was classified as mild (3 cases), moderate (3 cases) or severe (6 cases). Five patients recovered, 4 of them are alive and well, one died of pneumonitis at day +76; five patients died of multiorgan failure, two of concurrent infection. All but one had severe VOD.

In univariate analysis variables associated with an increased risk of VOD were: TBI based conditioning regimens, type of donor (unrelated versus related), GVHD prophylaxis with cyclosporine associated with methotrexate; in multivariate analysis unrelated transplant was the only variable predictive of VOD (RR 5.5; P 0.04). The incidence of VOD was lower than that reported by other authors. This study confirms that severe and moderate disease correlate with a high mortality rate.

P783

Acute respiratory failure, gross hemoptysis and severe hypotension after autologous peripheral blood stem cell infusion

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Introduction: Severe, life-threatening reactions related to the infusion of rapidly thawed peripheral blood stem cells (PBSC), although rare, have been reported. We present a case of acute respiratory decompensation, hemoptysis and severe hypotension immediately following autologous PBSC infusion.

Case report: A 12-year old boy had to perform an autologous peripheral blood stem cell transplantation for Hodgkin’s disease in partial remission, second relapse. The patient had a history of atopy, the cumulative dose of pre-transplant doxorubicine exposure was 160 mg/me2 and received no mediastinal radiation. The conditioning regimen was performed in conformity with the BEAM protocol. A few minutes after the patient received the third Bag of thawed PBSC (total CD34+ cells = 7.7×10^6/kg), he presented severe dyspnea, cough, gross hemoptisis, severe hypotension, tachycardia and decrease of SaO2. The chest roentgenogram showed opacity of reduced intensity, inhomogeneous in the middle and partial at upper and lower level of the left hemithorax; D-dimer test was negative. The patient was treated with oxygen, corticosteroids, diuretics, epinephrine and dopamine. He recovered clinically rapidly and the chest roentgenogram became normal in 8 days. The patient was discharged, being in complete remission after 4 months.

Conclusions: Although our presumed cause for this uncommon reaction was the pulmonary micro-thromboembolism, we propose and discuss an intricate mechanism including several other entities, transient left ventricular failure, acute respiratory distress syndrome (favoured by atopy), anaphylaxis due to dimethylsulfoxide and diffuse alveolar hemorrhage.

P784

Successful treatment of severe hemorrhagic cystitis by selective embolization of vesical arteries in HCT

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Hemorrhagic cystitis (HC) is a common and sometimes life-threatening complication of HCT occurring from 1 to 35% of transplant recipients. Its treatment is based on intensive hydration, transfusions, bladder irrigation and pain management. When these measures do not control the HC, numerous therapeutic approaches have been tested with low success. We report two HCT patients with severe HC successfully treated with selective embolization of vesical arteries.

Case 1.
A 56-year-old female with a NHL in 3rd PR underwent an autologous BM+PBSC transplantation conditioned with BEAC. Despite a correct neutrophil engraftment (day +7), she remained thrombocytopenic for a long time. On day +28 she developed severe HC. All cultures of urine including viral isolation were negative. In spite of supportive measures with hydration, continuous bladder irrigation and transfusions, the patient did not improved and on day +78 a selective embolization of both vesical arteries was performed. The HC resolved and the patient could be discharged without further complications; she remains well and alive 3 years after transplantation.

Case 2.
A 27-year-old male with refractory Hodgkin’s disease received an allo-PBSC from his HLA-identical brother conditioned with CY (120 mg/Kg) and fractionated TBI (12 Gy). On day +40 he started to complain of urinary frequency, and pain on micturition with gross hematuria and clots. Cultures of urine were negative but characteristic morphologic changes in the urinary sediment by electron microscopy of polyomavirus BK virus were seen. The PCR for polyomavirus BK was also positive. There was no improvement in symptoms after several days of hydration, continuous bladder irrigation and transfusions. On day +75 a selective embolization of the left vesical artery was performed. HC improved immediately. Three months after the procedure the patient is well.

In summary, we report two cases of severe HC successfully treated by the selective embolization of vesical arteries, a procedure that could be considered as a treatment option in cases of severe HC refractory to conventional treatment measures.

P785

Early liver dysfunction after unrelated donor cord blood transplantation (UD-CBt)


Introduction: The aim of this study was to analyse the incidence, characteristics and outcome of early liver dysfunction, including veno-occlusive disease (VOD), after UD-CBt.

Patients and Methods: Twenty-two patients with haematologic malignancies, median age 30 years (range 18-46), underwent UD-CBt at our institution between July/97 and February/01. Conduction of regimen consisted on thiotepa (10 mg/kg), busulfan (12 mg/kg), cyclophosphamide (120 mg/kg) and horse anti-thymocytic globulin (15 mg/kg/day for 4 days). Cyclosporin A and prednisone were given for GVHD prophylaxis. VOD was defined according to the Jones et al. criteria and all patients were daily tested for AST, ALT, bilirubin (BT), GGT and alkaline phosphatase (AF) before the start of conditioning until day +30.

Results: Five patients (23%) developed VOD (moderate in 3 cases and severe in 2). VOD was diagnosed at a median of 8 days after transplant (range 1-10). Maximum BT level was 10.9 mg/dL (range 2.7-56) and it was reached at day +17 (range 10-44). None of the analysed variables were associated with a higher risk of VOD. The probability of death before day +100 was significantly higher in patients with VOD (80% vs. 29%; P = 0.04). Significant changes from baseline levels were observed in the values of all liver function tests. Increases upper of double normal value occurred in 7 patients (32%) for AST; in 18 (82%) for ALT; in 15 (68%) for BT; in 18 (82%) for GGT; and only in 1 (5%) for AF. Maximum AST levels were significantly higher in patients with a previous haematopetic stem cell transplantation (P = 0.05) and maximum GGT and AF levels were significantly higher in older patients (P = 0.006 and P = 0.02, respectively). No other significant differences were observed. The patients who reached maximum AST levels upper double normal value had a higher probability of early death (71% vs. 20%; P = 0.01). According to Bearman et al. liver toxicity was graded as grade 0 in five patients (23%); grade I in 10 (46%); grade II in 4 (18%); grade III in 1 (5%); and grade IV in 2 (9%).

Conclusions: The incidence of VOD after UD-CBt in our series is in line with other studies. Cultures of liver function tests were constant and the mortality rate directly related to liver dysfunction was considerable.

P786

 Favorable outcome of isolated membranous nephropathy in an allogeneic stem cell recipient


A variety of factors can contribute to renal damage after allo-SCT. In either CsA-or-radiation-induced renal damage, endothelial cell injury leading to microvascular coagulation within the kidney is considered to be the primary pathogenesis. We present a case of membranous nephropathy (MN) presenting as nephritic syndrome after allo-SCT. A 34-year-old female with AML underwent allo-SCT from her HLA-matched brother after conditioning with Bu/CY. CsA-short MTX was given as GVHD prophylaxis. Engraftment occurred rapidly. As early complication, she experienced CY induced severe refractory haemorrhagic cystitis that resolved after hyperbaric oxygen therapy and heparin therapy. She was advised to HBV reactivation during tapering of CsA doses. On day+180, CsA was withdrawn and she acquired spontaneous immunity for HBV. Four months later, pedal edema developed. The urinary protein excretion was 6g/day. The serum albumin fell to 2.62g/dl. The patient appeared healthy. Serum creatinine clearance and hepatic function were within normal limits. She was in hematological remission and DNA analysis revealed complete chimerism. Renal biopsy showed thin granular deposits of IgG and C3 along the glomerular basement membrane, a characteristic pattern of MN. Tests for HBsAg, HBV DNA, HCV, antinuclear, anti-double stranded DNA, and antithyroidperoxidase antibodies were negative. Serum C3 and C4 levels were normal. Immune complexes, and rheumatoid factors were not found. Chest x-ray and ultrasonography of the abdomen were normal. In the absence of obvious underlying disease, atorvastatine and ACE inhibitor were given to reduce lipid levels and urine protein excretion, respectively. As disease-specific therapy, prednisone was given as a long course which was with no benefit. More aggressive therapy such as combined steroid/CsA was refused by the patient. Steroid was tapered and withdrawn. 18 months after the diagnosis of MN, the albumin level returned to normal and pedal edema disappeared whereas proteinuria persisted but less than 0.5 g/dl. This patient had no history of drug ingestion or of any disease that might cause MN. Therefore, it was speculated that the disorder was related with the autoimmune disease-like features of chronic GVHD. Unlike previously published case reports, she had no other accompanying organ involvement of chronic GVHD. The favourable outcome of this patient subjects the question whether the disease specific therapy is necessary in patients with low risk of progression.
P786
Low incidence of secondary myelodysplasia (sMDs) and acute myeloid leukemia (sAML) after high-dose chemotherapy and autologous bone marrow transplantation for breast cancer patients

We determined the incidence of sMDs or sAML in 364 node positive breast cancer patients who received high-dose chemotherapy followed by autologous stem cell support as adjuvant therapy between 1989 and 1997 and were reported to the EBMT registry. The median age of the patients was 45 years (range, 22-62). 291 patients received peripheral blood stem cells, and 55 patients received autologous bone marrow as stem cell support. The most frequently used conditioning regimen was the STAMP-V regimen (35%), followed by Melphalan/Thiotepa (23%) and Melphalan, Mitoxantrone and Cyclophosphamide (16%). The 5 year probability of overall survival is 71% (95% CI: 65-77%). After a median follow up of 48 months (range, 1-108) only one case of sAML was observed resulting in a cumulative incidence of 0.27%. This case of sAML was observed 18 months after a high-dose chemotherapy consisted of 3 cycles high dose epirubicin and cyclophosphamide and a cumulative dose of 960 mg epirubicine and 19 g cyclophosphamide (IBCSG 15/95) The FAB types of sAML was M4, and the cytogenetic analysis showed a translocation t(9;11). After complete remission following high-dose chemotherapy and 8% of patients without acute GVHD had neurotoxicity (P value=0.006) and also 18% of the patients developed grade I-II of acute GVHD and 30% with grade III-IV of acute GVHD had neurotoxicity (P value=0.021). All of the results were identified using Chi-Square test.

Conclusion: We conclude that significant predisposing factors for neurotoxicity of cyclophosphine include Allogeneic PBSCT, hypertension, Hyperlipidemia, hypomagnesemia, acute GVHD and grade III-IV of acute GVHD.

P787
Secondary neoplasms in pediatric patients after bone marrow transplantation: single center experience
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Secondary neoplasms are well-recognized complication of hematopoietic progenitor cell transplantation (HPCT). They usually occur five years or later after the procedure. The risk of developing secondary cancer increases in young children. We present three children transplanted in the Department of Pediatric Oncology and Hematology at Wroclaw University of Medicine who developed new neoplasm early after transplantation. Between 1994 and the end of 2000 in our institution 162 children underwent HCT.  Conditioning based on chemotherapy only - no TBI was applied. One hundred forty patients survived longer then 100 days including 61 girls and 71 boys with median age of 11 years (median follow up 20 months). Among them 44 underwent allogeneic and 96 autologous transplantation, ten patients were diagnosed with non-malignant diseases and 130 suffered from malignancies. Secondary neoplasm was diagnosed in 3 out of 140 children (2,2%). Characteristics of the children are summarized in a table. One patient (UPN 7) developed secondary AML M4 with t(4;11)(q12;q24) six months after transplantation. Therapy was unsuccessful and the girl died 30 days later due to overwhelming leukemia. In patient UPN 98 secondary NHL-T (mediastinal tumor with pleural involvement) was diagnosed 15 months after HPCT. She is treated according to NHL non-B FBM 90 protocol, now in CR with no evidence of disease and remains 3 months after transplantation still on maintenance therapy. Patient UPN 167 was diagnosed 6 months after HPCT with intraspinal astrocytoma (C2-C6) which was unoperative and due to localization impossible to be biopsed. The tumor was resistant to the radio and chemotherapy. The patient died in neoplasm progression due to hemorrhage and respiratory failure 14 months after transplantation.

Conclusions: secondary neoplasms are serious complications in children after HPCT and non-TBI conditioning. They may occur early after transplantation. Prior conventional chemotherapy and
radiotherapy may be significant cause of early incidence of new malignancies after HPCT.

### P791
**Autologous hematopoietic stem cell transplantation in patients over 60 years, low toxicity and transplant related mortality - Single center experience**

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High dose therapy with autologous hematopoietics stem cell transplantation (ASCT) is a widely used method of dose intensification in patients (pts) with hematological malignancies, however, patients over 60 are mostly excluded probably due to an anticipated high rate of transplant related mortality (TRM). We have evaluated high-dose therapy and ASCT in 58 cases involving 52 patients.

Patients and methods: Between September 1994 and 2001 we autotransplanted 52 pts (26 females and 26 males) with multiple myeloma (34), DLBCL (6), PTOL (4), MCL (3), FCL (1), CLL (3) and MALT-lymphoma (1) over 60 years (range 60-73, median 63). 6 pts with multiple myeloma received a double transplant. Pretreatment conditioning consisted of melphalan alone in 34 pts, BEAM in 15 pts and high-dose cyclophosphamide in 3 pts. Patients were eligible if they had a good performance status, normal cardiac, respiratory, hepatic and renal function (except of pts with multiple myeloma).

Results: All 52 pts engrafted with median of 11 (range 8-14) days to ANC over 500/ul and with median of 15.5 days (range 9-16) to reach platelets over 20.000/ul. 2 pts died within the first 100 days after transplantation (one of bronchopneumonia and one of cardiac failure) with an overall TRM 3.8%, 10 pts (19%) relapsed and died between 3-34 month (median 11.5), 1 pt died 6 months after ASCT from intracerebral ischemia and 1 pt died 7 months after ASCT from bronchopneumonia and hepatic failure. 38 pts (73%) are still alive with median of 16 months (range 5-69) after ASCT with the probability of 5-years survival of 59%.

Discussion: Although our cohort of pts is not very large and pts were selected according to their status before ASCT, we conclude that high-dose chemotherapy with ASCT is feasible in pts aged over 60 years with low toxicity and early transplant-related mortality. Elderly pts with hematological malignancies potentially curable with high-dose chemotherapy should be candidates for ASCT.

### P792
**Autologous stem cell transplantation in patients older than 60 years**

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We have analyzed our recent experience in HDT supported by peripheral blood stem cell transplantation in patients aged over 60 with hematological malignancies; we compared the feasibility and toxicity of the procedure with a group of younger patients underwent transplantation in the same period of time.

In the last three years, 18 elderly patients (10 males-8 females), median aged 65(60-70) entered in our autologous transplant program. Diagnosis was lymphoma (6 cases), myeloma (9), acute myeloblastic leukemia (2) and one myelodysplastic syndrome. The clinical status at transplant was 5 of 6 lymphoma patients were in completed remission (CR). 3 of 9 myeloma patients were in CR, and all AML patients and the MDS patient were in CR. The other patients were in partial remission and two were refractory.

Mobilization of CD34+ cells was achieved in 11 cases with chemotherapy and G-CSF, in 6 patients with G-CSF only, and in one case needed mobilization with SC-F and G-CSF.

We compared these group of patients with 34 patients younger than 60 years old, median age 51.5 (14-59), 16 males-18 females, with the following diseases: 17 lymphomas (12 in CR, 3 in PR and 2 in progression), 14 myelomas (3 in CR, 9 in PR and 3 in progression) and 3 AML, all in CR. They were mobilised with G-CSF in 19 patients (55.8%) and 15 patients needed chemotherapy and G-CSF. Myeloablative schemes included in elderly patients group: HD Melphalan in 9 cases, BEAM and BEAC in 6 cases, Cy-TBI in 2 cases and Bu-Cy in one patient. In younger group the conditioning regimen was 12 HD Melphalan, 14 BEAC or BEAM, 6 Cy-TBI and 2 Bu-Me.

No significant differences were observed in the number of CD34+ cells collected , but in the group of younger patients, most of them were mobilized with G-CSF only (55.8% vs. 33.3%). There were no differences in recovery of neutrophils, supportive care and day to discharge, but we found statistical differences in time to reach platelet>20 X 10^9/ul (median 16.5 vs. 14, p =0.01). The procedure appeared to be more toxic in older patients, oral mucositis >WHO grade 2 was more common in these patients but organ toxicity was similar. Two patients, one of each group, died in first 100 days after transplant, due to regimen related toxicity.

We conclude that high-dose therapy with ASCT is possible in elderly patients with normal performance status and can be used with tolerable toxicity.

### P793
**Feasibility of autologous stem cell transplantation (ASCT) in elderly patients (pts): a single center experience**

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Introduction: we report our experience in elderly pts undergoing ASCT for haematological malignancy in a single Institution.

Materials and methods: between March 1997 and October 2001, 31 pts aged 60 or more with haematological malignancies underwent 39 ASCT procedures in our Centre. ASCT was given as consolidation therapy as part of a first-line treatment in 26 pts; the other 4 were in second CR, 1 had a resistant T-LNH. Eligibility criteria were: a good performance status (ECOG 0-1), without relevant co-morbidity. Pts undergoing ASCT included: 15 multiple myeloma (13 in RP and 2 refractory); 1 Amyloidosis; 13 non-Hodgkin lymphoma (12 in CR and 1 refractory); 2 CLL (both in CR). The median age at transplant was 64 years (60-70). Double transplant was given in eight MM pts. The conditioning regimens included: high-dose melphalan (100-200 mg/m^2, as single agent) in 23 pts., BEAM in 7, BAVC in 1, Mito-Mel in 8.

Results: All pts. but one underwent PBSC collection, with a median yield of 8.6 X 10^6/Kg. CD 34+: The patient failing PBSC...
P794
Quantification of T-cell receptor excision circle DNA by quantitative PCR and the LightCycler system
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T cell receptor excision circles (TRECs) are generated during V(D)J gene recombination, a process responsible for diversity of the T cell receptor repertoire. They are circular, stable extrachromosomal DNA fragments which do not replicate with cell proliferation. Thus, the number of TRECs is a potential marker to estimate thymic output. Here we describe a sensitive, rapid and easy to perform real-time PCR assay based on the LightCycler technique for the quantification of TRECs among peripheral blood cells. We compared the number of TRECs found in cord blood to the number of TRECs in blood specimens (n = 52) obtained from healthy individuals with ages between 1 and 80 years and patients (n = 20) after allogeneic stem cell transplantation by LightCycler and by conventional PCR-ELISA. Furthermore, TREC levels in unFractionated PBMCs and in MACS-sorted CD45RA+ T-cells were analyzed. By LightCycler, a sensitivity of 20 copies of TREC-DNA was achieved whereas by PCR-ELISA, a detection limit of 2 copies was demonstrated. The LightCycler assay showed linearity between 2 x 109 and 2 x 101 copies. To demonstrate the reproducibility, amplification of serially diluted TREC DNA (2 x 106 to 2 x 101 copies) was repeated 30 times. Amplifying 2 x 106 copies, we obtained a median crossing point of 21.0 cycles (standard deviation 1.05 cycles), amplifying 2 x 101 copies, a median crossing point of 36.7 cycles (standard deviation 0.99 cycles) was achieved. This demonstrated the high reproducibility of the LightCycler assay. We also examined whether the number of TRECs in peripheral blood cells was associated with age. We achieved in blood samples from healthy individuals a median TREC count of 1.6 x 104 copies (range 2 x 101 to 2 x 105 copies). Twelve persons had levels of TRECs below the limit of detection from whom 4 were 70 years and older (median 55.5 years). In cord blood, we found a median TREC count of 1.45 x 105 copies (range 1.2 x 105 to 1.6 x 105 copies). No significant difference was found between unFractionated PBMCs (median count of 1.75 x 105 copies) and MACS-sorted CD45RA+ T-cells (median count of 1 x 105 copies). In conclusion, the LightCycler-based real-time PCR assay described offered a very sensitive, rapid, simple and inexpensive method for the quantification of TREC DNA. In the future, routine measurement of TREC levels with this test system may allow to indicate the potential for recovery of a damaged immune system.

P795
Altered immunity syndrome, a distinct entity in long-term bone marrow transplantation survivors?
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Current concepts of the pathogenesis of chronic graft-versus-host disease (cGvHD) are poorly understood but based upon the induction of responses to host tissue antigens. The similarity between cGvHD and autoimmune disorders is well described. However, a syndrome similar to connective tissue diseases but unrelated to cGvHD has been postulated in long-term survivors after allogeneic haematopoetic stem cell transplantation (HSCT). To further investigate this hypothesis, a retrospective analysis of 38 patients surviving HSCT for at least 10 years (mean 13.9 years) was performed. We screened for the presence of autoantibodies (anti-nuclear, anti-mitochondrial, anti-parietal cell, anti-heart muscle, anti-smooth muscle, anti-skeletal muscle, anti-parotid antibodies) and symptoms of a connective tissue disease (arthralgia, sicca syndrome, skin manifestations, reduced exercise tolerance, fatigue). A symptom score was calculated with a range of 0 to 5. Patients with autoantibodies (group I, n=20) had significantly higher score values (2.7 vs. 1.4; p<0.01) than patients without these antibodies (group II, n=18). The percentage of autoantibody positive patients increased with the symptom score (p<0.05). When analysed separately each scored symptom occurred more frequently in autoantibody positive patients. Our findings were independent of the presence of cGvHD. No significant differences between the two groups could be found regarding a number of standard parameters. We hypothesise that altered immune reconstitution may put long-term survivors after allogeneic HSCT at risk for late secondary autoimmune-like phenomena.

P796
Dendritic cells (DC1 and DC2) recovery after allogeneic and autologous stem cell transplantation

In peripheral blood (PB) two subsets of dendritic cells (DC) have been identified: DC1, myeloid, are lineage negative, HLA-DR positive, CD11c positive; DC2, lymphoid, are lineage negative, HLA-DR positive and CD123 positive. DC1 activate T lymphocytes, while DC2 seems to induce antigen-specific tolerance. We used a 3-colour flow cytometric assay to assess DC1 and DC2 reconstitution in 14 patients undergoing allogeneic transplantation for haematological malignancies: 8 patients (group 1) received CD34 positive selected PBSC plus 1x 10^6 CD3 positive cells from an HLA identical sibling donor, while 6 patients (group 2) received not manipulated bone marrow stem cells from a matched unrelated donor. 23 autologous transplant patients were used as control group. DC were identified in lysed whole blood as lineage negative (CD3, CD16, CD56, CD14, CD19, CD20), HLA-DR positive, CD11c positive (DC1) or CD123 positive (DC2). Prior to the beginning of conditioning regimen the mean number of DC1 and DC2 (per microliter) was 2.5±0.1 and 2.8±0.1 respectively. At admission of the patients after 6 months from transplant mean number of DC1 and DC2 (per microliter) was 2.4±0.1 and 2.4±0.1 respectively. In allogeneic patients instead, the mean DC1 and DC2 number was lower at day +7 the mean DC1 and DC2 number was lower than 0.02±0.001. All autologous patients recovered to the pre-transplant number of DC1 and DC2 within day +20 from transplant (3.0±0.1 and 2.7±0.1, reaching to normal numbers after 6 months from transplant (6.2±0.1 and 5.7±0.1). In allogeneic patients instead, the mean DC1 and DC2 number was lower at day +20 and day +60 in both groups and recovered to the pre-transplant number at day +90 (1.2±0.1 and 2.0±0.1-group 1; 2.4±0.1 and 2.4±0.1-group 2). One year after transplant mean
number of DC1 and DC2 was lower than in normal subjects in group 1 (1.4±0.1 and 1.1±0.1) but similar to normal subjects in group 2 (5.0±0.1 and 3.1±0.1). In conclusion, DC1 and DC2 recovery is markedly delayed following allogeneic transplant (especially in manipulated stem cells transplantations) compared with autologous transplant: this is probably linked to a delay in immune system reconstitution. Studies are in progress to evaluate the relationship between DC reconstitution and graft-versus-host disease.

P797
The quantitation of NK cells may be incorrect when measured as CD16/56+CD3- after transplantation

According to the US NCCLS it is recommended and now widely accepted for flow cytometric NK cell determination to use the combination of CD16 and CD56 and CD3 for T cell exclusion. Commonly, a gate is created around the lymphocytes and the compartments therein, i.e. B, T, and NK cells should result in a sum of 100%. However, in patients after transplantation this sum of the lymphocyte subsets sometimes significantly fails to reach about 100%. Obviously, there are myeloid/monocytoid cells within the gate and some of those share the CD16 antigen with NK cells. Therefore, we have included a CD33/CD16 combination of the gate and some of those share the CD16 antigen with NK cells. Moreover, we have measured in our patients’ sera the CD16 expression (NKc=NKu-CD33/CD16+) were 47.0% and 42.9%, respectively and differed statistically significant (P<0.0005). The difference between the NKu and NKc cells ranged from 0.2% to 25.9%. For concordance and plausibility we have created three check sums (cs): cs1= %B cells + %NKu cells + %T cells, cs2= %B cells + %NKu cells + %T cells + %CD33 cells, and cs3= %B cells + %NKc cells + %T cells + %CD33 (CD33+CD16-) myeloid cells. The mean values of cs1, cs2, and cs3 were 92.0%, 103.3%, and 90.2%, respectively. As expected, cs1 was significantly lower and cs2 was significantly higher than 100% (P<0.0005). Only cs3 was about 100% and represents therefore the accurate sum of the compartments of the lymphocyte gate. Our data suggest that the lymphocyte gate created in flow cytometric investigations for immune reconstitution after transplantation can hold up to about 26% myeloid cells and approximately a half of them can express the CD16 antigen. Therefore, the portion of CD16+ myeloid cells must be taken into consideration for accurate quantitation of NK cells.

P798
Phenotype, function and chimerism of monocyte-derived dendritic cells (moDCs) after allogeneic stem cell transplantation (SCT)
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Background: Antigen-presenting cells (APCs) such as DCs are essential for initiating T cell responses against either host- or leukemia-specific antigen. Patient and Methods: Immunephenotype, allostimulatory capacity, and chimerism of moDCs were analysed at various time points after allogeneic SCT in 17 pts. suffering from AL (n=9), CML (n=3), lymphoma (n=3), and MDS/MPS (n=2). MoDCs derived from their healthy sibling (n=10) or unrelated donors (n=7) served as control. Mature moDCs were generated from adherent PBMC cultured in the presence of GM-CSF and IL-4 for 7 days. and TNF-a for additional 72 h. Immunephenotyping was performed using the following directly conjugated MoAbs: CD1a-PE, CD14-FITC, CD40-Cy-Chrome, CD80-PE, CD83-PE, CD86-Cy-Chrome, HLA-class I-FITC, and HLA-class II-PE.

Results: Similar to mature moDCs of healthy donors patients’ pretransplant mature moDCs were negative for CD1a (5±2%), CD14 (2±1%), but expressed CD40 (42±5%), CD80 (40±5%), and CD83 (7±4%), and were highly positive for HLA class I (68±4%) and class II (82±6%). With the exception of the higher CD14 and lower CD80, CD83, and CD86 expression within the first 3 months posttransplant following RIC no significant differences in the antigenic profile between pre- and posttransplant mature moDCs were observed in pts. receiving myeloablative conditioning. The allostimulatory capacity of mature moDCs was only reduced early after SCT (d=14, stimulation index, SI, at a R/S ratio of 20:1, 2.0±0.7 vs 5.2±1.4 pre SCT ) reaching pretransplant or even normal values within 30 to 60 days posttransplant. After myeloablative conditioning mature moDCs were of 100% donor origin at any time point after SCT determined by VNTR analysis. In pts. receiving RIC two distinct patterns of chimerism were observed. MoDCs achieved full donor chimerism in one patient developing severe and finally fatal GVHD while in the other patient donor chimerism of mature moDCs rapidly decreased from 90% to 10% within one month prior to leukemia relapse.

Conclusion: The data presented although preliminary reveal a rapid phenotypic and functional reconstitution of moDCs after myeloablative allogeneic SCT, whereas a reduced maturation capacity of moDCs post RIC was observed. Chimerism analysis of moDCs following RIC might be helpful for identifying patients at risk for either GVHD or subsequent relapse.

P799
Late effects in children receiving a TBI-based preparative regimen for HSCT: 10 years of experience in a single center
M. Faraci, B. Cappelli, S. Dallorso, S. Barra, B. Podesta, G. Moreale, G. Dini, E. Lanino (Genoa, I)

Aim of the study is to evaluate late effects concerning eye, bone, lung, thyroid, ovary and secondary neoplasm in 168 children (67 F, 101 M) receiving TBI before HSCT (median age at HSCT 6 yrs, range 1-19) from 1985 to 1995. Underlying diseases were solid tumor (42%), AML (15%), ALL (34%), CML (3%) and lymphoma (6%) and 16% of patients had a history of previous RT. The median total dose of TBI was given over 3 days in 95 pts (330 cGy x 3 = BF) Patients TBI was given over 3 days in 3 fractions in 95 pts (330 cGy x 3 = BF) Patients TBI was given over 3 days in 3 fractions in 95 pts (330 cGy x 3 = BF) Patients TBI was given over 3 days in 3 fractions in 95 pts (330 cGy x 3 = BF). Patients TBI was given over 3 days in 3 fractions in 95 pts (330 cGy x 3 = BF). Patients TBI was given over 3 days in 3 fractions in 95 pts (330 cGy x 3 = BF). Patients TBI was given over 3 days in 3 fractions in 95 pts (330 cGy x 3 = BF). Patients TBI was given over 3 days in 3 fractions in 95 pts (330 cGy x 3 = BF).

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ANC > 0.5 G/l on day +9, PLT > 50 G/l on day +30.

Promising features, whereas the others still need substitution therapy. A secondary neoplasm (thyroid carcinoma 3, schwannoma 2, fibrosarcoma 1; breast adenoma 1, AML 1) was diagnosed in 8 pts after a median of 7 yrs (range 1-13) from TBI (MF 4%, BF 5%; <3 yrs 1%, > 3 yrs 5%). In conclusion, ocular, bone, thyroid and lung complications after TBI are more common and serious in young children. Our data support the current opinion of avoiding TBI in preparative regimen in children who are less than 3 yrs old. A prolonged follow up is recommended to all pts who received TBI to prevent and treat late events.

Additional abstracts to this topic

Cyclosporin-induced encephalopathy in five patients with major beta-thalassemia treated with related allogeneic bone marrow transplantation

M. Jahani, A. Ghavamzadeh, M. Iravani, M. Mojtabavi Naini, B. Bahar, H. Gholam Natanzi, S. Basiranpanah (Tehran, IR)

Cyclosporine (CSP) is the most frequently used immunosuppressive agent for prevention of graft versus host disease (GVHD) in allogeneic bone marrow transplantation (BMT). Some adverse effects such as hepatic and renal toxicity have been frequently encountered, but central nervous system (CNS) toxicity caused by CSP is rare. We report five patients with major beta-thalassemia who developed CSP induced encephalopathy under treatment for allogeneic BMT from siblings. Abrupt onset of mental confusion, disorientation and visual disturbance followed by seizure occurred on day 19 to 48. All patients had grade III GVHD at presentation of neurologic toxicity. The whole blood CSP level in all patients was low or lower limit of normal (between 25 to 279ng/ml) (Normal >100 to 300ng/ml). Magnetic resonance imaging (MRI) was, in some patients, normal and in others revealed high signal intensities in the right parietal lobe with predominant involvement of the cortical areas. 3 to 9 days after CSP was decreased, the patients recovered from the CNS toxicity. Unfortunately one patient died of respiratory infection 351 days after BMT.

When patients receiving CSP treatment for allogeneic BMT developed mental confusion, consciousness disturbance or seizure, CSP induced CNS toxicity should be taken into consideration.

Autoimmune thrombocytopenic purpura accompanys rapid B-cell and activated T-helper-cell induction recovery in children after allogeneic transplants from alternative donors


Late-onset autoimmune thrombocytopenic purpura (ATP) after allogeneic PBSCT remains rare, yet serious clinical problem. Two cases of ATP post transplant are reported and discussed.

10-month-old boy with Omenn Syndrome underwent haploidentical T-cell depleted PBSCT from HLA-mismatched mother in Oct. 1999. He was conditioned with Bu 20mg/kg, TT 10mg/kg, Flu 200mg/m2 and OKT-3. Hematological recovery was prompt: ANC > 0.5 G/l on day +9, PLT > 50 G/l on day +30. Neither GvHD nor other serious complications were observed until day +217 post transplant, when he developed ATP with PLT count of 31 G/l. Chimaerism analysis confirmed 100% donor engraftment. Immune studies revealed rapid recovery of CD19+ B cells (324/mcl, incl. CD5+CD19+ cells) in comparison with the previous examination two months ago (57/mcl) and peripheral expansion of CD3+CD4+ cells (> 100/mcl) incl. activated CD4+CD69+ cells. Different modalities for treatment of ATP were tried with transient success. Prednisone (4mg/kg) together with IVIG (0.4g/kg) resulted in ATP resolution lasting 2 months. PLT count dropped again to 51 G/l and five HD IVIG (2g/kg) courses were required to attain final ATP resolution 14 months post transplant.

Bone marrow transplantation-associated thrombotic microangiopathy (BMT-TMA): incidence and related factors

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BMT-TMA incidence varies with diagnostic criteria, modality, conditioning regimen, GVHD (and prophylaxis), and previous immunosuppressive and retransplantation. Reported prognosis and response to plasma exchange (TPE) or FFP infusion are poor. Eight patients (pts) from 3 hospitals with this complication, related factors, treatment and response are presented.

Were registered 2 cases among 355 auto and 6 among 42 allo BMTs (0.56% and 12.7% respectively). Median age was 40,4 years (19-65); 5 were women. Two pts had AML, 2 CML, 1 HD, 1 Mantle NHL, and 2 MM. AML patients received 4 previous chemotherapy lines; HD 3 lines and 1 MM 2 lines; the remaining, only 1. Allogeneic cases were HLA sibling-matched and were receiving CSP at diagnosis (dx). In 5 allo BMTs conditioning regimen consisted on Cy-TBI (two: unfractionated TBI); the 6th allo (with 1 previous auto-BMT) received non-myoeloblastic regimen. Auto-BMTs received scalded CBV (1) and BuMe (1). Two cases were preceded by acute intestinal GVHD (II and IV grade) and 1 case (pts. 2) had chronic hepatic GVHD immediately before. There were 3 bacterial and 4 CMV demonstrated infectious episodes.

Three patients appeared as conditioning-associated SHU (all > 120 days post BMT), 3 fulminant multifactorial purpura (49, 53 and 90 d) and 2 CSP-associated AHMA (29 d) and neurologic changes (100 d). Mean parameters at presentation were: leucocytes 5670 x 109/mL; hemoglobin 9.6 g/dL; platelets 55.375 x 109/mL; LDH 1.077 UI/L; schistocytes 3.8, BUN 72 mg/dL, creatinin 1.7 mg/dL.

Treatment first included CSP cessation (changing it by other IS) in allo-BMT. Six pts received TPE (0, 0, 1, 1, 115, and 154 days after dx) plus corticoids; 2 reached CR (both multifactorial purpura), 1 PR (but dead because multiorganic failure) and 3 were refractory (2 dead -multiorganic failure and lung hemmorage-, 1 developed terminal renal failure, but remained alive). Two pts received FFP infusion (4 and 0 d after dx), both with CR (CSP-AHMA and neurologic cases).

In our experience, BMT-associated TMA presented an overall incidence of 1.96%. Survival was 62.5%. Early dx and plasma therapy possibility played a favourable role. Fatal cases were associated with multiple previous chemotherapy lines, intense conditioning regimen, (specially single dose TBI) and previous GVHD.
**21. Aplastic Anemia**

**P800**

Nonmyeloablative conditioning without ATG and allogeneic peripheral blood stem cell transplantation in severe aplastic anemia

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Graft failure is one of the major problems in aplastic stem cell transplantation for patients with aplastic anemia (AA). High-dose cyclophosphamide (200mg/m²) is used in combination with ATG to prevent graft rejection. So far experience with dose reduced conditioning is limited in this disease.

We treated 4 patients (pts.) (2 females and 2 males) with AA. Three of them relapsed after previous immunosuppressive therapy and one pt. with prolonged pancytopenia after aggressive chemotherapy for secondary AML, with non-myeloablative conditioning and allogeneic stem cell transplantation. All pts. had failed at least one prior therapy and had received more than 10 transfusions. Two pts. received a combination of fludarabine (Flu)(3x30 mg/m²), cytoxan (120 mg/kg) and basiliximab, a humanized mouse anti-IL-2-receptor antibody (2x20 mg), followed by the infusion of mobilized peripheral blood stem cells from HLA-identical sibling donors. One pt. received a combination of Flu (3x30 mg/m²), cytoxan (2x50 mg/kg) and low dose TBI (2Gy) before transplantation of bone marrow from an HLA-matched unrelated donor (MUD). Another pt. was treated with Flu (3x30 mg/m²) alone and peripheral blood stem cells from a MUD while being aplastic and on mechanical ventilation for bilateral pulmonary mycosis with respiratory failure. All grafts were transplanted without futher manipulation. Prophylaxis for graft-versus-host-disease (GVHD) consisted in mycophenolate mofetil (MMF) and cyclosporine A (CsA) for those receiving peripheral blood stem cells and MMF and tacrolimus for the pt. receiving bone marrow.

Non hemotologic toxicity of the regimens described above was minimal (WHO<II). Three pts. did not need intravenous antibiotic treatment. All pts. showed complete engraftment after 14 days (range 9-16) for leukocytes >1000/µl and 14 days (range 11-17) for platelets >50.000/µl. The patient with respiratory failure being aplastic and on mechanical ventilation for bilateral pulmonary mycosis with respiratory failure. All grafts were transplanted without futher manipulation. Prophylaxis for graft-versus-host-disease (GVHD) consisted in mycophenolate mofetil (MMF) and cyclosporine A (CsA) for those receiving peripheral blood stem cells and MMF and tacrolimus for the pt. receiving bone marrow.

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We conclude that non-myeloablative conditioning therapy and allogeneic stem cell transplantation without ATG is feasible, even in heavily pretreated and pretransfused patients with severe aplastic anemia.

**P801**

The role of low dose busulfan (4mg/kg) with cyclophosphamide as a conditioning regimen for severe aplastic anemia

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Busulfan (BU) is an alkylating agent currently used in the myeloablative conditioning regimen for Bone Marrow Transplantation. Studies performed in animals demonstrated that marrow-lethal doses of BU have minor toxicity to the lymphoid system and cause little immunosuppression. However, successful allogeneic engraftment after the Tutschka regimen suggests that BU can enhance the immunosuppressive properties of Cyclophosphamide (CY). Graft rejection has been a problem in SAA who were conditioned with CY alone. Aiming to reduce graft rejection and to improve the immunosuppressive activity of CY, the authors used BU and CY at the doses of 4mg/Kg and 200mg/Kg, respectively, as a conditioning regimen in 81 patients with SAA. Methotrexate and cyclosporine were used in order to prevent GVHD. Patients were 3-53 years of age (median, 24), most of them heavily transfused. The previous transfusional number was 0-276 (median, 26), and 48% had therapy immunosuppressive before transplant. 14 out of 81 pts (17%) died before 4 weeks after transplant, most due to infection. At the end 67 pts were valuable for response. 12 patients rejected their transplants (15%) in a median time of 317 days (28-1001), 2 primarily (3%) and 10 (12%) presented a late rejection; 7 of them were successfully retransplanted. The probability of rejection was 22% (CI 10-33%). Acute GVHD grades 2 or 3 occurred in 20 out of 67 pts (31%) and chronic GVHD in 24 out of 61 pts (39%).

Currently, 70% are alive and well, and the actuarial survival rate at 2850 days was 68% (CI 57-81%). In the univariate analysis age, previous treatment, number of previous transfusions, time of cyclosporine use and acute GVHD were statistically significant for survival. Low dose BU (4mg/Kg) associated to standard dose of cyclophosphamide showed to be effective and safe as conditioning regimen for SAA with acceptable rejection and survival rates and may be an alternative for this category of patients, with low toxicity and cost.
P803
The treatment of severe aplastic anemia: The outcomes of allogeneic bone marrow transplantation and immunosuppressive therapy in Koreans
I. Kim, S. Yoon, S. Park, B. Kim, N. Kim (Seoul, KOR)

This is an analysis of 96 patients with severe aplastic anemia (SAA) treated in Seoul National University Hospital, Seoul, Korea between 1990 and 1999. Twenty-two patients were treated by allogeneic bone marrow transplantation (BMT) from HLA identical sibling donors and 74 with immunosuppressive therapy (IS) with antithymocyte globulin (ATG) or antilymphocyte globulin (ALG). There was no statistical difference between the 2 treatment groups about age, sex, disease duration before treatment and previous transfusion amount. In the BMT group, grade II-IV acute GVHD was developed in 10% and chronic GVHD occurred in 3%. Only 1 patient died from complication of transplantation (venoocclusive disease). Of 74 patients who received IS treatment, 45% achieved a complete or partial response. Twenty patients died among IS treatment group. Major causes of death were hemorrhage (40%) and Infection (55%). In the BMT group, the 5-year overall survival (OS) was 95% after a median follow-up of 42 months. In the IS group, the 5-year OS was 70% after a median follow-up of 48 months (p=0.04). The long-term survival rates of SAA in Koreans receiving BMT or IS were excellent. Further evaluation about prognosis of aplastic anemia in Asians should be recommended.

P804
Allogeneic bone marrow transplantation after liver transplant - case report

Few cases of hematopoietic stem cell transplantation (tx.) have been reported after liver rx. We describe a case of a 4-year-old girl referred to our BMT Unit with severe aplastic anemia after a liver transplant. In February 2001 she had fulminant hepatitis of unknown etiology (A, B and C virus - negative) with encephalopathy and underwent liver tx. 24 hours after admission. At this time she was neutropenic but the bone marrow was normal. Two weeks after this transplant, severe aplastic anemia was diagnosed and treatment with ATG was unsuccessful. Subsequent to persistent fever, hepatic micro-abscesses consistent with systemic candidiasis were found and treated with amphotericin B. On June 22 she was admitted at our Unit, with low performance status (Lansky 40%), severe malnutrition (< 5th percentile of weight and 10th percentile of height), fever, consolidation of left lower lobe of the lung. Bone marrow from a histocompatible sibling (total 4.5 x 10^8 nucleated cell/kg) was infused on 4 of July 2001 after conditioning regimen with cyclophosphamide (750 mg/m2 x 3), fludarabine (40 mg/m2 x 5) and ATG (2.5 mg/kg x 4). GVHD prophylaxis was done with cyclosporine and mycophenolate mofetil. Post-transplant period was complicated with persistent fever, maxillary sinus infection, and voluminous pericardial effusion without tamponade. On day (D) +16 an image compatible with lung abscess developed on the lower left lung lobe (aspergillosis?); trans-thoracic biopsy was not diagnostic. She was treated with broad-spectrum antibiotics, antifungal agents and granulocyte transfusions. She achieved neutrophils > 0.5 and platelets > 20 x 10 9/L on D +16 and +29, respectively. One month after transplant complete chimerism was present. She was discharged from the BMT Unit on D +35. At the last follow-up, 4 month after transplant, she is asymptomatic, with good performance. The final bone marrow histology was normal and normal liver function tests. The conditioning regimen contained Cyclophosphamide (50 mg/kg/daily IV), which was prescribed from day –5 to -2. But in conclusion in this high-risk group of patients the haematopoietic stem cell transplant is feasible with an acceptable morbidity and has a high curative potential. The question of duration of immunosuppression in this setting remains unanswered.

P805
HLA identical bone marrow transplant in children with severe aplastic anemia - a single center experience

Background: Severe aplastic anemia (SAA) is a life-threatening disorder of bone marrow failure characterised by pancytopenia and a hypocellular bone marrow, that can manifest itself at any age. Bone marrow transplantation (BMT) with marrow from a HLA-identical fully matched sibling donor is the best treatment option for SAA. This form of therapy has been undertaken for the past thirty years at the Department of Pediatrics of the Leiden University Medical Centre (L.U.M.C.), the major pediatric bone marrow transplantation centre for the Netherlands.

Method: The medical reports of patients who underwent an HLA-identical sibling-donor BMT for a confirmed diagnosis of SAA from 1971 to 2001 and who are at least 1 year in follow up (n = 44), were reviewed. The patients were divided into two groups: group A who were transplanted before 1989 (n = 24), and group B who underwent BMT in or after 1989 (n = 20). At that time, the graft versus host disease (GVHD) prophylaxis was changed to the combination of methotrexate and cyclosporin A. Age duration of aplasia, number of erythro-transfusions, initial therapy, conditioning regimen, infections, GVHD and its prolifeation in this setting remains unanswered.

Results: We found an increase in survival rates from 63% in the period before 1989 to 90% in 1989-2001. The risk of developing GVHD, with the combination of methotrexate and cyclosporin A has significantly decreased since 1989, with only one patient suffering from acute GVHD versus 13 before 1989 (p = 0,002). No patients after 1989 developed chronic GVHD versus ten before 1989 (p = 0,001). A shorter period between diagnosis and treatment, less blood transfusions and better maintainance was also observed.

Conclusion:
1. The outcome of allogeneic BMT for patient with severe aplastic anemia has considerably improved over the last ten years.
2. Young patients, grafted early after diagnosis from an HLA-identical sibling donor, have currently a 90% chance of long-term survival.
3. The most important factor in reducing the incidence of both acute and chronic GVHD is due to the change in prophylaxis.

Additional abstracts to this topic
Review of 29 patients with severe aplastic anemia who have undergone bone and marrow transplantation - Results and assessment
A. Ghavamzadeh, M. Iravani, B. Bahar, M. Jahani, F. Vafayizadeh, S. Gholibeikian, S. Samiee, B. Khoein, A. Mousavi, S. Basirpanah (Tehran, IR)

Introduction: Transplantation is the treatment of choice for patients with aplastic anemia. This study has been carried out on 29 patients with aplastic anemia who have undergone transplantation since 1992. We had 10 female patients (34.5%) with age range between 7-25 yrs (mean: 17yrs) and 19 male patients with age range between 4-33 yrs (mean: 19yrs).

Results: Mean average of platelets infused before transplant to the patients was 18.5 units (range: 0-60) and the mean average of packed cells infused was 11.5 units (range: 0-60). The conditioning regimen contained Cyclophosphamide (50 mg/kg/daily IV), which was prescribed from day –5 to -2. But in
patients who had received more than 5 units blood products transfusions, ALG one Amp for 10 kg body weight was added (day –5 to –3). GVHD prophylaxis contained cyclosporin 3 mg/kg IV (day –3 to +5) and after following that 12.5mg/kg PO till day +180. The mean average of MNC numbers infused for each transplant, assessed before processing, was 5.5X10^8 cells/kg (Min: 2.2X10^8 - Max: 11.25X10^8). Patients were transplanted on average 377.5 days after diagnosis. (Min:52 - Max:3650). Our patients 5 year overall and disease-free survival was 67%. On the average, GVHD occurred 10 days after transplantation in 21 patients (12 days after bone marrow transplants and 8 days after peripheral blood transplants). 7 patients were with grade I [24.1%], 8 patients with grade II [27.6%], 6 patients with grade III [20.7%]). We had 8 recurrence cases [27.6%]; also 9 patients have died to date [31%].

Conclusion: This study shows that Cyclophosphamide and ALG prepared a good conditioning regimen for patients with severe aplastic anemia, going to be transplanted even if high-risk.

Graft failure and boost treatment in adult with severe aplastic anemia


Graft failure after allogeneic bone marrow transplantation(BMT) for severe aplastic anemia(SAA) is a significant clinical problem with a high risk of mortality. Reinfusion of donor stem cells may be an accepted therapeutic approach, but second transplantation with intensified conditioning would probably be associated with toxicity. So, our patients received reduced conditioning for their bone marrow with minimized toxicity. We report a favourable outcome of a boost with reduced conditioning in SAA patients in whom graft failure occurred following an HLA-identical sibling transplant. Ten consecutive patients received a boost treatment. The conditioning regimen for first BMT consisted of cyclophosphamide, antithymocyte globulin(ATG) and procarbazine. The GVHD prophylaxis was a combination of cyclosporin and short-term methotrexate. In order to monitor donor cell engraftment, the evaluation of chimerism status was done. All patients except primary engraftment failure revealed mixed chemirism or complete donor chimerism. Median interval from BMT to graft failure was 58 days(range, 0 to 354). Bone marrow(n=1) or G-CSF mobilized peripheral blood stem cell(n=9) was used as the source of stem cells. Total nodal irradiation(TNI) and ATG was given prior to the boost for 5 patients, while 3 received TNI+steroid, 1 fluocytarnine + ATG and 1 ATG. Post-boost GVHD prophylaxis consisted of cyclosporin for all patients. Eight(80%) of 10 patients are alive with a normal graft function and achieved complete donor chimerism. Two patients did not obtain an engraftment : one patient died from cerebral hemorrhage after 6months of the boost and another is live but still dependent on transfusion. The boost related toxic death was not observed. After the boost, 2(20%) of 10 patients developed acute GVHD of overall grade II and 2(20%) of 10 patients developed a limited type of chronic GVHD. In conclusion, the reduced pre-boost conditioning was well tolerated and accompanied by a high incidence of engraftment. Incidence of severe acute and/or chronic GVHD did not develop. Based on the excellent outcome seen in these patients, the boost treatment with reduced conditioning may be feasible for SAA patients with graft failure. More study is needed to determine whether the pre-boost conditioning carries the low risk of short/long term sequelae.

Successful engraftment of second haploidentical peripheral stem cell transplant (Psct) for amegakaryocytic thrombocytopenia


A 10 month old boy with congenital amegakaryocytic thrombocytopenia and dependent on platelet transfusions had a haploidential PsCT from his 3/6 matching father as he had no HLA-identical sibling on family member donor or MUD. The conditioning regimen consisted of Fludarabine (Flu) 125 mg/m2 + Cyclophosphamide (CTX) 200 mg/kg+Busulphan (Bu) 14mg/kg + TGN(rabbit) 25mg/kg. GVHD prophylaxis was not given. CD34+ cells of the graft: 18.62x10^6 /kg, and CD3+: 0.76x10^5 /kg. Engraftment was on the 12th day (WBC>1,0x10^9 /L, Plt>30x10^9/L). Mixed chimerism (recipient-donor) was detected. During his follow-up VOD and septicemia due to catherer related MRSA developed and treated with tPA+antibiotics. But on the +30th day WBC fell (<1x10^9/L) and chimenism study showed only the recipient was detected and 2nd PSCT was decided from the father with a conditioning regimen consisting of Flu 125mg/m2 + ATG (rabbit) 25 mg/kg and cyclosporine A (CSA) 1 mg/kg for GVHD prophylaxis. CD34+ cells (graft) was 18.62x10^6 /kg and engraftment was achieved on the +12th day for WBC (>1x10^9/L) and +17th day for platelets (>30x10^9/L). On the +14th day GVHD (grade II) developed, treated with steroids. A mixed chimenism (donor dominant) was achieved. The bone marrow aspirate was normocellular and showed enough megakaryocytes. At +4th month, he is still alive and well with a CBC of WBC: 6,5x10^9/l, Hb:13,5g/dl, Hct:36%, platelet: 169x10^9/l. In conclusion, amegakaryocytic thrombocytopenia is always a candidate for stem cell transplantation.

22. Stem Cell Source

P060
Enhancement of the anti-tumor efficacy of allogeneic transplantation with use of blood-derived stem cells: 5-year follow-up of a prospective study comparing marrow and blood allografts


39 adult patients with hematologic malignancies underwent blood stem cell transplantation: 20 marrow(n=20, PBSC) or marrow(n=19, BM) stem cell transplants from HLA-identical siblings in randomized, blinded study after standard myeloablative conditioning regimens between 6/95-8/97. BM and PBSC were collected from donors. Study remained blinded until a year after the last patients transplant. Both patient groups were comparable. GVHD prophylaxis comprised cyclosporin-methotrexate. All patients received all 4 doses of methotrexate without dose modification. The quantities of CD34+ cells and T lymphocytes received by PBSC recipients were 2-10 times higher than BM recipients. PBSC group had significantly faster reconstitution of neutrophils, platelets, lymphocytes, CD4+ and CD25+ cells. Incidence of acute GVHD was comparable. 13 patients died of treatment-related causes at 15-733 d (median 57). 6 patients relapsed at 117-1233 d (median 149)-all in BM group. No other factor affected the probability of relapse significantly. 1/6 relapsing patients is alive in remission after receiving originally harvested PBSC from donor as cell-mediated immunotherapy and 1 is alive in relapse. 5-y probabilities of developing chronic GVHD were not significantly different (P=0.8) for BM (40%) and PBSC (50%). As of 7/01, 9 BM recipients are alive at 42-70 mo (median 63) vs 13 PBSC recipients at 51-74 mo (median 63). 1 patient in BM group has mild pulmonary chronic GVHD and 1 patient in PBSC group has severe pulmonary chronic GVHD. The 5-y probability of relapse in BM vs PBSC group was 44 +/-14% (n=6) vs 0%, P=0.005; DFS 37 +/-11% (n=7) vs 65 +/-11% (n=13) P=0.1; OS 47 +/-12% (n=9) vs 65 +/-11% (n=13) P=0.3 respectively. Since the publication of our preliminary observation of reduced relapse with PBSC allografts (Powles et al. Lancer 2000;355:1231-37), another prospective study has also reported lower relapse rates with PBSC allografts (N Engl J Med 2001;344:225-31). We observed a significant rate (0/100) of our observation of reduction in relapse has remained valid with a median follow-up exceeding 5 y suggesting that benefits associated with PBSC transplantation persist. Superior DFS
associated with PBSC in our study is not statistically significant due to small patient numbers. We have not found a higher incidence of chronic GVHD among PBSC recipients possibly due to rigorous GVHD prophylaxis. We recommend using PBSC as preferred source of stem cells for allografts from HLA-identical siblings with rigorous cyclosporine-methotrexate GVHD prophylaxis.

**P807**

Peripheral blood vs. non-cryopreserved bone marrow for autologous transplantation in malignant lymphomas

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The goal of the study was to assess retrospectively hematopoietic recovery and outcome after autoPBSCT compared to autoBMT in malignant lymphomas. In contrast to similar studies performed earlier, in our centre bone marrow was not cryopreserved but stored for 24 h at 4 deg C. Conditioning regimen used for autoBMT was CBV, for autoPBSCT = CBV or BEAM. We analyzed 236 patients (age 31(13-64) y; NHL n=108, HL n=128) who were treated with autoHSCT (PBSCT n=149, BMT n=87) in our department between 1992 and 2001. There were no significant differences between PBSCT and BMT group in terms of diagnosis, disease stage at BMT, pre-treatment nor age. Median NRM time was 3.6(1.1-15.5)x10^8/kg for PBSCT and 2.9(0.9-7.8)x10^8/kg for BMT (p=0.006), median CD34+ cells equalled 4.0(0.4-5.5)x10^6/kg and 2.4(0.3-8.5)x10^6/kg, respectively (p=0.001).

Recovery of neutrophils >0.5 G/L was faster for PBSCT compared to BMT by 2 days (median 15(10-73) vs. 17(11-46) d., p=0.001). However, there was no statistically significant difference in terms of PLT-50 G/L recovery; median 17.6(8-161) d. for PBSCT and 19.5(11-148) d. for BMT; p=0.11. Median hospital stay since the day of autoHSCT was 19(12-77) d. in the PBCT group vs. 21(11-87) d. in the BMT group (p=0.006). There were no significant differences in the occurence of severe adverse events, blood products transfusions, iv antibiotics nor cytokine administration between both groups. Early mortality (until d. 100) equaled 2/146 (1.3%) for PBSCT and 2/87 (2.4%) for BMT. The overall survival (OS) rate after 8 y. was 49% and 82% (p=0.05), respectively, with the median follow-up of 1.8 y.

Conclusions: PBSCT compared to non-cryopreserved BM results in 2 days shorter time of haematopoietic recovery in malignant lymphoma patients. This difference is markedly smaller than those found in the studies where BM was cryopreserved and does not translate into improved mortality or reduction in intensity of supportive treatment. The significant difference in OS rate in favour of BMT needs further confirmation with longer follow-up and within more homogenous subgroups.

**P808**

Improved outcome after peripheral blood stem cell (PBSCT) compared to bone marrow transplantation (BMT) from unrelated donors using a uniform GVHD-prophylaxis regimen

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PBSC are increasingly used in allogeneic transplantation in recent years. Since November 1995 we have transplanted 159 patients 8,49 years, 8 patients had hematological malignancies (ALL n=4, AML n=2, MDS n=2) and 15 had Fanconi’s anemia. All donor pairs were matched on class I A,B, 22/23 were matched on class II DR (1 difference n=1), 21/23 were matched on class II DQ (1 difference n=1, 21/23 were identical on class II DP (1 difference n=13, 2 diff. n=5). The conditioning regimen consisted of chemotherapy in all patients ,TBI n=21 and ATG n=23. GVHD prophylaxis consisted of ciclosporin A with corticosteroids or methotrexate. Median total nucleated cells 0.03X10^8/kg (0.007-1.8) and CD34 cells 0.05x10^3/kg (0.001-4).

Results: Median follow up was 37 months (8,7-70,4), the incidence of acute GVHD grade II-IV was 31% (p=0.009) and that of chronic GVHD was 29% (p=0.09), the probability of neutrophil engraftment at D+60 was 91% (p=0.114) and that of platelets at D+180 was 88% (p=0.2866). 17/23 patients were considered in complete remission and 6 showed relapse or rejection. Negative factors were used alone in 2 patients giving complete remission in one , and associated with chemotherapy in 1 relapsed giving no response. 12/23 died, of them 9 from TRM and 3 from relapse or progression. The principal causes of TRM were rejection n=4, GVHD n=3 and fungal infection n=2, the probability of TRM at D=100 was 13% (p=0.0115). Overall survival at 3 years was 46% (p=0.014).

Conclusion: This study showed a significant role of selected CD34+ cells to assure engraftment, minimal GVHD. Reinforcement of the negative fraction evaluation needs more patients and follow up.
P810
Mismatched family donor transplants in children using high doses of positively selected CD34+ cells: update of the Hannover Medical School experience
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In the years 1997 to 2001, 16 mismatched family donor (haploidentical) transplants were performed at the Hannover Medical School in children aged 4 months to 17 (median 7) years. Diagnoses were ALL in 3, Lymphoma in 3, VSAA in 2, congenital amegakaryocytic thrombocytopenia in one, Hurler's disease in one, and AML in 6. Three of the AMLs were secondary to congenital disorders; one each Schwachman-Diamond syndrome, severe congenital neutropenia, and Fanconi anemia.

One to four mismatches (A, B, DR, DO) were present between donors and recipients: 5 had 1/8, 2 had 2/8, 3 had 3/8 and 4 had 4/8 mismatches. Conditioning regimens for leukemias and lymphomas were TBI-based, included ThiopEPA, and in addition Cyclophosphamide (CPM), VP-16 or Fludarabine. Only the 1 year old patient received Busulfan, Fludarabin and Melphalan. The children with secondary leukemias and the nonmalignant conditions received, depending on diagnosis, TLI or Busulfan in combination with Fludarabin and/or CPM.

All patients were transplanted with positively selected CD34+ cells (CliniMACS, Miltenyi Biotech) ranging in amount from 6.5 to 71.8 (median 24.4) x 10^6/kg BW. The transplanted CD3 cells doses ranged from 0.02 to 6.5 (median 2.3) x 10^6/kg BW. Donor lymphocyte infusions (DLI) of 3 x 10^6/kg D3 cells/kg BW were given in 3 patients. Only two patients developed GVH; one grade III acute GVH after a DLI, and one de-novo extensive chronic GVH without having received DLI. Ten of 16 patients survive with a follow-up of 1.5 months to 4.5 years (median survival 43.5 months). Causes of death were transplant-related in 3/14, and leukemia relapse in 3. Taking into account the small patient numbers, there was no relation between the degree of mismatch and outcome.

Conclusions: Haploidentical transplants using large doses of highly purified CD34+ cells are a valuable therapeutic alternative for many children that lack an HLA-identical donor.

P811
A single center experience of unrelated umbilical cord blood transplantation (Cbt) in childhood myelodysplastic syndromes (Mds)

UCB represents a source of hematopoietic stem cells successfully used for unrelated transplantation primarily in pediatric patients, showing a probability of survival comparable to that obtainable with other source of stem cells as unmanipulated or T-cell depleted bone marrow. Allogeneic stem cells transplantation seems to be the only therapy consistently proved to cure patients with MDS; however, available data in childhood MDS are limited. We present our experience of unrelated UCB transplant in five children affected by MDS, who lacked an HLA identical sibling. All patients received a CBT from a mismatched donor (1 locus 4 pts; 2 loci 1 pt); 3 CB units came from GRACE (Italy) and 2 from New York. There were 2 patients with Refractory Anemia, 2 with RAEB and 1 with Juvenile Myelomonocytic Leukemia (JMML). The median age of the recipients was 2.9 years (range 1.3 – 6.6). Conditioning regimen consisted of TBI or BUS + CTX + VP16 in 4 patients and Fludara + Ara-C + VP16 + Thiotepa in the JMML child who had already failed a mismatched (1 locus) alloPBSCT transplant from the mother, using BUS + CTX as preparative regimen. ATG was added in all cases and GVHD prophylaxis consisted of CSA and steroids. Median dose of NC, CD34 and CFU-GM infused after thawing were 5 x 10^7/kg, 2.7 x 10^5/kg and 2.35 x 10^4/kg, respectively. Four patients engrafted, achieving PMN count > 500/mm3 at a median time of 26 days (range 23-30); PLTS recovery was documented in 2 cases after 34 and 40 days. Full donor chimerism was documented in 4 cases on day 28, 1 child showed mixed chimerism and died of sepsis on day 30. Three patients developed grade II or grade IV aGVHD, one patient was limited to grade I and one patient died for grade IV aGVHD. Two patients (1 RA and 1 JMML) are alive and well at median follow up of 3.5 years, three patients died of early related transplant causes (1 VOD + Pseudomonas sepsis, 1 heart failure and 1 aGVHD). In conclusion, CBT appears to be a promising approach for childhood with MDS; the early transplant related mortality remains, however, a major problem of this transplant procedure.

P812
Umbilical cord blood for allogeneic transplantation in children and adults with hematological malignancies
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Objectives: The aim of this study was to investigate engraftment, survival, and GVHD after transplantation of one or two units of allogeneic cord blood for the treatment of hematological malignancies in children and adult patients.

Patients and Methods: 18 patients were enrolled in this study. The median patient age was 14.1y (range 3.5-37y), and median body weight 42 kg (range 12.5-95kg). 11 patients were transplanted for good-risk status, 7 at poor-risk leukemia. 13 patients received one unit containing average nucleated cells (NC) 4.25 X 10^6/kg (range 1.73-12 X 10^6/kg ), and 5 received 2 units consecutively with NC of each unit less than 2 X 10^6/kg. The patients transplanted with one unit UCB were HLA matched at 5/6 (n = 6) and 6/6 (n = 7) loci, 5 patients received 2 units of banked UCB which were mismatched at 1 or 2 loci. Conditioning regimens consisted of BU/CY for 14 patients, CY/TBI for 3 patients. Most patients also received ATG at a dose of 15-20mg/kg/d for 3 days. GVHD prophylaxis consisted of CsA, MMF and methylprednisolone for most patients.

Results: 15 patients survived for more than 70 days were evaluated for engraftment, GVHD, LFS and EFS. By day 60 after UCBT, 93%(14/15) patients with ANC>500/mm3 was 20.7 days (range 11-60), and 73%(11/15) with platelet more than 20,000/mm3 was 37.5 days (range 20-68). 5 patients developed grade II and 1 grade IV aGVHD. Up to November 25, 2001, 12 patients still survive with median follow-up of 360 days (range 90’C1000 days). For patients with good-risk leukemia, LFS is 91%(10/11), and EFS 73%(8/11). However, for 7 patients with poor-risk leukemia, only 2 patients survive without relapse, and EFS is 14.3%(1/7). For 4 adult patients transplanted with 2 units of UCB, 3 had sustained engraftment with only one unit of UCB and have survived for 18, 13 and 6 months, respectively.

Conclusions: HLA-matched or 1-2 loci-mismatched CBT is a feasible procedure to cure a significant proportion of children or adults with leukemia, especially if conducted in a favourable phase of disease. 2 units of CBT were feasible for adult patients if the cell number of one unit is not enough.

P813
Cord blood cell quality is not affected by volume reduction utilizing a new cell washer device
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Introduction: Stem cells derived from cord blood (CB) are routinely employed as source for transplantation to treat different oncology, haematological and inherited disorders. Volume reduction permits to reduce not only the storage requirements in large scale CB banking, but also the DMSC amount for cryopreservation, leading to evident economical and clinical advantages.

Materials and Methods: We processed 24 cord blood units to verify the effectiveness of the Baxter Cell Washer device (CytoMate) and the quality of the final product. The mean pre-
Processing value of volume, hct, TNC, MNC, CD34+ cells and vitality were: 86.95 ml (49-127), 40.99% (31.4-50.5), 1.013x10^9 (0.641-1.723), 526.45x10^6 (306-939.4), 204.07 x10^6 (125-465.5), 96.5% (90-98.9), respectively. Every sample was tested for microbial contamination and short-term cultures (STC) before and after processing. The processing time was always recorded.

Results: After processing the mean value of volume, hct, TNC, MNC, CD34+ cells and vitality were: 55 ml (25-69), 33% (19-46), 0.707x10^6 (256-1266), 474x10^6 (200-694), 170x10^6 (86-339), 92.8% (87-98.8), respectively. The recovery (%) for TNC, MNC and CD34+ cells was: 74.5, 90.8, 83.3, respectively. No microbial contamination was detected after manipulation. STC showed no difference on CFU-GM, BFU-E, CFU-Mix growth. The mean working time was 20 minutes.

Conclusions: Our preliminary data demonstrate that CytoMate cell washer is effective in reducing cord blood volume, allowing a good recovery, especially in terms of MNC and clonogenic cells, without changing the quality of the units to be banked. The time required for the entire procedure is cost effective. On the contrary, the washing procedure doesn’t permit to reduce significantly the red cell contamination of cord blood units without utilizing an appropriate lysing solution.

**P814**

**Evaluation of different cord blood treatment methods**

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It is known umbilical cord blood (CB) may be used as a reach source of stem cells for bone marrow reconstitution in recipients. The clinical banking of cord blood requires volume reduction and red blood cell depletion for cryopreservation.

Objective of the investigation was making clear the best method of CB treating which might be used for clinical banking.

Materials and Methods: We have used 1) 3% Gelatin and 2) 6% Hetastarch (HES) for red cells depletion and 3) density gradient separation using Histopaque-1077. The Gelatin Method: CB was mixed with an equal volume of 3% Gelatin, the double sedimentation was performed at 22°C for a maximum 25 minutes. HES Method: CB was mixed with 6% HES in proportion as 4:1, sedimentation was performed at 22°C for a maximum 25 minutes. In both probes cells were washed by centrifugation and analyzed.

Density gradient Method: CB was mixed with RPMI in proportion 2:1 (CB/RPMI) and layered onto Histopaque in proportion 1.5:1. The gradients were centrifuged for 30 minutes. The layer of light-density cells was washed by centrifugation and analyzed.

Results: The treating by Method 1 (n=26) got recovery 83.8±10.1% of NC, 81.4±14.1% MNC, 1,34±0,81x10^6/3 CFU-GM/ml. The recovery by Method 3 (n=26) yielded 30,1±10,2% NC, 1,34±0,81x10^3 CFU-GM/ml and 98,4±1,2% Red cell depletion.

Conclusion: The Gelatin and HES Methods gave the better recovery of NC and MNC then Histopaque (p<0,01). But recovery of CFU-GM was higher after Gelatin sedimentation (p<0,05). We have come to conclusion the Gelatin Method might be used for clinical banking as effective, simple and inexpensive procedure of CB treatment.

**P815**

**Evaluation of important factors on nucleated cell and volume of cord blood collected in 35 cesarean deliveries**

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Introduction: Umbilical cord blood (CB) has been shown to contain sufficient progenitor cells to provide durable engraftment. The number of nucleated cells in CB transplant is the most important factor affecting the engraftment. In order to maximize the yield of nucleated cells in a CB unit, we studied whether maternal and neonatal characteristics have an effect on the nucleated cell counts.

Results: We studied the characteristics of the mother and babies including the mothers’ age, parity, gestational duration, and the birth weight, birth height and sex of the babies. The mean maternal age was 27 (range 21-35) years. 17% of mothers were primiparous. 41% had her second parity, 24% the third and 17% the fourth. The mean gestational duration was 38.8 (range 37.4-40) weeks. 56% of the newborn were female and 43% male. The mean birth weight and birth height were 3121 grams (range 2250-5000) and 50 cm (range 47-53), respectively. The mean CB volume and nucleated cell count were 83 ml (including 16 ml of ACD-A) and 7867/mm3 with a range of 50-140 and 3720-12,700, respectively. The viability of all samples was over 98%. The volume of collected CB was positively correlated with the birth weight of the newborns (p=0.01). The male babies with a mean weight of 3388 gm were significantly (p=0.04) heavier than females with a mean weight of 2917 gr. The CB volume and the nucleated cell count were significantly higher in male babies than females (p=0.02, P=0.07). The outcome measures were not influenced by maternal age and gestational duration, whilst parity had a significant positive effect on the nucleated cell count (p=0.007).

Conclusion: Our data suggests that CB belonging to the higher birth weight newborn male and the multiparous woman are more suitable for CB banking and transplantation.
patients who are candidates to an allogeneic transplant the donor search should simultaneously be addressed towards both the BMDWW Registry and CB Banks

P817

Use of anti-BDCA-2 antibody for detection of dendritic cells type-2 (DC2) in allogeneic hematopoietic stem cell transplantation

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Th2-inducing dendritic cells (DC2) are commonly identified as negative for lineage markers and positive for HLA-DR and CD123 expression. More recently, normal blood DC2 were shown positive also for BDCA2 and BDCA4 antigens. The aim of this study was to evaluate whether BDCA2 expression on DC2 is impaired in patients undergoing an allogeneic hematopoietic stem cell transplantation (HSCT) and in healthy donors treated with G-CSF for HSC mobilization. Flow cytometry assays for DC2 detection using either a triple staining with anti-HLA-DR PerCP, anti-Lin+anti-CD34 FITC and anti-CD123 PE monoclonal antibodies (mAbs) (Becton Dickinson), or a double staining with anti-HLA-DR PE (B.D.) and anti-BDCA2 FITC (Miltenyi Biotec) mAbs, were compared in: blood samples from patients who underwent an allogeneic HSCT (n=11), excluded from the donors before (n=11) and after (n=8) G-CSF mobilization, as well as in healthy donors' leukapheresis products (n=12) or bone marrow (n=4). median values of CD123+/ DC2 and BDCA2+ DC2 were not statistically different in the blood of patients previously treated with chemotherapy, nor in the blood or bone marrow of healthy donors. Also, a 5 days course of G-CSF treatment did not affect BDCA2 expression on healthy donors' blood DC2 significantly, nor modified BDCA2+ cells expression of HLA-DR, CD54 and CD38. Consequently, the selection procedures with the new SW 2.5CE we achieved higher median capture efficiency of the selection procedures (72.5% vs. 92.9%; p<0,05). Additionally, in the first group we observed 1.3 alarms per procedure and in one case the procedure was terminated because of a spinner malfunction. In the second group we did not recorded any alarm or troubleshooting.

Conclusion: CD34+ cells can be effectively isolated from allogeneic and autologous grafts using the automated magnetic technique. The selection efficiency of procedures performed on the Isolex 300i device was improved after the new 2.5CE computer SW version was introduced into practice.

P818

Selection efficiency of the CD34+cells with the new software on the Isolex300i device

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Objectives: The magnetic selection of CD34+ cells from mononuclear collections prepared from the peripheral blood of mobilized donors/patients is being increasingly used for allogeneic or autologous haematopoietic cell transplantations. The variables that may influence the effectiveness of CD34+ cell selection are under investigation.

Methods: The efficiency of the selection procedures using two different software versions of magnetic cell separator Isolex 300i (Nexell) were compared. Apheresis collections of mononuclear cells were performed on CS 3000 (Fenwall,Baxter) Ten CD34+ cell selections from peripheral blood progenitor cells (PBPCs) (2 allogeneic and 8 autologous) were performed using the old software version 1.12 and four selections (1 allogeneic positive / negative and 3 autologous) using the new software version 2.5 CE. The results of yield, purity, capture efficiency as well as number of troubleshooting were compared and analysed.

Results: There were no statistical significant differences in cell counts between the apheresis products used for CD34+ cell selection. In the first group (SW 1.12) the median cell counts were: TNC: 5.4x1010 (Ly 61%, Mo 11%, PMNG 28 %) with CD34+: 510x106 per product and PLT: 861 x 109/L. The apheresis products selected with the SW 2.5CE showed median cell counts: TNC : 6.2 x1010 (Ly 75%, Mo 11%, PMNG 14 %), CD34+: 340x106 per product and PLT: 907x109/L. By using the new SW 2.5CE we achieved higher median capture efficiency of the selection procedures (72.5% vs. 92.9%; p<0,05).

P819

Result of a multicenter randomized study comparing low doses of hemopoietic growth factors for PBSC mobilization in autologous transplantation - 3 days of GMSCF and 2 days of GCSF versus 5 days of GCSF


Introduction: This study was based on previous results which showed the feasibility of low doses of GCSF and/or GMSCF (5 micro-grams/kg/day) for peripheral stem cell (PBSC) mobilisation. In an open multicentric randomised study, the potential interest of low doses of GCSF for 3 days(d) followed by GCSF for 2d. (Arm B) was tested versus GCSF(Arm A) alone for 5d. for PBSC mobilisation.

Patients: Over a 3 year period, 143 patients with solid tumours and non myeloid haematological malignancies and eligible for intensive therapy followed by autologous PBSC rescue were enrolled.

Method: The first apheresis was scheduled 14 to 21d. after a standard chemotherapy. 75 patients were in Arm A and 68 in arm B, GCSF was pursed to obtain a minimal result of 2x10E6 CD34+/Kg.

The principal objective was the number of apheresis to obtain this CD34+ number, the secondary objectives were : CFU-GM numbers, length of aplasia and hospitalisation and global treatment tolerance according to WHO monitored for 3 months after transplant.

Results: Both arms had equivalent general characteristics. Among 143 patients, only 125 were autotransplanted. A statistical difference was shown between the 2 arms regarding CD34+ (7.20 x10E6/Kg vs.4.44x10E6/Kg, p<0.03) and CFUGM (169 vs.93.6 x10E4/Kg, p<0.01) in favour of the arm A; the number of apheresis was equivalent. No significant difference was demonstrated either for the tolerance related to growth factor treatment or for the haemopoietic recovery (PMN>0.5G/l :13.4 vs.13.8, Platelets >200G/l: 9.8 vs. 10.2), the hospitalisation length (16.6d. vs. 17.7d) and the toxicity post transplant.

Conclusion: This study confirms the feasibility and the performance of PBSC mobilisation using low doses of growth factors, without any previous specific chemotherapy. Although the CD34+ PBSC content was better in the arm A, there was no difference concerning toxicity and/or recovery after transplant. The question remains of the exact significance of the CD34+ PBSC content in terms of post-transplant recovery.

P820

Uncontrolled-rate freezing spares primitive hematopoietic progenitors and guarantees successful engrafing capacity

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Uncontrolled-rate freezing procedures represent an attractive alternative to controlled-rate cryopreservation, which is time consuming and requires high level technical abilities.
Peripheral blood stem cells (PBSC) from 104 patients with hematologic malignancies (25 AML, 20 NHL, 1 NHL-HIV, 12 HD, 2 HD-HIV and 44 Multiple myeloma) were cryopreserved, without rate-controlled freezing, in a cryoprotectant solution consisting of phosphate buffered saline (80%, v/v), human serum albumin (10%, v/v), dimethylsulfoxide (10%, v/v) and stored in liquid nitrogen. The cryopreservation procedure required on average 1-1.5 hour. The mean storage time was 54 days, (range 27-298).

PBSC bags were tested before cryopreservation and at thawing for primitive (LTC-IC) and committed hematopoietic progenitors (CFU-Mix, BFU-E, CFU-GM), by means of long- and short-term culture assays, respectively. Although thawing was associated with a statistically significant reduction of the absolute number of nucleated cells (21 x 10^9 vs 17 x10^9, P<0.004), recovery of LTC-IC (17 x 10^5 vs 18 x 10^5, P<0.6), CFU-Mix (12.3 x 10^5 vs 9.2 x 10^5, P<0.08), BFU-E (143 x 10^5 vs 131 x 10^5, P<0.3) and CFU-GM (437 x 10^5 vs 324 x 10^5, P<0.1) was not affected by the freezing/thawing procedures. No adverse effects were reported at reinfusion of cryopreserved PBSC; hematopoietic recovery was obtained in all patients with a mean time to neutrophil (>0.5 x 10^9/L) and platelets recovery (>50 x 10^9/L) of 10 days (range 8-14) and 19 (range 10-77), respectively. In conclusion our cryopreservation procedure is as fast as easy, allows recovery of primitive and committed hematopoietic progenitors and guarantees rapid hematopoietic recovery after myeloablative therapy. Our study strongly supports technical improvements aimed at cost reduction and feasibility of routine freezing procedures.

P821

Selective cytotoxicity of a new synthetic pentapeptide towards K 562 leukemic cell line but not towards normal hematopoietic precursors - A new purging agent


Relapse after Autologous Bone Marrow Transplantation can originate from neoplastic contamination of bone marrow harvest and therefore to reduce relapse rate, purging agents have been proposed.

A purging agent is required to have a selective cytotoxicity against leukemic cells while sparing normal marrow cells so that engraftment of treated bone marrow cells is not affected.

A syntetic pentapeptide has been recently shown (G.A. Fichera: CFU-GM /5x10^4, pentapeptide: 35 CFU-GM /5x10^4, differences compared to controls, colony formation: controls: 38, PBSC: 37) as a new purging agent effective in vitro against many neoplastic cell lines (Yoshida’s tumors, neuroblastoma, Ehrlich’s tumor).

We evaluated, using a colony growth assay, the differential cytotoxicity of this new agent against normal BM progenitors and leukemic cell line K-562.

Mononuclear Bone Marrow cells from normal donors or K-562 cells were incubated in presence of this pentapeptide, used at the concentration of 10 microg/ml (test), or in presence of saline (control), for 5-20 hours at 37°C; cells were thereafter plated in a IMDM methylcellulose medium using Leucocyte Conditioned Medium as growth factors; K-562 were plated at concentration of 2.5x10^4 cells/plate and normal BM plated at concentration of 5x10^4 / plate, colonies were scored after 10 days of incubation at 37°C and 5% CO2.

After incubation in presence of pentapeptide, normal BM cells retained compared to controls, colony formation: controls: 38 CFU-GM /5x10^4, pentapeptide: 35 CFU-GM /5x10^4, differences were not significant ( paired t-test: P = 0.7).

On the contrary colony growth of K-562 cells was greatly reduced to 95% of the initial value after pentapeptide incubation. In control plates K-562 cells grew a mean of 80 colonies per plate, while after preincubation with pentapeptide, K-562 cells had a mean growth of only 3 colonies per plate, difference was statistically significant ( paired t-test: P = 0.02).

This new pentapeptide could be usefull as Bone Marrow purging agent before autologous Transplantation.

PB82

PBSC grafts provide greater suppression of host anti-donor responses when compared to primed or unprimed bone marrow grafts


The kinetics of hematopoietic stem cell engraftment differ between bone marrow and mobilized peripheral blood stem cell grafts. Recently, differences in gene expression of the CD34+ cells within these grafts have been reported. In this study we tested the hypothesis that the stem cell source might also differentially affect host anti-donor responses. MHC-mismatched cynomolgus monkeys were conditioned with high dose thymoglobulin, fludarabine, and melphalan to test three sources of stem cell grafts: bone marrow (BM, n=2), G-CSF primed bone marrow (PBSC, n=2), and CD34+ selected, G-CSF and stem cell factor mobilized peripheral blood (PBSC, n=2) for effects on engraftment and host anti-donor responses. Mean CD34+ cell contents/kg were BM, 3.0±2.5 x 10^6, PBSC, 7.3±8.2 x 10^6, and PBSC, 5.9±0.8 x 10^6. Mean CD34 contents/kg were BM, 1.7±2.3 x 10^7, PBSC, 4.2±3.9 x 10^7 and PBSC, 1.2±0.2 x 10^5. No recipient developed GVHD. Post-transplant host anti-donor lymphoproliferative activities were compliant with the pre-transplant values via stimulation indices and found to be reduced>50% in PBSC and PBMC recipients but increased in BM recipients.

Cytotoxic anti-donor antibody development was observed in all recipients of BM and PBSC grafts but was barely assayable in one PBSC recipient. PBSC, but not PBM, conferred a survival advantage to the donor-derived kidney allograft when placed 2-4 months after hematopoietic engraftment (PBSC, 145, BM, 32±1 and PBM, 13±3 days). To determine whether host lymphoid reconstitution could account for the differences observed in host immune responses, we examined the number of days required to achieve pre-transplant levels of host CD2+, CD4+, CD8+, CD20+ and CD56+ cells and found no significant differences between the three groups. Although mixed chimerism was observed in all recipients, (2-16%) PBSC recipients had significantly longer periods of detectable CD34+ and CD8+ donor derived cells when compared to BM and PBSC recipients, (399±88 vs. 103±37 and 67±25 days, respectively). As levels of host T and B cell depletion were similar in all recipients, this is not likely that lymphoid depletion could solely account for altered anti-donor immune responses. Further, the suppression of anti-donor responses was not dependent on higher contents of CD34+ or CD3+ cells, or degree of lymphoid depletion. These observations indicate that the type of stem cell graft plays a large role in altering anti-donor responses in the host.

PB83

Hematologic reconstitution after PBPC autotransplantation: comparison between programmed-rate freezing and uncontrolled-rate freezing at -80°C


The most widely used system for PBPC cryopreservation is the programmed-rate freezing. Some studies indicate the feasibility of uncontrolled-rate freezing (URF) at -80°C, but its clinical impact has not been sufficiently studied yet. We compared two consecutive groups of patients autografted with PBPC cryopreserved according to two different methods: group A, 69 patients autotransplanted with PBPC cryopreserved with PRF; group B, 192 patients autotransplanted with PBPC cryopreserved with URF at -80°C. The same cryogenic mixture (DMSO 10%-Albumin 4%, final concentration) and the same storage system (-196°C in liquid nitrogen) were used. The two groups were matched for the main clinical characteristics except than for CFU-GM x 10e4/Kg reinfused: 73 (0-1390) in group B and 32 (0-286) in group A (p<0.0001). After thawing the median CFU-GM
recovery (tested in the bags before the reinfusion) was similar in the two groups: 32% (0-74) in group A and 24% (0-100) in group B (p=ns). All 261 patients showed stable engraftment, but URF cryopreservation was associated with a slower HR: 10 (8-14) days in group A and 11 (9-21) days in group B for ANC > 500/ml, 12 (8-37) days in group A and 13 (8-35) days in group B for platelets count > 20,000/ml, 15 (9-51) days in group A and 19 (10-95) days in group B for platelets count > 50,000/ml, 22 (10-90) days in group A and 26 (12-160) days in group B for platelets count > 150,000/ml. The median duration of neutropenia with ANC < 100/ml was 5 (2-10) days in group A and 6 (0-50) days in group B (p=0.01), we did not observe any significant difference as regards the duration of fever and antibiotic therapy, days of hospitalization and RBC or platelets transfusions. On multivariate analysis four parameters were significantly associated with slow engraftment: CD34 x 10e6/kg reinfused < 6 for ANC > 500/ml (p=0.001) and for all parameters of platelets engraftment (p=0.03, p=0.0001; p=0.04); URF for ANC > 500/ml (p=0.0006), platelets=20,000/ml (p=0.01) and > 50,000/ml (p=0.0022); mieloablative (including TBI or busulfan) conditioning regimen for platelets > 50,000/ml (p=0.004); previous chemotherapy regimens > 3 for platelets > 150,000/ml (p=0.01). No graft failures or MDS were observed in the two groups, the TRM was 2.9% (2 patients) % in the group A and 2.7% (5 patients) in the group B. In conclusion our data confirm, in a large cohort of patients, that URF technique is safe and allows sustained engraftment without increasing the risks of the transplant procedure, but the kinetics of engraftment after URF is significantly slower both for neutrophils and for platelets.

P825
Improvement of CD34+ recovery, purity and T depletion using isolex 300i with software versions 2.0 and 2.5 in allogeneic transplantation
P. Accorsi, T. Bonfini, M. Dell’Isola, V. Catinella, G. Di Girolamo, R. Giancola, P. Di Bartolomeo, A. Iacone (Pescara, I)
Peripheral blood progenitor cell immunomanipulation techniques have been developed in order to obtain an optimal dose of CD34+ cells and T-depleted cells in allogeneic transplantations. The Isolex 300i, upgrade software versions 2.0 and 2.5 have proven to give an excellent outcome for graft manipulation. In this report the performance of 81 immunoselection procedures with apheresis products from matched and mismatched related donors mobilized with G-CSF 10µg/kg/d have been evaluated using 3 different softwares. In order to reach a target dose of 6 and 10 CD34+ x 10e6/kg bw the number of selection procedures/patient was 1.6±0.5 and 2.4±0.7 for matched and mismatched transplants, respectively. Overall data are summarized in Table 1. The results of both CD34 positive and plus/minus selections are shown in Table 2. The CD34+x10e6/kg and CD3x10e4/kg doses were 7.2±1.4 and 100 in matched transplants and 9.7±3.0 and 3.2±4.1 in mismatched transplants respectively.

The upgrade of the software versions 2.0 and 2.5 increased the recovery of 22% and the CD34+ cell purity. Furthermore, in mismatched transplant, the T depletion was significantly higher and the CD3x10e4/kg infused did not exceed 5.0 (p=0.005).

Table 1. Overall data according to software upgrading.

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<th>CD34+ Purity</th>
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Table 2. Results of positive and plus/minus selections according to software upgrading.

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<th>CD34+ T Depletion</th>
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P824
Phenotypic and functional changes observed in peripheral blood chemotherapy plus G-CSF mobilized patients
Aim: To study, in peripheral blood, immunological (phenotypic and functional) changes in T, NK and dendritic cells (DC) induced by chemotherapy plus G-CSF in autologous transplant patients.

Patients and Methods: 16 patients underwent a chemotherapy plus G-CSF containing regime (9 multiple myeloma, cyclophosphamide+G-CSF; 7 Non-Hodgkin lymphoma, DHAP+erythropoietin+G-CSF). Venous samples were drawn before starting chemo and just prior to apheresis. T, B, NK and DC populations were analyzed by flow cytomtery. DC populations were divided in DC1 (myeloid origin), DC2 (lymphoid origin) and DC16 (coexpression of CD16). PHA-induced T cell activation and proliferation were studied in vitro. T-cell Fas-dependent apoptosis was also measured.

Results: No significant changes were observed in the percentage of B, T and NK subpopulations. DC1, DC2 and DC16 increased 3, 4.5 and 50 times with mobilization (p<0.01 for DC2 and p<0.0001 for CD-16). After mobilization T cell PHA-induced activation (determined by CD69n expression) diminished significantly (p<0.05). In T populations, no changes were observed either in Fas expression or Fas-dependent apoptosis.

Conlusion: Chemotherapy+G-CSF mobilization schemes in autologous transplant patients induce preferently an increase in DC2 and DC16 populations (as it has been described in the allogenetic setting). PHA-induced T cell activation decreases with mobilization. T cell Fas-dependent apoptosis does not change.

P826
Long-term follow-up of peripheral blood stem cell transplant (PBSCT) recipients: graft-versus-host disease, transplant-related mortality and survival
Between 1995 and 2001 sixty patients with various diseases (CML n=15, AML n=22, NHL n=8, ALL n=7, SAA n=1, MDS n=3, myelosarcoma n=1, myeloma n=2, CLL n=1) and a median age of 37, range, 18-55) years received unmanipulated peripheral blood stem cell (PBSCT) transplants from HLA-identical sibling (n=47), HLA-identical unrelated (n=7) or 1-antigen mismatched unrelated (n=6) donors. Twenty patients were considered to be standard risk patients, whereas 40 patients had high risk disease. Myeloablative therapy consisted of cyclophosphamide (CY) and total body irradiation in 47, and high-dose chemotherapy alone in 13 patients. For graft-versus-host disease (GVHD) prophylaxis 50 patients were given cyclosporine A and methotrexate and 10 patients cyclosporine A alone. PBSCT were mobilized by granulocyte colony-stimulating factor (G-CSF) given at 10µg/kg b.w./day for four days. Leukapheresis started on day 5 using a Cobe Spectra Cell Separator. In 48 donors (80%) one leukapheresis was sufficient whereas in 12 donors a second one had to be performed. A median of 7.19 x 106 CD34+ cells/kg b.w.
(range, 1.65-21.5) was obtained. Twenty-two patients received G-CSF (5μg/kg) after PBSCT until neutrophil recovery. Absolute neutrophil counts (ANC) >0.5 x 10^9/L and ANC >1.0 x 10^9/L were reached after a median of 14 (range, 9-32) and 15 (range, 10-34) days after PBSCT. Unsupported platelet counts >20 x 10^9/L and 50 x 10^9/L were reached after 15 (range, 7-33) and 17 (range, 11-40) days, respectively. Incidence of acute GVHD was 52%, including 23% of grades III-IV. In patients with sibling and unrelated donors 44% and 61% of acute GVHD was observed. Chronic limited GVHD occurred in 32%, and extensive in 28% of patients. The incidence of chronic GVHD in patients with sibling and unrelated donors was 62%, and 50%. Transplant-related mortality was 23%, with no significant difference between standard and high risk-group. Relapse rate was 35% in the high risk was 23%, with no significant difference between.

### P827
**The safety and efficiency of stem cell apheresis with the MNC and aPBSC program in children**
K. Drabko, I. Woznica, T. Grenczewska-Chmiel, B. Wojcik, J. R Kowalczyk (Lublin, PL)

In new developed pediatric BMTC unit in Lublin we have performed 115 peripheral stem cell collection for auto peripheral stem cell transplantation in 58 children aged 4-20 since 1999. Procedures were done on CobeSpectra separators using MNC program (n=91) or aPBSC program (n=24).

The aim of the analysis was to estimate the safety of procedures and to compare efficiency of MNC and aPBSC program.

Children were diagnosed lymphoma (n=27), leukemia (n=3), neuroblastoma (n=7), Sa Ewing (n=11) another solid tumors (n=10) and they were qualified to apheresis when the CD 34+ count in peripheral blood was > 20/ul and general clinical condition was good. In most cases single lumen central catheter in femoral vein was placed as access line. Existing central catheters or peripheral vein catheters as the return line were used. Only 3 (2%) procedures were not completed due to problems with access pressure (2 with access peripheral vein and 1 central line). In 2 cases procedures were repeated after replacement of catheter.

During procedures patients were substituted with calcium gluconatum in boluses before and in the middle of apheresis according to serum levels.

Children were not transfused with erythrocyte concentrates during procedures. If the hemoglobin level was less then 8g/dl patient were transfused before apheresis.

No serious clinical adverse effects of the procedures were found. In all cases decrease of hemoglobin level was observed: in MNC procedure decreased 16% in average and for aPBSC procedure 12.8%. Trombocytopenia and hipokalemia were also observed in majority of children.

We concluded that stem cell apheresis are safe procedures, even in children with low body mass.

In our materiel apheresis with the MNC program is more efficient then with aPBSC program (86% versus 61 % of CD 34 cells in peripheral blood were collected, respectively).

### P828
**Ancestorm (stem cell factor) in combination with filgrastim can mobilize sufficient peripheral blood progenitor cells (PBPC) to support high-dose salvage chemotherapy and late intensification in relapsing and refractory aggressive non-Hodgkin’s lymphoma**

High-dose therapy with autologous stem cell transplantation is the standard treatment in patients (pts) with recurrent aggressive non-Hodgkin’s lymphoma. A maximal response before intensification is required for long term survival but hematological toxicity of this chemotherapy is severe. In order to decrease hematological toxicity of this procedure we hypothesized that adding SCF (20mg/kg/d) to filgrastim (5mg/kg/d) at d3 after the mobilization regimen (Cy 4.5g/m2, VP16 450mg/m2 at d2) might increase the number of collected CD34+ cells thereby allowing to support both the 2 courses of VIM-AraC (VP16 150mg/m2 d1-d3, Iloksamid 1.5g/m2 d1-d3, Mitoxantrone 10mg/m2 d1-d2, AraC 2g/m2 d3-d4) delivered (60% at d30 and d60 (25 % of the total PBPC yield reached at d6 of each course) and late intensification by a BEAM regimen at d90 (50 % of the total yield reinfused at d8). Both cytokines were administered once daily until aphereses have been completed. Primary objective was to reach an optimal yield of 12x10^6 CD34/kg. In previous study with G-CSF alone, median number of collected CD34+ cells and of aphereses was 8x10^6 (0.9-50) and 2 (1-6), respectively. From 11/98 to 12/00, 39 pts were enrolled (median age 48y; 1st relapse 2, partial response 2, large B-cell T 8, anaplastic large cell 3). 36 of 39 pts had at least one apheresis (3 pts not collected: abdominal pain 1, infection 1, low CD34+ in peripheral blood 1). The median number of CD34+ cells was 17x10^6/kg (3-133) with a median of one apheresis (1-5). In 28 pts (78%) the optimal yield of 12x10^6 CD34/kg was obtained. There were no serious allergic reaction to Ancestim. As of 11/01, 28 pts completed the salvage procedure (no collection 3, insufficient collection 1, progression on therapy 4, infectious complications 3). Median recovery delay of ANC>1x10^9/L and of platelet >20x10^9/L after salvage (1), 1st course (2), 2nd course of VIM-AraC (3) and after the BEAM (4) were: (1) d12 (10-20) and d14 (12-30), (2) d11 (8-15) and d10 (6-19), (3) d11 (8-16) and d11 (6-19), (4) d11 (8-14) and d8 (6-15), respectively. No hematological failure of recovery and no toxic death were observed. We conclude that mobilization of PBPC is enhanced by co-administration of SCF allowing more patients to achieve sufficient CD34+ cells to support salvage high-dose therapy in aggressive lymphomas and resulting in reduced number of aphereses. Outcome of the 39 patients will be presented.
Patients and methods: Data of 2156 patients who received HSCTs for AML 1CR from year 1994 to 2000 were analyzed. Median age was 35 both in patients receiving BM and PB. Stem cell source: BM in 1316 cases, PB in 838. Interval diagnosis to CR1, interval CR1 to HSCT, number of WBC at diagnosis, cytogenetics, FAB type, sex of donor and patient, donor age, number of nucleated cells infused, methods of GVHD prevention, use of TBI, grade of GVHD were considered.

Results: In multivariate analysis factors affecting outcome were: for transplant related mortality (TRM) in BM recipient: Age >35 (p=0.01, RR 1.5); for PB recipients: (i) Age > 35 (p=0.003; RR 1.9) and (ii) F -->M (p=0.001; RR 2.35). For relapse incidence (RI) nucleated cell dose (>2.6 x10^8/kg) (p=0.006; RR 0.6) for BM recipients and for PB recipients F-->M (p=0.04; RR 0.5). For leukemia free survival (LFS): for BM recipients (i) interval CR to transplant (p=0.03; RR 1.5), (ii) cytogenetics (p=0.02; RR 1.5), (iii) nucleated cell dose (p=0.007; RR 0.65), (iii) FAB M5-6-7 (p=0.025; RR 1.5); for PB recipients (i) age > 35 (p=0.02; RR 1.4).

Conclusions: These data suggest that prognostic factors affecting transplant outcome differ according to the stem cell source utilized.

P830
Dendritic cells (DC1 and DC2) subsets detection in bone marrow and G-CSF priming peripheral blood harvest from allogeneic stem cell donors
R. Stocchi, D. Damiani, A. Sperotto, M. Cerno, A. Geromin, P. Masolini, A. Michelutti, C. Skert, C. Filì, M. Baccarani, R. Fanin (Udine, I)

In peripheral blood (PB) and in bone marrow (BM) two subsets of dendritic cells (DC) have been identified: DC type 1, myeloid, are lineage negative, HLA-DR positive and CD11c positive; DC type 2, lymphoid, are lineage negative, HLA-DR positive and CD123 positive. DC1 activate T lymphocytes, while DC2 seems to induce antigen-specific tolerance. Since the number of infused DC can influence the kinetic of the immune system reconstitution and the development of graft-versus-host disease, we counted DC1 and DC2 in the PB and in the BM of allogeneic stem cell donors using a 3-colour flow cytometric assay. DC were identified in lysed whole blood or bone marrow as lineage negative (CD3, CD16, CD56, CD14, CD19, CD20); only CD11c positive, CD123 positive (DC1) or CD123 positive (DC2). In PB the mean DC1 number (per microliter) was higher after G-CSF mobilisation than prior to G-CSF administration (4.1±0.1 versus 7.3±0.2, p=0.03); the mean number of DC2 was also significantly increased after G-CSF administration (3.4±0.1 versus 20±0.2, p=0.002). In BM the mean number of DC1 was similar to that found in PB before G-CSF administration (4.1±0.1 versus 4.2±0.2, p=0.94), while the mean number of DC2 was higher than that found in PB before G-CSF administration (3.4±0.1 versus 16±0.2, p=0.06) but similar to that found in PB after G-CSF administration (20±0.2 versus 16±0.2, p=0.08). BM harvests (n=6) contained a mean of 0.04±0.01x10^6/kg recipient DC1 and 0.31±0.01x10^6/kg recipient DC2 while PBSC harvests (n=8) contained a mean of 0.3±0.01x10^6/kg DC1 and 1.2±0.1x10^6/kg DC2. In conclusion, in standard condition the mean number of DC1 is similar in PB and in BM, while the DC2 number is higher in BM than in PB. However, G-CSF administration increases the number of DC, in particular of DC2 and, for this reason, the number of DC1 and DC2 infused is higher in PBSC harvest in comparison with BM harvest.

Additional abstracts to this topic

CD34-selected sibling allogeneic peripheral blood stem cell (PBSC) transplants for acute myeloid leukaemia (AML)
N. Butt, N. McGinnity, R. Clark (Liverpool, UK)

An increasing proportion of allografts use PBSC as the graft. This raises concerns over a higher donor T-cell dose, with reports of an increase in late GVHD. Positive CD34 selection offers a means of T-cell depletion of the graft. We report our experience of CD34-selection for matched sibling PBSC transplants performed for AML. Eleven patients (7F, 4M) with AML, in remission, (9=CR1, 2=CR2) were transplanted between March 2000 and August 2001. Their median age at transplant was 29 (range 17 – 45) years. Transplant conditioning was with standard cyclophosphamide and TBI and post-BMT cyclosporin and short methotrexate. Following donor G-CSF priming, the mean total cell dose harvested was 10.68 (range 6.75 – 21.59) x10^8/kg. CD34 selection was performed by immunomagnetic separation using the Isolox 300i Magnetic Cell Separator. The mean CD34 cell dose infused was 4.25 (range 1.40 – 8.09) x10^6/kg post selection with a mean residual T cell dose of 2.63 (range 0.92 – 13.18) x10^6/kg post selection. This represents a depletion of about 2 logs of T-cells. All patients survived at least 100 days. The median times to neutrophil (>0.5x10^9/l) and platelet (>50x10^9/l) engraftment were 15 and 20 days respectively. Three patients experienced acute grade I (cutaneous) GVHD (at days +22, +26 and +56), with no cases of more severe acute or chronic GVHD. Three patients died of disease relapse at days +140, +262 and +347. One of these patients was transplanted in second CR. The remaining eight patients are well. The median FU is 280 (range 68 – 581) days with overall actuarial disease free survival of 59% at 581 days post-BMT. CD34-selected PBSC allografting for AML is a safe technique which offers excellent prophylaxis against GVHD, with an acceptable disease relapse risk.

Methods of PBSC harvesting for auto- and allogeneic transplantation
L. Fregatova, A. Golovacheva, M. Estrina, E. Babenko, E. Zueva, B. Afanasyev Stem cell source

Quality of the graft is the basis for successful transplantation of haemopoietic stem cells (HSCT). We have observed 23 patients (pts) with a variety of malignant and hematological disorders (I gr.) in whom PBSCs were collected for autologous transplantation (12-62, f/m - 10/13, NHL-11, HL-4, sol.tum. - 3, MM-3, autoomm. diseases-2) and 11 relative donors of PBSC (II gr.) (age 15-62, f/m - 7/4). These groups were mobilized with G-CSF 5 mcg/kg SC for two days and conditioned with group III gr.) (n = 26) treated with single-dose exposure of CD34-SF (10 mcg/kg SC) 19-67, f/m - 16/10, NHL-10, HL-3, sol.tum.-8, MM-5). Both mobilization regimens were generally well tolerated. Procedures of LVL were performed using a COBE "Spectra" blood cell separator (version 4.0, 5.1). An average of 5-6 total blood volumes (TBV) (15 - 33 L) were processed (I gr. and II gr.), compared to 3 TBV (7 - 12 L) in group III gr.. In cases of LVL anticoagulation was accomplished by ACD-A and low-molecular-weight heparin "Clexane" (Aventis). The blood: ACD-A ratio was 20:1 with a i.v. injection of "Clexane" (10 mg/kg) in the return line at the beginning of procedure, as a fixed bolus dose.

Results: The mean yield of MNC/kg per procedure was in I gr. 17,3± -3,6 x10^6/kg (3,6 -38), 53,3± -8,6 x10^4/kg (0,4-22,9), in II gr. 16,6+ -2,2x10^8/kg (4,2 -36), CD34+- 12,6+ -5,7x10^6/kg (1,7 -30). The blood: ACD-A ratio was 20:1 with a i.v. injection of ''Clexane'' (Aventis) in the return line at the beginning of procedure, as a fixed bolus dose. Such approach was resulted in to significantly lower mean of CFU by ACD-A and low-molecular-weight heparin ''Clexane'' (Aventis). The mean nucleated cell dose (10 mg/kg) in the return line at the beginning of procedure, as a fixed bolus dose.
MNC, CD34+ and CFU-GM per 1 procedure of LVL was enough for transplantation. Conclusion: Use of the split doses of G-CSF for progenitor cells mobilization and increase of the TBL processing during LVL makes enable to collect adequate dose of PBSC in one procedure, makes this process more convenient for pts and less time consuming for the stuff.

**BFU-E** contains in the inoculum correlates with early hematologic regeneration after allogeneic peripheral blood stem cell transplantation

K. Halaburda, M. Bieniaszewska, W. Knopińska-Posluszyńska, K. Lewandowski, J.M. Zauda, J. Balon, A. Hellmann (Gdańsk, PL)

60 patients were transplanted using allogeneic peripheral blood stem cells from HLA matched family donors. 58 patients were transplanted for malignant diseases and 2 for SAA. The conditioning regimen in 58 cases of neoplastic diseases was BuCy120 and in 2 cases of SAA was ATG/Cy. Uniform aGHD prophylaxis consisting of CsA/Mtx was employed in all cases. The grafts were analyzed for the numbers of WBC, MNC, CD34+ cells, CFU-GEMM, CFU-GM and BFU-E. They were calculated per kilogram of recipients' body weight. Early hematologic regeneration was evaluated in days necessary for the patients to reach ANC>0,5G/L and ANC>1,0G/L. Although the correlation for CFU-GM was marginally stronger than BFU-E for the time to reach ANC>0,5G/L and ANC>1,0G/L only the latter was significant for both the ANC and PLT regeneration in the early posttransplant period.

**Is there a correlation between peripheral blood CD34+ cells and peripheral blood erythocyte precursors in healthy G-CSF stimulated donors and the effect of G-CSF and apheresis on complete blood count**


The aim of this study was to evaluate the correlation between peripheral blood CD34+ cells and hyper-lucent reticulocytes (HLR), immature reticulocyte fraction (IRF) and reticulocytes in G-CSF stimulated allogeneic peripheral blood stem cell donors. The effect of G-CSF and apheresis on CBC indexes was also analyzed. Between May 2000 and February 2001, 12 healthy peripheral blood stem cell donors for patients with different hematologic malignancies were studied. Median age of the donors was 31 (19 – 50). M/F ratio was 10/2. The donors were the HLA-identical siblings of the patients. All donors were given G-CSF (Neupogen, Roche) 5µg/kg/s.c twice daily for 5 days and stem cell collection was performed on 5th day 2 hours after the last injection. Four consecutive (pre-G-CSF, pre-apheresis, harvest material and post-apheresis) blood samples were collected and tested for CD34+ cells (FACSort, Beckton Dickinson, USA), CBC, HLR, IRF and reticulocyte (Coulter GenS) counts. Median leukopheresis cycles in order to collect >0.5x106/kg CD34+ cells were 2 (1 – 3). A positive correlation was detected between the number of preapheresis peripheral CD34+ cells and the ones detected in the harvest material (p=0.0022). No correlation was found between the CD34+ cell counts and the HLR, IRF and reticulocyte numbers during G-CSF treatment (p>0.05). Peripheral blood HLR, IRF, reticulocyte, WBC, MCH, MCHC, neutrophil, lymphocyte, monocyte, eosinophil and basophil counts significantly increased during G-CSF administration. On the contrary platelet counts significantly decreased. Apheresis cycle also markedly decreased reticulocyte, hemoglobin, hematocrit, leukocyte, platelet, MPV, neutrophil, lymphocyte, monocyte and eosinophil counts.

**Presence of tissue factor (TF) antigen expressing cells within allogeneic peripheral blood progenitor cell (PBPC) harvest**


Thromboembolic complications are one of the early post-transplant complications seen in bone marrow transplant patients that cause morbidity and mortality. We hypothesized that PBPC express procoagulants that contribute to these complications. TF is the primary initiator of physiologic coagulation and known as prothrombotic. There were no sufficient data available about any role of TF positive cells in the harvest material and clinical course of PBPC transplantation. We assessed tissue factor antigen (TF:Ag) expression on PBPC collected from 19 HLA identical sibling donors for allogeneic PBPCST, following s.c. injection of 10mcg/kg/d x 5d G-CSF. TF:Ag expression (American Diagnostica, Greenwich) was detected by flow cytometry on PBPCs. We evaluated whether there was any correlation between the amount of TF infused per patient’s body weight and engraftment kinetics, the frequency and severity of acute and chronic graft versus host disease, and thromboembolic complications. The median TF:Ag expression on mononuclear and myeloid cells was 22% (range, 0%-74%). We were not able to demonstrate any co-expression of TF: Ag neither with CD34+ cells nor lymphoid cells. The mean value of the TF:Ag expressing cells infused was 1.52X106/kg±2.77. We did not find any impact of TF:Ag positive cells on engraftment kinetics and incidence of both acute and chronic GVHD. There were nine thromboembolic complications at peri-transplant course (VOD=7, DVT=2). Though patients with VOD received more TF:Ag positive cells in comparison to patients without VOD, 2.26±3.55x106/kg and 1.29±2.5x106/kg, respectively. Pearson correlation analysis showed no statistical significance. The gender of the donors had no significant impact on TF:Ag expression. Male donors’ PBPC yield included significantly (p=0.002) more TF:Ag positive cells in comparison to female’s (2.73 vs 0.29, respectively). Though, our donor pool is limited and estimation technique for TF:Ag expression is undistinguished, we were able to show the presence of TF: Ag expression on infused PBPCs and to quantify the number of them. Further analysis will be performed using LPS and myeloid cells was 22% (range, 0%-74%). We were not able to demonstrate any co-expression of TF: Ag neither with CD34+ cells nor lymphoid cells. The mean value of the TF:Ag expressing cells infused was 1.52x106/kg±2.77. We did not find any impact of TF:Ag positive cells on engraftment kinetics and incidence of both acute and chronic GVHD. There were nine thromboembolic complications at peri-transplant course (VOD=7, DVT=2). Though patients with VOD received more TF:Ag positive cells in comparison to patients without VOD, 2.26±3.55x106/kg and 1.29±2.5x106/kg, respectively. Pearson correlation analysis showed no statistical significance. The gender of the donors had no significant impact on TF:Ag expression. Male donors’ PBPC yield included significantly (p=0.002) more TF:Ag positive cells in comparison to female’s (2.73 vs 0.29, respectively). Though, our donor pool is limited and estimation technique for TF:Ag expression is undistinguished, we were able to show the presence of TF: Ag expression on infused PBPCs and to quantify the number of them. Further analysis will be performed using LPS for the induction of the cells within the harvest and for further improvement of TF:Ag expression. Male donors PBPC yield did contain significantly more TF:Ag expressing cells than the female’s. Further studies are necessary to draw a firm conclusion about the contribution of TF to thrombotic complications after transplantation.

**Evaluation of umbilical cord blood bank inventory accounting to TNC, CD34+ and CFU-GM content of units**

T. Bonfini, E. Liberatore, G. Di Leve, T. Rotondo, V. Catinella, F. Di Penta, L. Costarelli, A. Iacone (Pescara, I)

Banking and transplant programs with umbilical cord blood (UCB) are continuously increasing. Volume and total nucleated cell (TNC) count of CB units (CBUs) are the major banking criteria. Nevertheless, despite an apparent linear correlation between NC and progenitor counts, there is a high variability in progenitor concentration (up to more than 1 log) within a range of TNC.
counts. Hence, CBUs with a good TNC count may turn out to have drastically lower than expected progenitor content and CBUs which do not reach the NC banking threshold could contain a sufficient cell dose of progenitors.

Aim of our study was to evaluate, under a practical approach, the inventory of our banked CBUs; applying an arbitrary transplant threshold dose of $2 \times 10^7$ NC/Kg, $0.5 \times 10^5$ CD34+/Kg and $1 \times 10^4$ CFU-GM/Kg, several cellular Transplant Index (TI) were desumed (TINC=2x10e7/Kg, TI CD34+=0.5x10e5/Kg, TI CFU-GM=1x10e4/Kg) and 3 categories of putative recipients (<50 Kg, >50=70 Kg and >70 Kg) were considered. Categories which per se do not contain a sufficient number of progenitors; 2) in the TI TNC=2x10e7/Kg >70 Kg category, approximately 20% of units do not contain a sufficient number of progenitors; 3) in the TI TNC=2x10e7/Kg <50 Kg, >50=70 Kg and >70 Kg were 44%, 40% and 16%, respectively. Data on the pattern of distribution of progenitor content, accounting to TNC categories are shown in graphics 1 and 2.

Concerning the results, two major issues are to be considered: 1) in the TI TNC=2x10e7/Kg >70 Kg category, approximately 20% of units do not contain a sufficient number of progenitors; 2) in the TICD34+=0.5x10e5/Kg or TI CFU-GM=1x10e4/Kg >70 Kg categories, the proportional contributes were respectively 30%, 44% and 26% from TITNCx10e7/Kg categories <50 Kg, >50=70 Kg and >70 Kg were 44%, 40% and 16%, respectively. Data on the pattern of distribution of progenitor content, accounting to TNC categories are shown in

Evaluation of haematopoietic progenitor cells of CBB inventory for transplant, having an adequate progenitor content.

Concluding remarks: Although the low number of SCT evaluated preclude definitive conclusions, some remarks can be made 1) Despite the higher cost of CD34+ selection, the final cost of this procedure is similar to that observed in allo-PBSC transplants due to lower.

23. Selected Aspects

**P831**

**Toxicity of salvage therapy in patients relapsing after an autologous hematopoietic progenitor transplant (HPT)**

M. Hermosilla, T. Büchner, C. Ferrà, M. Encuentra, D. Gallardo, J. Sarrà, J. Berlanga, A. Grañena (Barcelona, E)

Therapeutic options for patients relapsing after autologous HPT are limited and often they are associated with severe treatment-related toxicity. The toxicity of rescue in relapsed patients after HPT was retrospectively evaluated in a group of 53 patients between 1993 and 2000. The median age was 46.5 years (18-74). All the 53 patients received chemotherapy, in 18 (34%) patients it was associated with radiotherapy and in 12 (23%) patients it was associated with biological modifiers (interferon, interleukin-2, mabthera,...). Some patients received more than one treatment sequentially administered. The primary disease were NHL in 25 patients (47%), Hodgkin’s disease in 9 patients (17%), myeloma in 8 patients (15%), ALL in 6 patients (11%) and AML in 5 patients (9%). The incidence of grade III-IV toxicity (WHO) was: hematological in 28 patients (53%), infectious in 5 (9%), gastrointestinal in 4 (8%), hemorrhagical in 3 (6%) and neurological in 1 patient (2%). There was no detected cardiac complication nor hemorrhagical cystitis.

The planned salvage therapy was reduced or omitted due to toxicity in 15 patients (28%). The mortality related to the salvage therapy was 26% (14 patients): 7 patients due to infection, 4 due to hemorrhage, 2 bronchospasm and 2 instertitial pneumonias.

Conclusion: Salvage treatment is associated with a high morbidity and mortality. The treatment-related toxicity forced to interrupt therapy in some cases and mortality was 26%. Patients should be carefully evaluated previous to consider salvage therapy.

**P832**

**Prospective analysis of direct hospital medical costs in several modalities of allogeneic and autologous stem cell transplantation (SCT)**


Objectives: To calculate the hospital expenses in SCT programs and to compare the cost of different SCT modalities.

Patients: All consecutive SCT performed in our centre between 1/1/01 and 30/6/01.

Type of analysis: Cost-identification study.

Perspective of the analysis: Hospital derived expenses.

Type of costs: Direct medical costs until day +100 after SCT.

Data collection methods: Prospective by a single observer.

Results: During the study period 53 SCT were performed (20 allo and 33 auto-SCT). The median (range) age of the patients was: 44 (17-68) years. SCT was performed due to an acute leukaemia in 13 cases, chronic leukaemia in 10, multiple myeloma in 11, lymphoma or Hodgkin disease in 9, multiple sclerosis in 3 and other diagnosis in two. Two patients had a non-myeloablative SCT and two an unrelated donor SCT. In 23 cases conditioning regime included total body irradiation. All but five SCT received peripheral blood stem cells (PBSC). Seven autologous SCT were managed at home. Total hospital expenses of these SCT were 908,258 Euro (463,442 to 442,816 for allo-SCT). Mean (+SD) cost of auto and allo-SCT were 15,014 (+7,078) and 22.140 (+8,458) Euro, respectively. The cost (x 103 Euro) of main SCT modalities was:

Conclusions: Although the low number of SCT evaluated preclude definitive conclusions, some remarks can be made 1) Despite the higher cost of CD34+ selection, the final cost of this procedure is similar to that observed in allo-PBSC transplants due to lower.
hospitalisation expenses. 2) At home auto-SCT implies a significant reduction in hospital medical costs.

<table>
<thead>
<tr>
<th>Type of SCT</th>
<th>Allogeneic</th>
<th>Autologous</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMT</td>
<td>PBSC</td>
<td>CD34+</td>
</tr>
<tr>
<td>Number</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>SC collection</td>
<td>0.8 (±0.1)</td>
<td>1.1 (±0.5)</td>
</tr>
<tr>
<td>Hospitalisation</td>
<td>11.1 (±1.6)</td>
<td>13.2 (±6.6)</td>
</tr>
<tr>
<td>Blood products</td>
<td>2.1 (±1.0)</td>
<td>3.6 (±4.0)</td>
</tr>
<tr>
<td>Pharmacy</td>
<td>52 (±4.9)</td>
<td>12 (±1.0)</td>
</tr>
<tr>
<td>Lab &amp; image</td>
<td>2.9 (±0.1)</td>
<td>3.9 (±1.9)</td>
</tr>
<tr>
<td>Out-patient</td>
<td>111 (±55)</td>
<td>56 (±3.4)</td>
</tr>
<tr>
<td>Other</td>
<td>0.9 (±0.1)</td>
<td>0.8 (±0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>24.1 (±6.3)</td>
<td>24.2 (±9.5)</td>
</tr>
</tbody>
</table>

**P833**

**Development of a hospital-based Cell Factory**


Rapid developments in the biotechnology field have raised the need of specific facilities for cell manipulation, built to avoid microbial contamination of the product and to protect the operator and the environment. A recently developed Cell Factory suitable for the scaling-up of cell therapy procedures. To this aim, collection of referenced documents was performed using the web sites of the Italian Istituto Superiore di Sanità (http://www.iss.it), the European Agency for the evaluation of Medicinal Products (http://www.emea.eu.int), the USA National Institute of Health (http://www.nih.gov), the Food and Drug Administration (http://www.fda.gov) and the Center for Disease Control (http://www.cdc.gov). The laboratory staff met periodically with the project engineers and, during the validation of the facility, an experienced microbiologist was consulted. As a result, a 78 sqm Cell Factory was designed, including two BL3 sections, one for therapeutic cell production and the other for viral vector manipulation, completely separated and individually sterilizable. Each section is provided by self-closing, double door access and separate clothes-change rooms. The cleaning class, according to US federal standard 209, is 10,000 for the working area and 100,000 for the clothes-change room. Regarding the laboratory for viral manipulation, we followed the containment characteristics corresponding to Biosafety Level 3 described in the Manual of Biosafety in Microbiological and Biomedical Laboratories of the US Center for Disease Control and Prevention. In particular, in the working area, a negative pressure into the laboratory was produced. Moreover, HEPA-filters were installed in the input and exhaust air ducts and recirculation of the exhaus air was avoided. In conclusion, we developed a Cell Factory complying both with the requirements of a clean room facility and with that of a microbiology laboratory for viral vectors manipulation. This result was obtained with a close co-operation between the laboratory and project staff. The facility has been approved by the Italian Ministry of Health and given the code MI/Cl/IMP/J/00-02.

**P834**

**Critical analysis concerning the availability of full match sibling donors and feasibility of allogeneic bone marrow transplantation in Brazil**

K. Eid, E. Miranda, A. Vigorito, F. Aranha, G. Oliveira, C. de Souza (Campinas, BR)

The feasibility of allogeneic bone marrow transplantation (BMT) in a developing country was not demonstrated until now. Many hypotheses were severe aplastic anemia and hematological malignancies in all cases; 497/1138 (43.6%) candidates had full match donors; 352/1138 (30.8%) eligible candidates for allogeneic BMT. Only 235/352 (66.7%) were transplanted; 123/235 (52.3%) patients had 1 to 8 degrees of scholarship; the monthly familiar income ranged from US$ 60 (7%) to more than US$ 400 (36%); Overall Survival for CML, SAA and AML, 58%, 60% and 30% respectively. In conclusion, Overall Survival for most frequent hematological diseases is similar to those reported from International Registry, except for AML. This descriptive and explorative analyses confirms the feasibility of allogeneic BMT in a developing country like Brazil.
to define patterns of care. In two consensus meetings the indications for autologous transplants are defined (Ann Oncol 1994:5:19 and Ann Oncol 1999; 10:13). The Typhon registry was used to analyse the percentage of transplants adhering to these recognised criteria and also to assess the outcome of the patients not fulfilling these criteria. From 1980 to 2000 a total of 1604 cases was reported with in 1481 sufficient data: AML: 325, ALL: 155, CML: 10, NHL: 608, Hodgkin’s disease: 170, Myeloma: 188, MDS/secondary leukaemia: 25.

Of patients with AML 84%, ALL60%, CML 0%, NHL 55%, Hodgkin’s disease 97%. Myeloma 45% and MDS/secondary leukaemia 50% fulfilled the criteria set. Comparing the outcome for the patients with recognised criteria with those not fulfilling the criteria, we found no difference for ALL (HR 1.3 CI 0.8-2.1), Hodgkin’s disease (HR 0.26 CI 0.4-1.9) and Myeloma (HR 1.5 CI 0.86-2.5). However, patients transplanted for AML (HR 1.7 CI 1.2-2.4) fared significantly better if fulfilling the consensus criteria. For AML, these differences are partly explained by a significantly increased treatment related mortality in later stages. Although patients with earlier stages of ALL also had a higher treatment related mortality, this did not translate in a reduced outcome compared to CR-1 patients. Also, patients with NHL did not better if fulfilling the consensus criteria (HR 0.8 CI 0.6-1.1). In the non-consensus cohort 23.8% was transplanted in first CR, 18.1% had low grade NH and 49% was transplanted within 12 months from diagnosis, making this a relatively good risk cohort. However, for a third cohort defined as patients with T cell NHL, mantle cell NHL and primary refractory NHL did significantly worse (HR 1.4 CI 1.0-1.99). For MDS and CML numbers of transplants were insufficient for analysis.

From these data we may conclude that the majority of patients (64%) transplanted in the Netherlands fulfilled the criteria set by the consensus meetings. However, significant numbers of patients, even with NHL, did not fulfil these criteria. Transplanting ALL patients in CR-1 does not translate in better outcome. This study suggests that registry data can be used to analyse protocol adherence.

P837

The profile of erythrocyte antigens after allogeneic hematopoietic stem cell transplantation


The difference of erythrocyte antigens (EA) between donor and patient has been studied since 1980s in order to detect engraftment, chimerism and to define allotransplantation criteria. In this study, 31 patients were followed for the difference of EA to evaluate the profile of EA and engraftment. Apart from major ABO groups, 21 different EA (C,c,E,e,Fya, Fyb, Jian, jkb, K, k, Lea, Leb, M, N, S, s, and P1) representing 8 minor blood groups were used. All these antigens were typed for the donor and patient before the transplant and the different antigens that the patient carry but not the donor or visa versa was followed for engraftment. The RBC transfusions given during the peri- and posttransplant period were carefully selected not to mix up the antigens followed. Patients who received blood transfusion for the last three months before the transplant were not eligible for the study. 20 (64.5%) patients received ABO identical, 8 (25.8%) received ABO minor mismatched and 3 (9.7%) received ABO major mismatched grafts. 13 patient received bone marrow derived and 18 patients received peripheral blood derived stem cells. One patient who did not received suitable transfusion and another one who showed an early relapse after transplant was excluded from the study. Remaining 29 patients were followed for a median of 12 months (6-16) after transplant. Median EA and reticulocyte engraftment were observed on days 35 (28-70) and 18.6 (12-50) respectively. Three patients showed EA and reticulocyte engraftment on the same day (+14, +14 and +29). One months after transplant 65% of the patients expressed donor type EA. Patients who received peripheral blood stem cells showed significantly faster EA and reticulocyte engraftment than patients who received bone marrow stem cells. Patient type EA were disappeared at a median of 80 (21-130) days after the procedure. Appearances of donor type antigens were significantly faster than the disappearance of patient type EA (p<0.0001). Table shows the antigens followed ant their median appearance and disappearance days after transplant. The earliest donor type EA detected was from Rh and KIDD system.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Appearance of donor type EA (days)</th>
<th>Disappearance of patient type EA (days)</th>
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<tbody>
<tr>
<td>K</td>
<td>42 (28-70)</td>
<td>32 (21-30)</td>
</tr>
<tr>
<td>Fy</td>
<td>46 (28-70)</td>
<td>44 (28-70)</td>
</tr>
<tr>
<td>Jk1</td>
<td>33 (21-30)</td>
<td>25 (12-50)</td>
</tr>
<tr>
<td>Jk2</td>
<td>22 (12-30)</td>
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</tr>
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<td>Kj</td>
<td>38 (28-70)</td>
<td>31 (20-40)</td>
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<tr>
<td>Kk</td>
<td>100 (50-130)</td>
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</tr>
<tr>
<td>Ll</td>
<td>70 (21-130)</td>
<td>71 (40-90)</td>
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<td>P1</td>
<td>90 (28-130)</td>
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</tr>
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<td>F</td>
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<td>71 (40-90)</td>
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<td>Le</td>
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</tr>
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<tr>
<td>E</td>
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Additional abstracts to this topic

First activity report on allogeneic bone marrow transplantation from Lebanon

A. Ibrahim, T. Jar, A. Mugharabi, N. Yassin, R. Jailouli, G. Nasoul, S. Mawakkil, L. Abs, E. Fadel (Beirut, LBN)

Lebanon is a small country of about 3.5millions citizens. Makassed General Hospital, one of the major hospitals in Lebanon established the first comprehensive program of hematopoietic stem cell transplantation (HSCT). Among 60 patients (pts) who received HSCT between January 1997 and October 2001, 23 underwent allogeneic bone marrow transplantation (BMT) for various diseases. Median age was 22 years (5-49). There were 10 females and 13 males. Indications and characteristics of allogeneic BMT: Allogeneic BMT was performed for AML de novo in 8 pts (CR1:4 pts, CR2: 4 pts), secondary AML in one patient (pt); ALL in 3 pts (refractory: 1 pt, CR2: 2 pts); CML in 5 pts; Thalassemia major in 5 pts and Fanconi’s anemia (FA) in one pt. Twenty two pts received allogeneic BMT from an HLA-identical sibling and one pt (with CML) from HLA-identical mother. Conditioning regimens combined Busulfan (Bu) (16mg/kg) and Cyclophosphamide (CTX) (120mg/kg) in 21pts; one pt (with refractory ALL) received Bu (16mg/kg), Cytosine-Arabinoside (12g/m2) and Melphalan (140mg/m2) and the pts with FA received ATG and CY (10mg/m2). Results of allogeneic BMT: Hematological reconstitution was obtained in all pts. Prophylaxis of GVHD combined Ciclosporin and Methotrexate (days 1, 3, 6 and 11) in 21pts; the other 2 pts (Thalassemia major: 1 pt, FA: 1 pt) received Ciclosporin alone. Acute GVHD occurred in 4 pts (17%). CMV antigenemia was transient positive in 8 pts (35%). Transient hemorrhagic cystitis occurred in 3 pts (13%). Seven of the 9 pts allografted for AML are alive in CR at a median of 29m+ (2-52); one pt is alive with disease at 18m+ and one pt died due to toxicity at 8m.Two of the 3 pts allografted for ALL died of toxicity (hemorrhage: 1 pt, VOD:1 pt) and the other is alive with disease at 13m+. Three of the 5 pts allografted for CML are alive in CR at 1m+, 4m+ and 24m+ respectively; one pt is alive with disease at 35m+ and one pt died of toxicity at 6m. Three of the 5 pts allografted for Thalassemia major are alive. Transplantation independent at 19m+, 31m+ and 38m+ respectively; one pt is alive with disease at 30m+ and one pt died of GVHD at 3m. The pt allografted for FA is alive and well at 9m+.
In the period between January and November 2001 in our center were performed 30 autologous PBSC transplantsations. Malignancies to be treated were: MM- 7, HD-13, NHL-2, breast cancer- 5, Wilms sarcoma-1, germ cell tumor-1. Thirty patients (2 MM, 1 HD) received double transplantsations. All patients with lymphomas were heavy pretreated or had refractory disease. Characteristics of patients: 11m/14f, median age- 35 years (range 16-62).

Mobilization methods included specific for underlying malignancies high-dose salvage regimens + G-CSF. Cy (2-6 g/m2) + G-CSF, G-CSF alone. Collections of PBSC were performed using blood cell separator COBE Spectra. Sufficient cell dose > 2x10^6 CD 34+ cells/kg was reached during 1-4 collection procedures. The mean volume of blood processed per procedure was 13.9 L (range 5.6-23.1).

High-dose chemotherapy for conditioning included: MM - melphilan 200 mg/m2; HD – BEAM; NHL - Bu + VP + Cy; ES - Bu + Mel; breast cancers - CPB; germ cell tumor - ICE. G-CSF 5 mg/kg was used (not for all pts) from d +7. All patients showed good hematological recovery with neutrophil count > 0.5x10^9/L on day +10 (range +8-+14) and platelets count > 20x10^9/L on day +12 (range +9-+14). 45% of patients didn’t need any transfusions of blood products. More frequent complication was febrile neutropenia (90 %) with one confirmed bacteremia (Staph. epidermidis). Treatment related toxicity rate was low and there were no deaths related to transplant procedure during 100 days. Post transplant therapy included radiotherapy for HD and immunotherapy with IL-2 for ES. The follow-up is very short but 5 pts already have evidence of disease progression.

Our experience shows that HDT with PBSC rescue is a good therapeutic strategy for patients with advanced malignancies but remission status and volume of previous treatment significantly influent outcomes.

**Platelet volume and platelet distribution width in differential diagnosis of thrombocytopenia of leukemia and ITP**

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Introduction: Traditionally, thrombocytopenia is divided into states of increased platelet destruction or loss, generally associated with increased production, and conditions of decreased platelet production. Included in the evaluation of thrombocytopenia have been the and the mean platelet volume (MPV ). Every particle under 20 fl will be counted as a thrombocyte.

Purpose: Comparing the MPV and platelet distribution width (PDW ) in differential diagnosis of thrombocytopenia in immune thrombocytopenic purpura (ITP ) and leukemia.

Methods and materials: We studied complete blood counts (CBC ) of 29 patients with leukemia and 36 patients with ITP from 1995 to 2000 in the Shahid Gazi Hospital. Their CBCs was done by Technicon H1 system, and their platelet count, MPV and PDW was determined.

Results: The mean of MPV in leukemia was 5.67 and in ITP was 4.58. The mean of PDW in leukemia was 79.40 and in ITP was 93.45. The mean of mode platelet volume in leukemia was 1.64 and in ITP was 0.61.

Discussion: Although MPV has been used in the evaluation of thrombocytopenia, but it can be influenced by a variety of artifacts such as erythrocyte and blastic cells debris. MPV is increased in ITP and myeloproliferative disorders, but remains normal in decreased production of thrombocytes such as aplastic anemia.

Our study reveals that the mean MPV in leukemia is greater than ITP. The mean of PDW in ITP is greater than leukemia. Therefore we conclude that PDW is more sensitive than MPV in differential thrombocytopenia in leukemia and ITP.
oral Amphotericine as prophylaxis for one year after transplantation. Furthermore, 3 pts who had a history of fungal pneumonitis before transplant received Amphotericine B intravenously at the dose of 1 mg/kg/day from day 0 to engraftment. Sixteen pts (6.3%) developed oropharyngeal infections; 10 pts had pneumonia, which was complicated by pneumonia-pericardium in 2 cases; 5 pts developed a systemic infection and 1 had a suspected CNS infection. Microbiological evidence was obtained in 9/16 cases, demonstrating Candida albicans or spp in 7 pts and Aspergillus in 2. IFI occurred before day +100 in 9 pts, and after day + 100 up to day +833 in 7 pts; late infection was associated with chronic GVHD in 67.7% cases. None of the patients with a previous history of fungal infection developed IFI after transplant. All pts were treated with Amphotericine B intravenously at the dose of 1-3 mg/kg/day. Three pts died of fungal infection; 10 pts recovered: seven of them subsequently died of GvHD or CMV infection or relapse, the remaining 3 are alive and well.

The incidence of IFI after allogeneic stem cells transplantation was low as compared to that reported by other Authors, with a mortality rate of 18.7% despite specific treatment.

**P840**

Factors affecting acute toxicity in 294 autologous transplant procedures: a single center multivariate analysis


We retrospectively analysed the toxicity of different conditioning regimens infused in our Departments in 268 patients, aged in median 49 years (range 16-79), affected by haematological malignancies. BEAM (23.4%), Melphalan +/- other drugs (37%), Bu7Cy2 +/- other drugs (18.8%), and other preparative regimens (14%) were administered as conditioning followed by the infusion of a median of 5.9x10^6/Kg CD34+ cells (range 1-36). 26 patients received a double transplant for a total of 294 procedures. Day +30 toxicity was assessed along WHO criteria. Time to platelet (>20,000/ml) and neutrophil (>500/ml) recovery, grade III-IV oral mucositis and infectious complications, use of analgesic opioid therapy were analysed as events. Pre-transplant patient and disease characteristics, conditioning regimen and duration of neutropenia were analysed as risk factors. Amifostine was infused at 740 mg/sm in 78 patients before melphalan infusion. A neutrophil (>500/ml) and platelet recovery (>20,000/ml) was achieved in a median of 8 (range 2-65) days and 13 (range 9-66) days respectively. We observed 188 febrile episodes, 43.5% microbiologically documented, (58% Gram+, 32% Gram -, 2.4% virus, 4.8% fungal). Grade III-IV oral mucositis was observed in 94 procedures (32%). Day +90 TRM was 3.1% and did not differ between young and elderly patients (> 60 years). Multivariate analysis showed that the infusion of a dose of CD34+ cells>5x 10e6/Kg significantly delays both neutrophil (RR= 3.3, p=0.001) and platelet recovery (RR= p=0.01). MM patients had also a slow neutrophil recovery (RR=3.3, p=0.02).

Severe oral mucositis was influenced by the kind of conditioning administered and the use of Amifostine. BEAM+Amifostine was associated with the lowest risk of severe oral mucositis (RR=1). BEAM alone was correlated with an higher, but not significant risk of mucositis (RR= 3.7, p=0.12), while Busulfan (RR= 12.1, p=0.003) and Thiopeta (RR=20.9, p= 0.0006) containing regimens were both associated with a high risk of mucositis. Severe infections were more frequent in patients with a >8 days long severe neutropenia (RR=1.98, p=0.04). Patients in progression (RR= 1.7, p=0.05) and those receiving conditioning different from BEAM+Amifostine (RR= 1.6, p=0.0006) received more analgesic opioid therapy. DFS was not different in patients affected by MM and HG NHL treated or not with Amifostine. In conclusion conditioning regimens, pre-transplant status, duration of neutropenia and dose of CD34+ cells infused are the main factors influencing respectively extrahaematological and haematological toxicity. Aging did not show any increasing of toxicity, this confirms the safety of transplant procedure in elderly patients. Amifostine, when administered with BEAM, may reduce oral mucositis and analgesic opioid administration, apparently without reduction of antitumor efficacy.

**P841**


From 1985 to 2004 285 patients were treated with allogeneic stem cell transplantation for malignant disease/severe aplastic anemia. We have examined the incidence of invasive fungal disease/fungemia. The diagnosis was based on blood culture findings, biopsy or autopsy material or findings in bronchoalveolar lavage together with radiographic changes. 86% of the patients were conditioned with Bu4Cy2. Graft versus host disease(GVHD)/prophylaxis was cyclosporine A with conventional short course of i.v. methotrexate. 96 patients (34%) were transplanted with an unrelated donor, 92(32%) developed GVHD grade II and needed immunoospressive treatment with steroids and/or T cell antibodies. Routine fluconazol prophylaxis was not given.

Results: Fungal isolates were identified in 35 patients, in 3 of these patients two different species were found. There were 24 candida infections in 22 patients (8% of the total material)(18 candida albicans, 4 non-albicans, 2 not further characterized) and 14 aspergillus infections(5% of the total material)(8 aspergillus fumigatus, 6 aspergillus not further characterized). In 1 patient candida and aspergillus species were isolated. Repetetive findings of the same fungal isolates are not included here. 14 patients developed infection in the neutropenic phase(granulocytes <,5x10^9/L; 13 candida, 1 aspergillus)while 21 patients were diagnosed later(8 candida, 12 aspergillus, 1 candida/aspergillus).

In the latter group 17 patients were treated for aGVHD. Of the patients transplanted with a family donor, fungal infection was identified in 10%, and of the patients receiving graft from unrelated donor, fungal infection was identified in 18%. 80% of the patients with fungal infection had one/more of the following possible risk factors at time of diagnosis: 1)GVHD; 2)Treatment with steroids; 3)Anti-Thyocyt-Globuline (ATG)/monoclonal antibody OKT3 as treatment/part of the conditioning regimen; 4)Total Body Irradiation (TBI)/cranial irradiation as part of the conditioning regimen. 30 of the 35 patients with fungal infection died. The diagnosis was made post mortem in 13 patients; of whom 10 had candida and 3 had aspergillus infections.

Conclusion: In this retrospective single centre study, deep fungal infections and fungemaes were found in 12% of the patients. 30 patients died(86% versus 41% in the total material). GVHD and/or treatment with steroids/ATG/TBI were identified as possible risk factors for developing fungal infections.

**P842**

Invasive mold infections (IMI) in allogeneic hematopoietic cell transplant (allo-HCT) recipients


IMI are an important cause of morbidity and mortality in allo-HCT recipients. We analyzed all allo-HCT cases with IMI recorded in our Unit among 151 patients allo-transplanted over 1990-6/2001. IMI cases were defined after EORTC-IFICG criteria as proven, probable or possible. IMI developed in 23/151 patients (15.2%); IMI cases were diagnosed later(8 candida, 12 aspergillus, 1 candida/aspergillus).

In the latter group 17 patients were treated for aGVHD. Of the patients transplanted with a family donor, fungal infection was identified in 10%, and of the patients receiving graft from unrelated donor, fungal infection was identified in 18%. 80% of the patients with fungal infection had one/more of the following possible risk factors at time of diagnosis: 1)GVHD; 2)Treatment with steroids; 3)Anti-Thyocyt-Globuline (ATG)/monoclonal antibody OKT3 as treatment/part of the conditioning regimen; 4)Total Body Irradiation (TBI)/cranial irradiation as part of the conditioning regimen. 30 of the 35 patients with fungal infection died. The diagnosis was made post mortem in 13 patients; of whom 10 had candida and 3 had aspergillus infections.

Conclusion: In this retrospective single centre study, deep fungal infections and fungemaes were found in 12% of the patients. 30 patients died(86% versus 41% in the total material). GVHD and/or treatment with steroids/ATG/TBI were identified as possible risk factors for developing fungal infections.
Central nervous system aspergillosis in allogeneic stem cell transplant (SCT) recipients

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Invasive aspergillosis is rather common in allogeneic SCT recipients. Lungs are the most common site but central nervous system (CNS) involvement is also observed in this setting. We have retrospectively studied 14 cases (sibling donor 9, unrelated 5) of CNS aspergillosis found in a cohort of 455 allogeneic SCT recipients (incidence 3%). All patients except one had experienced acute GVHD treated with high-dose corticosteroids and in eight patients (57%) also with ATG. The median time to diagnosis was 124 d (49-347 d) from SCT. Prior diagnosis of pulmonary aspergillosis had been made in four patients (29%). Neurological symptoms were observed in 11 patients (79%) a median of 7 d before the diagnosis of CNS aspergillosis. The most common initial symptoms were hemiparesis (4 pts) and convulsions (4 pts). Neuroradiological studies (9 pts) most commonly revealed single (3 pts) or multiple (4 pts) focal lesions. Although hemorrhage was observed in some patients. Despite strong clinical suspicion, the specific diagnosis was done only in one patient during life. Twelve patients (86%) received amphotericin B or liposomal amphotericin B. Despite therapy all patients died 0-27 d after the initial CNS symptoms. CNS aspergillosis is not uncommon in allogeneic SCT recipients. Neurological manifestations are dramatic and the prognosis is dismal. Earlier and more effective treatment for invasive aspergillosis is needed to prevent dissemination of the infection into CNS.

Microbiological evaluation of isolated pathogens in pediatric stem cell transplantation: are surveillance cultures necessary?


Although the understanding of the deficits in host defences and spectrum of infections that occur at various intervals after SCT have improved a great deal, Infections continue to be the major cause of morbidity and mortality in marrow transplant recipients. Between November 1992- August 2001,152 pediatric SCT patients (98 Allo, 47 Auto, 1 Syngeneic, 4 Haploidentical) with underlying 98malign and 54 non-malign diseases with a mean age of 8.17 ±2.18 years(3 mo-24 years) were evaluated for bacterial and fungal infections retrospectively. All the cultures were evaluated in our microbiology department Out of 6235 surveillance cultures from throat, nasopharynx, hemoculture, catheter exit site, urine, sputum, stool, lesion 312 (~5%) were found to be positive for any grow.312 documented grows in cultures Gram (+) bacteria was isolated in 111(46.4%)cultures, Gram(-) bacteria was identified in 128(53.5%) cultures and in 73 (23.3%)cultures a fungus was isolated. The distribution of isolated pathogens for Gram (+) cocci were as follows Staph.Aureus: 45 (40.5%), Coagulase negative staph.51 (45.9%), Streptococci :15 (13.5%),Gram (+) rods:2 (2.1%). Of the Gram (-) bacteria 41(32%) were Pseudomonas spp.28 (21.8%) were Klebsiella spp, 20 (15.6%) were non-fermentative gram (-) rods and the others consisted of 19 (14.8%). The main fungal pathogen was yeast in 65 (89%)fungus and mold in 8(11 %) isolated fungi. Out of 6235 cultures the grow in the taken cultures according to sites were 5% for throat,3 % for nasopharynx,5% for hemocultures,4 % for catheter exit site, 4.3% for urine,11 % for sputum, 5% for stool and 15% for lesions. The documented infection rate in our febrile neutropenic group is 59%. The total cost of taking surveillance cultures is 82.000 dollar for 152 patients. The cost is not so high but it added no benefit and loaded the usage of more antibiotics. When we add the clinically not needed antibiotic costs the total cost is 192.000 dollar.

In conclusion in our pediatric SCT unit the surveillance cultures had no benefit. To take the cultures when needed (in any case of clinical necessary or fever is more relevant.

Invasive fungal infection in hematologic stem cell transplant recipients at a Brazilian university hospital

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To assess the frequency and risk factors (RF) for invasive fungal infections (IFI) in allogeneic and autologous haematologic stem cell transplant (HSCT) recipients at Campinas State University Hospital, Brazil.

Patients & Methods: Retrospective study of 115 patients (ptts) who underwent HSCT from Jan/97 to Dec/99. IFI were classified as proven, probable or possible. RF were evaluated by an unmatched case-control study.

Results: The incidence of IFI was 13% (15/115), with no difference according to the graft (P=1.00). IFI were: 8 (53%) fungemia; 4 (27%) sinusitis; 2 (13%) pneumonia and 1 (7%) disseminated. Eight (53%) ptts had proven IFI and 7 (47%) ptts had possible IFI. Of the proven cases, Candida spp. were the most frequent (63%), followed by Fusarium spp. (25%) and Trichosporon inkin (12%). C.parapsilosis accounted for 60% of Candida isolates. Paracoccidioides brasiliensis was isolated from 1 case, but it was excluded of the analysis. RF for IFI were bone marrow as source of cells (P<0.01), total body irradiation (TBI) (P=0.01), prolonged neutropenia (P=0.03) and urinary catheter (P=0.05). In the multivariate analysis, prolonged neutropenia (P<0.001; OR=1.2 [95%CI=1.0-1.3]) and TBI (P=0.02; OR=6.3 [95%CI=1.3-33.3]) were independent RF for IFI. IFI was not associated to the number of CD34+ cells, grade =>2 mucositis, parenteral nutrition, hemodialysis, mechanical ventilation CMV infection, fungal colonization, failure of engraftment and acute Graft versus Host Disease. The mortality among IFI ptts was 33% (5/15). There was no difference in the proportional cumulative survival Kaplan-Meier) between ptts with and without IFI (P=0.173). The frequency of IFI was 13%. Although yeasts were the most frequent pathogens associated with IFI, emerging and endemic pathogens were also diagnosed. Prolonged neutropenia and TBI were independent RF for IFI. The AM due to IFI was 20%.
Combination of fungal PCR and serology for detection of invasive fungal infections in stem cell transplanted patients

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Objective: To see if a combination of fungal PCR(1) and serology (Platelia(R)Candida antibody (Cab), Platelia(R)Candida antigen (Cag) and Platelia(R)Aspergillus (Aag), Bio Rad) may contribute to early detection of invasive fungal infection (IFI) in patients receiving stem cell transplantation (SCT).

Introduction: Invasive fungal infections (IFI), mainly caused by Candida and Aspergillus spp., are associated with high morbidity and mortality in immunocompromised patients such as stem cell transplanted (SCT) patients.

Materials and methods: 10 SCT patients (6 females and 4 males, age range: 4 - 53 years) were included. 6 of them suffered from Invasive Candida infection (ICI) (n=1 C. albicans, n=2 C. glabrata, n=3 Candida spp.) and 1 from Invasive Aspergillus infection (IAI). ICI and IAI were proven by autopsy and/or blood cultures (Bio Merieux). 3 patients had no evidence of IFI.

Pan-fungal PCR was performed on blood and other body fluids according to a method earlier described(1,2).

Result: Serology and/or PCR were positive before blood or other cultures in 45/58 patients (in whom samples had been taken before first positive culture) with verified IFI (3 Candida and 1 Aspergillus). PCR were positive in 3/5 patients (1 Aspergillus and 2 Candida) and serology were positive in 3/5 patients (3 Candida) before blood or other cultures, in whom samples had been taken before first positive culture. In 2 patients PCR samples and serology were taken when IFI already had been confirmed. Both patients were tested positive with either of the tests (2 Candida).

Conclusion: A combination of fungal PCR and Platelia(R)Candida antibody, Platelia(R)Candida antigen and Platelia(R)Aspergillus may contribute to an early diagnosis of IFI in transplant recipients.

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Prospective comparison between the NucliSens Basic Kit (NASBA) and a PCR-ELISA for the detection of Aspergillus species

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Invasive aspergillosis is a major cause of morbidity and mortality in immunosuppressed patients. Early diagnosis is mandatory for appropriate, successful therapy. In this study, a prospective comparison between a NucliSens-NASBA- (Nucleic Acid Sequence Based Amplification) based assay (detection of Aspergillus-RNA) and a previously published protocol for the amplification of fungal DNA by PCR-ELISA was performed. Blood samples from patients (n = 21) receiving an allogeneic stem cell transplantation (SCT) were analyzed twice weekly by both assays. Serially diluted Aspergillus fumigatus conidia were used for sensitivity testing of the new molecular-based method. Primers and a specific probe were designed within the fungal 18S rRNA gene region. Blood specimens (n = 245) from SCT recipients were compared subsequently by PCR and NASBA assays. Isothermal amplification combined with electrochemiluminescence were performed by using NucliSens Basic Kit reagents (Organon Teknika/biomérieux, Boxtel, Netherlands) as described previously. The NASBA assay showed a detection limit of 1 CFU/ml blood (PCR: 10 CFU/ml). Thirteen patients were positive by both assays whereas 5/21 showed a positive result only by the NASBA test. Two patients suffered from documented invasive aspergillosis and received antifungal therapy. They were both NASBA-positive over 3 weeks prior to radiological documentation of pulmonary infiltrates and remained positive for up to 31 days. In contrast, PCR remained positive only for 4 days. In patients with pulmonary infiltrates or amphotericin B therapy (n = 10), a mean number of 6.4 NASBA tests (range 1-13 tests) was positive. In patients with febrile neutropenia (n = 6), a mean number of 2.3 NASBA-positive tests (range 1-5 tests) was documented.

The NASBA Basic Kit technology is a highly sensitive tool for the detection of Aspergillus-RNA in blood specimens of immunosuppressed patients.

Diagnosis of invasive fungal infections by nucleic acid amplification in pediatric and adult patients with hematological malignancies

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Introduction: In immunocompromised patients with fever, the distinction between bacterial, viral or fungal infection is difficult. In the setting of BMT or PBSCT, the mortality of invasive fungal infections (IFI) is up to 85%; therefore many patients with fever of unknown origin (FUO) after BMT are treated with antifungoc prophylactically or preemptively. Here, PCR based technologies (NASBA, 13 tests) was positive. In patients with febrile neutropenia only (n = 21) negative by both assays whereas 5/21 showed a positive result only by the NASBA test. Two patients suffered from documented invasive aspergillosis and received antifungal therapy. They were both NASBA-positive over 3 weeks prior to radiological documentation of pulmonary infiltrates and remained positive for up to 31 days. In contrast, PCR remained positive only for 4 days. In patients with pulmonary infiltrates or amphotericin B therapy (n = 10), a mean number of 6.4 NASBA tests (range 1-13 tests) was positive. In patients with febrile neutropenia (n = 6), a mean number of 2.3 NASBA-positive tests (range 1-5 tests) was documented.

The NASBA Basic Kit technology is a highly sensitive tool for the detection of Aspergillus-RNA in blood specimens of immunosuppressed patients.

Discussion: During the study, the PCR assay showed no false positive results in patients with FUO. In patients with IFI, fungal DNA could be amplified in blood samples taken during the early phase of IFI and once during treatment with subtherapeutic iraconazole levels. After prolonged antifungal therapy, no positive PCR-result was seen.
Detection of Candida and Aspergillus antigens in allogeneic stem cell recipients

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Fungal infections are a serious and relatively frequent complication in allogeneic stem cell transplant recipients, and the mortality is high. To improve early diagnosis, we are studying the clinical utility of Candida and Aspergillus antigen detection. Thus far 44 allogeneic stem cell recipients have been studied. Their median age is 46, range 19 to 60 years. 17 patients have AML, 14 CML, 3 ALL, 3 CLL, 2 MM, 2 MDS, 1 NHL, 1 SAA, 1 MF. 27 donors were HLA-identical siblings and 17 unrelated. 26 patients received a BM and 15 a PBSC graft. All grafts were unmanipulated. 3 patients were retransplanted due to rejection or no-take. The conditioning consisted of cyclophosphamide 60 mg/kg x 2 and fractionated TBI 12 Gy (lungs 10 Gy) in 43 cases and cyclophosphamide 50 mg/kg x 4 in one patient (SAA). ATG (Sangstat) 2mg/kg x 3 was given if the donor was unrelated. GVHD prophylaxis consisted of CyA and a short course of MTX. Low-dose methylprednisolone was given on days +8 - +110 to patients with a sibling donor. No systemic prophylaxis for fungal infections was used. Serum samples were taken biweekly after the transplantation, weekly for the first 3 months and then every 2 to 8 weeks until one year. They were analysed for Candida and Aspergillus antigens by a sandwich ELISA (Bio-Rad). Mouth and nasal swabs for fungal cultures were taken weekly during hospitalisation. The follow-up is 22-316 (median 151) days. None of the 335 samples for Aspergillus antigen, taken from the 44 patients, were positive. Of 488 Candida antigen samples, 22 samples taken from 12 patients were positive (1 to 4 positive per patient). 17% (53 out of 313) of mouth samples revealed Candida albicans. Of 655 nasal samples, 9 samples from 4 patients were positive for either Aspergillus or Candida. Only one systemic fungal infection has been diagnosed so far. This patient died of Candida albicans septicemia 75 days after the first (41 days after the second) transplantation. Four out of seven Candida-Ag tests were positive, the first one 48 days before the septicaemia. No confirmed Aspergillus infection has taken place among the studied group of patients. It is too early to draw conclusions about the clinical utility of Candida and Aspergillus antigen testing. No false positive Aspergillus antigen results have thus far been observed.

Early high-resolution computed tomography (HRCT) scans allow prompt diagnosis and efficacious treatment of invasive pulmonary aspergillosis (IPA) in immunocompromised patients with hematologic malignancies


Prognosis of IPA occurring in neutropenic patients remains poor. In the haemato-oncology unit at RMH, IPA requiring high-dose lipid formulations of amphotericin were a rarity and were used in less than 5% of haemato-oncology patients as diagnosis of proven IPA was difficult and done by exclusion in unresponsive patients with pulmonary infiltrates on Chest X-ray. By using prompt HRCT scanning, we have been able to explore the possibility of early diagnosis and better response to therapy in our patients. Probable and Proven IPA was diagnosed according to EORTC criteria. HRCT scans were performed in the presence of pulmonary signs if in a neutropenic patient, temperature did not resolve after more than 96 h of empiric antibiotic therapy. Response was categorised as complete (CR) if there was resolution of all signs, symptoms, microbiological evidence if present and HRCT abnormalities (completely or to scars) and partial (PR) if there was improvement in baseline HRCT findings by at least 50%. Between 11/94 and 7/01, 34 immunocompromised patients (16-73 y median 50; 7 F, 32 M; 24 acute leukaemia, 9 myeloma, 1 myelofibrosis) received 39 courses of antifungal therapy (AFT) for IPA after autologous (n=11) or allogeneic (n=6) stem cell transplantation or chemotherapy (n=22). AFT comprised liposomal amphotericin 3-5 mg/kg (n=27), amphotericin B colloid dispersion 4mg/kg (n=5), amphotericin B lipid complex 5 mg/kg (n=4) and conventional amphotericin 1mg/kg (n=9). IPA was difficult and done by exclusion in unresponsive patients less than 5% of haemato-oncology patients as diagnosis of proven IPA was difficult and done by exclusion in unresponsive patients. Aspergillus specific PCR may be quite important tools for early diagnosis but they have not been standardised yet for mass practical use. HRCT is a practical non-invasive technique and helps in early start of AFT. We recommend that HRCT availability be a mandatory requirement for large active haematopo-oncology units undertaking intensive therapy as early start of efficacious AFT leads to high response rates and better survival.

Early infectious complications after stem cell transplantation: a retrospective analysis of first-line antimicrobial therapeutic strategies


Prolonged duration of neutropenia exposes patients undergoing both autologous (Auto) and allogeneic (Allo) stem cell transplantation (SCT) to a high risk of fungal and bacterial complications. In this study, we retrospectively analysed first-line antimicrobial therapeutic strategies from 1992 to 1997 (Phase 1) and from 1998 to 2001 (Phase 2). All patients received quinolone and anti-fungal prophylaxis. During Phase 1, 43 cases received an AlloSCT. Seventy-seven percent of cases (151/195) experienced at least one febrile episode. In almost all cases (96.5%), first-line antimicrobial approach consisted of a combination therapy containing either an aminoglycoside or a glycopeptide or both. Response rate was 75.5%. Longer fever duration was significantly associated with the grade of mucositis (1 d versus 3 d versus 4 d for respectively grade I, II and II-IV mucositis, p<0.0001) and with the type of neoplastic disease (1 d for solid tumors versus 3 d for haematological malignancies, p=0.0117). These results induced us to add the aminoglycoside or glycopeptide or both to a broad-spectrum beta-lactam antibiotic in selected cases. In this regard, we stratified patients into low-risk (cases with an expected neutropenia less than 7 days, those undergoing non-myeloablative AltoSCT, solid tumors and all cases with no clear signs of shock) and high-risk categories. In this Phase 2, we performed 329 transplants with a fever incidence of 77.2%. One hundred and fifty-one cases were evaluable for response to first-line anti-microbial therapy. Patients were treated either with a broad-spectrum single antibiotic (79 cases, regimen A) or a broad-spectrum antibiotic associated with either the aminoglycoside or the glycopeptide (25 cases, regimen B) or with all 3 antibiotics (47 cases, regimen C). Overall response rate was 74.8%. In particular, 79%, 90% and 70% of cases responded to regimen A, B and C, respectively. Moreover, fever duration was 3 d for regimen A, B and 5 d for regimen C. Overall these results indicate that i) a significant percentage of patients presenting neutropenic fever after SCT was over-treated in Phase 1 group, ii) monotherapy is able to successfully cover a significantly high proportion of low-risk patients and iii) a more aggressive first line anti-microbial therapy should be tailored for patients with a really high risk of early infectious complications.
Prevention of invasive aspergillosis (IA) in immunocompromised patients by high-efficiency air filtration

MedicCleanAir devices


Introduction: IA is still a major problem in patients(pts.), undergoing aggressive chemotherapy: in allogeneic BMT settings it accounts for 10% of TRM

Material and Methods: Between 01/01/2000 and 31/08/2001 we performed 385 ELISA Platelia tests for Galactomannan (Gal) twice a week in 42 immunocompromised hosts admitted in our non-conditioned, semi-intensive ward, undergoing allo/autoBMT, or chemotherapy for acute leukaemia. Results were considered positive (confirmed test) in a single patient if at least 2 consecutive positive tests were obtained. Air-borne Aspergillus (Asp.) contamination was monitored for by CFU-C assay in the air, in the dust and on the furniture. Similar surveys performed in the same clinical ward rooms some months later, on 22/01/2001 and 19/09/2001, and 8,1 and 3/mq per hour on the furniture. During 2000 5 patients with proven and probable IA were treated with L Amb with an overall cost of 161627 euros, while the following year no single patient was treated.

Conclusions: In this study we stress that the use of MedicCleanair (R) Forte devices for air filtration was able to dramatically reduce the incidence of IA, saving an overall expense of more than 161000 euros/year. Such approach appears to be easily feasible even in poor economic resources countries.

Low-dose (50 mg/day) fluconazole prevents invasive candidiasis in allogeneic-HCT

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Background: The optimal dose of fluconazole to prevent fungal infection in transplanted patients has not been formally revealed. Although the most widely employed dose is 400 mg/day there has been some indication that lower doses could be as effective as higher doses.

Methods: The incidence of invasive fungal infection (IFI) was retrospectively evaluated in all consecutive adult patients with hematologic malignances submitted to allo-HCT from an HLA-identical sibling donor.

Results: In 2000 Gal assay was positive in 18 out of 193 blood tests, for 5 confirmed tests in 5 (2 proven and 3 probable) IA out of 23 pts; in 2001 there were 8 single positive out of 192 tests, with no positive confirmed test and no patient out of 19 developed IA. Air room Asp. survey performed on 01/08/2000 revealed 1 colony/2 m³ of air, 140 colonies/m²/h in the dust and 51 colonies/m²/h in the furniture. Similar surveys performed in the same clinical ward rooms one year later on (day 22/01 and 19/09/2001) revealed respectively 6 and 0 Asp. colonies/9 m³ in the air, 0/m³/h in the dust both on 22/01 and 19/09/2001, and 8,1 and 3/m³/h on the furniture. During 2000 5 pts were treated with L Amb, while in the following year no single patient was treated; the fungal TRM was 13%/3/23 in 2000 and 0/0/19 in 2001.

Conclusion: The use of Medic Clean Air Devices dramatically reduced the incidence of IA as shown by a triparametric (clinical, laboratory and microbiological) survey.
Caspofungin (CSF) after allogeneic stem cell transplantation (SCT): A single center experience of 16 patients (pts) treated for fever of unknown origin (FUO) or invasive mycosis (IM)


Caspofungin is the first in a new class of antifungal agents that interfere with fungal cell wall synthesis by inhibition of glucan synthesis. A complete or partial response to CSF therapy was seen in about 40% of immunocompromised adults with invasive aspergillosis who did not respond to, or did not tolerate, other antifungal agents in a noncomparative multi-center study. Here, we report 16 pts treated with CSF after allogeneic SCT. We introduced CSF as a second line antifungal agent in case of IM (n=8) and FUO (n=8). First line therapy following antifungal decontamination included Amphotericin B (n=10), liposomal Amphotericin B (n=3), Fluconazole (n=2) and Itraconazole (n=1). Among the pts with IM, we diagnosed 4 proven, 2 probable and 2 possible invasive fungal disease according to the EORTC criteria. Median time of treatment with CSF was 14 days (6-32). 11/16 pts were neutropenic. The median duration of neutropenia (mean ANC <100/µl) was 11.5 days (3-108) before starting CSF. All pts had additional immuno-suppressive therapy: Cyclosporin A (CSA) (n=15), Prednisolone (n=3), T-cell depletion (n=1) and OKT3 (n=1). In 8/15 pts treated with CSF, this drug was interrupted prior to CSF therapy because of renal impairment. In 7/8 pts CSA could be restarted under CSF medication. CSA was given without interruption in 7/15 pts. No major acute side effect was noted. GPT raise was significant but acceptable (mean GPT tstart = 8.5 IU/l and tend = 22.4 IU/l). We documented a normalization of serum creatinine as well as a significant reduction of CRP. Complete remission was obtained in 5/8 pts with IM. 3/8 died in the context of severe acute GvHD. 5/8 pts with FUO could be discharged, which grew Fusarium species in the third month of her treatment. One patient (5%) died due to infection (candida sepsis) on day 9 during neutropenia. In all other pts preexisting infections were controlled by GTX. Serial CT-scans showed decreasing sizes of lesions in all but one of 12 patients with radiological evidence of fungus. Nonrelapse mortality on d+100 was 3/17 (17%). another 2 pt. died on d+ 52 due to idiopathic IP and on d+ 85 due to GvHD. Late non-relapse mortality was seen in 2 pts due to bacterial pneumonias on d +42 and d+531 (noncompliance due to alcoholism). Eight pts. relapsed after a median of 96 (range 32 – 765) days. Four pts. with proven preexisting pulmonary fungus are in CCR after a follow-up of median 781 (range 109-857) days.

Conclusions: Preemptive GTX in combination with antifungal therapy prevents progression of fungal lesions during posttransplant neutropenia, resulting in an acceptable d+100 mortality. In addition, granulocyte transfusions save platelet transfusions. Long term outcome in this high risk group of older patients with advanced leukemia appears to be related to factors other than preexisting fungal infections.
Granulocyte infusions two weeks before her Bone Marrow Transplant (BMT). She went on to receive a Matched Unrelated Donor BMT with Fludarabine, and Cyclophosphamide conditioning. In vivo T cell depletion was achieved by Campath 4H. Initial infection caused by Fusarium oxysporum was treated with Voriconazole and Granulocyte infusions which would have previously prevented her BMT.

**P859**

**Lipopolysaccharide Binding Protein (LBP) is a very early marker of infection in patients undergoing stem cell transplantation**

L. Hambach, R. Lichtinghagen, E. Dammann, M. Eder, A. Ganser, B. Hertenstein (Hannover, D)

Objective: Early and sensitive parameters of infection might help to improve the outcome of patients undergoing stem cell transplantation. Lipopolysaccharide binding protein (LBP) has been reported to be an early mediator in the inflammatory cascade especially related to the endotoxin of gram-negative bacteria. It might therefore be useful as an early diagnostic marker of infection but has not yet been evaluated in immunocompromized patients.

Patients and Methods: Morning blood samples were collected prospectively in a cohort of 10 patients (median age 42 yrs., range 19-64) undergoing stem cell transplantation (5 patients autologous, 5 allogeneic). LBP, soluble interleukin 2 receptor (sIL-2R), IL-6, IL-8 and C-reactive protein (CRP) were measured in the stored samples starting 3 days before the beginning until 3 days after the end of the first febrile episode.

Results: Febrile episodes were due to bacteremia (gram-positive: n=4, gram-negative: n=1), FUO (n=3) and administration of ATG (n=2). Fever was associated with an increase of LBP, sIL-2R, IL-6, IL-8 and CRP levels in 100% of the patients. Median maximum levels during bacteremia, FUO and ATG-administration were 46 (range 14-57), 46 (20-129) and 51 ng/l (30-72) for LBP, 1951 (1610-4375), 3817 (3490-11561) and 5148 U/l (250-1416) for sIL-2R, 216 (97-366) and 218 mg/dl (169-268) for CRP respectively. Median levels before increase of the markers were 6 ng/l (range 3-17) for LBP, 903 U/l (250-1416) for sIL-2R, 5 ng/l (5-11) for IL-6, 8 ng/l (5-35) for IL-8 and 8 mg/dl (5-44) for CRP. During bacteremia and FUO an increase of LBP, sIL-2R and IL-6 levels was detected 36 hours (median) before onset of fever, while compared to these parameters an increase of IL-8 and CRP was detected with a delay of 17 and 24 hours respectively. There was no significant difference for any of the analyzed parameters during fever in aplasia and no aplasia.

Conclusions: LBP was a highly sensitive and like IL-6 and sIL-2R a very early marker of infection (including gram-positive bacteremia) in our study. In this respect it was superior to the established marker CRP. However, the increase during ATG-administration reveals that elevated LBP levels are like the other parameters not only related to infectious reasons.

**P860**

**Use of FK 463, a novel echinocandin analog in patients undergoing stem cell transplantation (SCT) for hematologic malignancies, a matched pair comparison of biochemical toxicity with historical controls**


Invasive fungal infections by Candida and Aspergillus spp are an increasing cause of morbidity and mortality amongst recipients of SCT. Currently available antifungal agents are hampered by serious infusion or drug-related toxicity. FK 463 is an echinocandin analog with activity against candida and aspergillus.

In another study, maximum tolerated dose (MTD) and safety of FK463 was assessed in doses up to 8mg/kg. (Data submitted for EBMT 2001). To further evaluate safety of FK463, data from MTD study was used for matched-pair analysis. Analysis compared the alterations in the haematologic and biochemical profile for patients on FK 463 with those who have undergone a SCT at the Royal Marsden Hospital (historical controls). 36 patients (median age 47.5 y; range 19-62; 23M, 13F) undergoing SCT received FK463 for 8-28 d (median 18) as part of Phase I/II study in 4 cohorts of 3, 4, 6 patients had myeloma (all autologous), 7 chronic leukaemia (all autologous), 12 chronic lymphocytic leukaemia (all autologous), 8 chronic leukaemia (12 autologous, 4 allogeneic). Patients received FK463 for minimum of 7 d or till neutrophils recovered to 0.5x109/L. Maximum treatment period was 28 d. None of the patients had Grade 3 or 4 adverse event (AE) related to the drug. There were no infusion-related AEs, though phlebitis was reported in 3 patients related to the study drug. Each study group patient was matched with two historical controls for diagnosis, type of SCT (autologous or allogeneic ) and age at SCT for 2 historical controls. Historical controls were chosen by the computer from prospectively maintained RMH database. Maximum bilirubin, alkaline phosphatase (Alk phos), alanine transaminase (ALT) , creatinine, urea post-SCT while the patient was on study drug were compared with those of historical controls for similar duration. Median values in study group patients vs the historical controls are as follows- creatinine 94.6micromol/L(58-323) vs 102 (51-750) P=0.2; Urea 7.2 mmol/L(3.9-119) vs 8.7 (3.5-56.6) P=0.2; bilirubin 23mmol/L(11-241) vs 26(11-246) P=0.5; Alk Phos 91U/L (42-446) vs 80 (34-386) P=0.4; ALT 48 U/L (14-154) vs 55 (20-751) P=0.2 respectively. Engraftment was comparable in both groups. These data indicate that FK463 is very well tolerated and is not nephrotoxic or hepatotoxic at the dose range studied.

Additional abstracts to this topic

**Attributable mortality due to invasive fungal infection among hematologic stem cell transplant recipients in a Brazilian university hospital**

P. Trabasso, A. Vigorito, C. De Souza, M. Branchini (Campinas, BR)

To assess the attributable mortality (AM) due to invasive fungal infection (IFI) among hematologic stem cell transplant (HSCT) recipients in the Campinas State University Hospital, Campinas, SP, Brazil.

Patients and Methods: We performed a matched case-control study of patients (pts) who underwent HSCT from Jan/97 to Dec/99 and developed IFI during the pre-engraftment period. All cases were proven or probable IFI. Controls were selected from the same population. Selection criteria were: sex, age (± 5 years), underlying disease, graft and temporal relatedness of the transplant (± 6 months). Death was evaluated on discharge and in the day +100; matching was made without the knowledge of this
information. The difference between mortality among cases and mortality among controls was considered as attributable to IFI. Results: The overall matching success rate was 87%. Success rate for matching in each criteria was: graft, 93%; sex, 87%; underlying disease, 87%; age, 80%; relatedness of the transplant, 80%. Five out of 15 pts died during in the first period (Crude Mortality Rate of Cases=33%); in the control group, 2 out of 15 patients died (Crude Mortality Rate of Controls=13%). The attributable mortality due to IFI was 20% (P=0.39; OR=2.5 [95%CI=0.6-10.9]). In the day +100 evaluation, 7 out of 15 pts with IFI had died (Crude Mortality Rate of Cases in day +100=47%); in the Control group, 2 out of 15 patients had died (Crude Mortality Rate of Controls in day +100=13%). The attributable mortality due to IFI in day +100 was 34% (P=0.11; OR=3.5 [95%CI=0.86-14.18]). Attributable Mortality due to IFI among HSCT recipients was 20% in the pre-engraftment period and was 34% in day +100 evaluation.

Treatment of confirmed and suspected invasive fungal infections with liposomal amphotericin B in patients with a hematological malignancy in a tertiary care hospital in 1991-2000

J.H Salonen, K. Remes, T.T. Salmi, J. Nikoskelainen (Turku, FIN)

Objectives: To evaluate retrospectively the efficacy and safety of liposomal amphotericin B (Ambisome) in patients with a hematological malignancy with confirmed or suspected invasive fungal infection.

Patients and Methods: Hospital records, laboratory data and results from imaging studies were evaluated from 133 patients (94 males, 39 females) with a hematological malignancy treated with Ambisome in Turku University Central Hospital in 1991-2000. Mean age of the patients was 48±17 years. 108 (81%) of the patients were neutropenic (neutrophils < 500/mm3) and 57 (43%) had received a stem cell transplantation (27 allogeneic and 30 autologous). Underlying diagnosis was AML in 45, ALL in 14, CML in 3, CLL in 5, multiple myeloma in 20, non-Hodgkin lymphoma in 28, MDS in 8, aplastic anaemia in 4 and other hematological malignancy in 6 patients. Invasive fungal infection was confirmed in 27 (29%) and suspected in 106 patients (80%). 40 patients (30%) had a refractory infection and 49 patients (37%) were intolerant to the prior antifungal therapy.

Results: The mean duration of Ambisome therapy was 18.1 ± 23.4 days and the mean daily dose was 127 ± 59 mg/day. The mean cumulative dose was 2.6 ± 5.0 g. The overall success rate was 72% (96/133). 52% of patients with confirmed invasive fungal infection (50% in invasive candidiasis and 55% in invasive aspergillosis) and 90% of patients with fever of unknown origin responded successfully. Treatment was discontinued for severe side effects in only 3 patients: 1 patient had severe back pain and 2 had highly elevated ALT and AFOS values. Most common side effects were: hepatotoxicity (44%), nausea (31%), fatigue (23%), hypokalemia (19%) and elevated serum creatinine values (14%). Most of the side effects were mild and reversible not leading to discontinuation of therapy. Altogether 58 patients (44%) had laboratory abnormalities suggesting hepatotoxicity. This was particularly common in stem cell transplant recipients (50%). In 30 of 58 patients (52%) signs of hepatotoxicity emerged only after initiation of Ambisome. In 34 of 58 patients (59%) laboratory abnormalities were persistent and 22 of these patients (65%) died. Conclusions: Liposomal amphotericin B is effective as salvage therapy of confirmed or suspected invasive fungal infections in neutropenic patients. In patients receiving stem cell transplantation the risk of hepatotoxicity is increased.

Treatment of suspected and proven systemic mycoses in neutropenic patients with a combination of liposomal amphotericin B and caspofungin

M. Lindauer, K. Kolbe, C. Huber, A.J. Ullmann (Mainz, D)

Systemic mycoses in neutropenic patients treated with amphotericin B have a mortality rate of 75-100%. We report on the tolerance and clinical responses of proven or probable fungal infections in neutropenic patients to a combination treatment with caspofungin and liposomal amphotericin B in four cases.

Case 1: A 24-year-old woman, ALL-relapse. History of suspected fungal infection of liver and lung, lesions resolved under Caspofungin B treatment. After allogeneic bone marrow transplantation 14 months later relapse of hepatic lesions. Liposomal amphotericin B was administered instead of fluconazole. Candidemia with C. krusei developed, and caspofungin was added. Candidemia could not be cleared, despite frequent changes of all iv lines. The patient died due to graft failure two weeks later.

Case 2: A 45-year-old woman, AML-relapse. History of probable invasive pulmonary mycosis. After allogeneic PBSCT new pulmonary infiltrates with fungal spores in BAL-fluid. The patient needed artificial respiration despite treatment with ambisome. Caspofungin was added, followed by clinical improvement. The patient died due to graft failure three weeks later.

Case 3: A 15-year-old boy, AML-relapse. Pulmonary infiltration refractory to broad spectrum antibiotics. Temporary improvement under treatment with liposomal amphotericin B, then the pulmonary infiltrates expanded again. In BAL-fluid, fungi were proven. Under a combination treatment with caspofungin/liposomal amphotericin B, the pulmonary infiltrates regressed during neutropenia. He underwent allogeneic PBSCT. Two weeks after discharge from hospital he had a relapse and died one week later.

Case 4: A 49-year-old man, multiple myeloma, pretreated with an autologous and an allogeneic PBSCT. After a second allogeneic PBSCT the patient developed an inflammation of the orbita, CT and MRT scans showed inflammatory infiltration of the sinus ethmoidales and parts of the orbita without bone destruction. Treatment with ambisome and caspofungin, improved the clinical symptoms and findings by CT and MRT.

Summary: Combination treatment of proven or suspected mycoses with liposomal amphotericin B and caspofungin was well tolerated. All patients had an increase in serum bilirubin grade II in two patients, grade III toxicity in the patient with hepatic candidiasis. In three of the four cases, treatment was effective in controlling the infection during neutropenia.

Hematopoietic recovery and infectious complications after tandem autograft with CD34+ selected peripheral blood progenitor cells (PBPC): a comparison between breast cancer (BC) and multiple myeloma (MM)

L. De Rosa, G. Anghel, A. Pandolfi, M. Riccardi, R. Amodeo, I. Majolino (Rome, I)

Autograft with CD34+ selected PBPC is often associated with prolonged recovery time and higher incidence of infections, however most of reports concerns patients(pts) with lymphoproliferative disorders, while in BC results are contradictory. Aim of our study was to compare the hematopoietic recovery and infectious complications of 19 BC pts with 17 MM pts entered in a high dose therapy (HDT) program of tandem autograft with CD34+ selected PBPC. PBPC were collected after mobilizing chemotherapy (VAC in MM, HD-FEC or DE in BC plus G-CSF) and processed for positive selection using CEPRATE-SC* or Isolex 300i*. Forty-two (24 BC and 18 MM) CD34+ selections were performed. After selection a median of 54% CD34+ cells were recovered with a median final purity of 92% with no significant differences between MM (55% and 92%) and BC (53% and 89%). A median of 5.4x10⁶/Kg MNC (4.6 BC, 6 MM), 4.5x10⁶/Kg CD34+ (4.4 BC, 5.4 MM) and 18x10⁴/Kg CFU-GM (21 BC, 16 MM) were reinfused after each HDT. Twenty-six pts (10 MM and 16 BC) received tandem autograft, 10 pts received only one autograft for low collection(5),clinical conditions(3),refusal(2). The HDT included HD-melphalan and ICE regimen in BC pts; HD-melphalan, IOBV and/or TBI+VP16+CY in MM pts. We found a significant prolonged recovery time for neutrophils>500/ul in MM patients (13 vs. 10 days, p<0.005), while for platelets>20,000/ul the differences were not significant in the two groups (13 vs. 12 days, p=NS). The type of HDT regimen did not influence the hematopoietic recovery. No late engraftment failure and no toxic
deaths were observed. All patients received similar anti-infectious prophylaxis for 3 months after transplant (ciprofloxacin, acyclovir, itraconazole and Ig7S). The incidence of extra-hematological toxicity was similar in the two groups. After 12 months of observation we found a statistically significant higher incidence of bacterial infections in MM pts (63% vs 28%, p<0.005), while fungal infections were similar in both groups. Among viral infections 2 pts for each group had HZV (11% in BC and 12% in MM), while CMV infection was observed in 3 pts (18%) with MM and none with BC. Our experience demonstrates a prolonged recovery time of neutrophils and higher incidence of bacterial and viral infections in MM as compared to BC pts. This observation suggests that the altered immunity of pts with lymphoproliferative disorders may play a role in the incidence of infections after CD34+ selected transplants.

**Surveillance study for the identification of nosocomial infections (NI) following autologous and allogeneic PBSCT and BMT**

H. Bertz, M. Dettekofer, W. Ebner, R. Babikir, J. Finke, F.D. Daschner (Freiburg, D)

In a prospective, 54-month study at the Haematological Stem Cell Transplantation Unit of Freiburg University Hospital, we investigated the overall and site-specific incidence of nosocomial infection (NI) rates following autologous or allogeneic BMT or PBSCT. Ward staff were consulted and charts and microbiology reports reviewed twice a week by an infection control nurse. NI were defined according to Centers for Disease Control and Prevention (CDC) definitions and modified for neutropenic patients (WBC<1x10^9/l). A total of 351 patients undergoing allogeneic (n=316) or autologous (n=35) BMT or PBSCT were investigated. These pts. stayed on the ward for 14,256 days; of these, 5026 were neutropenic days (35.3%). During the study period 239 NI were identified in 169 pts. (120 pts with 1 NI, 33 pts with 2 NI and 16 pts with >3 NI). The pathogens isolated were mainly coagulase negative staphylococci (36.3%), clostridium diff. (20.4%) and enterococci spp. (10%). 171/239 NI (71.5%) occurred during neutropenia. The site-specific incidence also showed an increase during neutropenia. Comparison of NI/1000 neutropenic days vs NI/1000 non-neutropenic days revealed: 13.8 vs 1.6 blood-stream infections, 11.9 vs 1.8 cases of pneumonia and 1.6 vs 0.3 urinary tract infections.

A significant change in the overall or site-specific incidence rates of NI was not observed during the study period.

Conclusion: After transplantation, nosocomial infections mainly occur during the neutropenic phase, and neutropenia is a major risk factor. To prevent NI, and to improve the quality of care in these critically ill patients, future multicenter surveillance programmes should focus on data on the occurrence of these infections.
### Data Management

**875**

**Data management systems: commercial or in-house?**

*P. Naik (London, UK)*

Summary: A single centre experience to evaluate the values of various database systems tried and tested.

Situation: Prior to April 2000 The London Clinic had no in-house database systems. All transplant registrations were sent in to the various registries by post in paper format. In April 2000 a commercial system, StemSoft, was bought to house all data for the Stem Cell Transplant Unit. The purpose of this system was to store all in a readily accessible system, reduce repetitiveness and paperwork and simplify the registering of transplants. The database was designed so that all relevant information to the various registries was easily stored and sent electronically via e-mail or disk. However, the limitations were that, although as a generic system it works very well, the scope for fine tuning it to existing systems in The Clinic was limited and dependent on the in-house IT department and the software dealers; this made it a very expensive option.

The last year saw the launch of a web-based database, PROMISE, accessible to all registered centres. Again this system dispensed with the paper format so that all data could be entered directly onto the database. The limitations were that it was specifically for registry information and was dependent on Internet access.

**Results**

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<th>Method of Data Collection</th>
<th>Time taken to collect Information</th>
<th>Time taken to reach Registry</th>
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<tbody>
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<td>Paper Format, StemSoft</td>
<td>Med A (100 day follow-up)</td>
<td>Med B</td>
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<tr>
<td></td>
<td>One day to week</td>
<td>One month, laborious</td>
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<td>Instantaneous</td>
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**Conclusion:** There are advantages and disadvantages to all systems. From a very small centre's perspective the commercial option is a ready-made instant solution. PROMISE, although taking time, is ideal for an instant access to input and view data for the registries. The ideal option would be for the in-house IT department to work with all the various departments which generate our data; Clinicians, nursing staff, microbiology, radiology, immunology, HLA typing lab, Stem Cell Laboratory, Oncology, Haematology, Blood Bank, Pharmacy as well as the Registries. Until StemSoft works well as a system that addresses more than just the registry needs and the PROMISE gives us instant access to up-to-date Registry information.

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**876**

**The Royal Marsden Hospital Leukemia-Myeloma database: a research resource for planning new drug trials in Europe and the US**


It is becoming increasingly difficult to test drugs due to stringent demands from drug regulatory bodies and ethic committees overseeing the interest of patients. Delays place large financial burdens on pharmaceutical companies which can partly be avoided by predicting enrolment into trials using models testing entry and exclusion criteria. Multi-institutional databases are not population based (unselected consecutive patients) or prospective, not having serial clinical data (IBMTR, EBMT) which only offers a limited glimpse of the course of the disease. Since 1978, comprehensive data has been collected prospectively on 3500 plus consecutive unselected population based patients referred to our Leukemia/Myeloma units. For each patient, up to 600 separate data fields are recorded and multiple, longitudinal prospective values for each (eg serial blood counts recorded on all the days that they are done—"sparse array") on all aspects of disease and therapy. Database comprises a definition collection and query/analysis package written in MUMPS, and presently contains 2 x 107 data items. A defined patient population may be analysed at any instant in time (snapshot) or over a given period (panorama). For example, a Myeloma snapshot is (a) number of living patients, (b) number in CR, (c) number on IFN therapy, (d) number on induction, (e) number with renal failure, (f) number on erythropoietin. A myeloma panorama (last year) is: (a) number of new patients seen, (b) number of failing initial therapy, (c) number of relapsing after the first autograft, (d) number of failing post-autograft salvage therapy, (e) number with renal failure, etc. Thus in a practice of 40 new population-based myeloma patients/year, prediction of how many patients can be recruited for a study over a period of time is possible, using specific criteria, for eg a proposed one year study of a new drug for patients relapsing after the first autograft with normal renal function would accrue 83 patients almost immediately (the number alive today with disease relapsing after a first autograft) and 21 more over the next year (based on the number relapsing last year). By changing the inclusion to any relapse, the total number increases to 112, and by eliminating the renal function criterion, to 114. Similar analyses can be done for leukemia and strength of this analysis is that it can be extrapolated to other centres. This database can hence be used to plan multicentre studies and predict power of these studies.

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**877**

**The need for more reliable evidence on the effectiveness of stem cell transplants**

*S. Richards (Oxford, UK)*

Randomized trials comparing treatments have been done for over 50 years. Publications from as long ago as 20 years show that historical or non-randomized concurrent controls are a less reliable way than using randomized evidence. This applies to transplants as much as any other treatment. For autologous transplants, randomization between transplant and standard therapy is possible, and some trials have been done, although
very few are large enough to give really clear evidence. Over-optimism on the basis of non-randomized evidence leads to trials which are too small to establish whether moderate but worthwhile benefits exist - eg in breast cancer. For related donor transplants, for a population where transplant is recommended, many authors have demonstrated that cause-specific cumulative incidence (CI) functions are best suited to estimate the subdistribution functions of interest, by taking into account the existence of other events within a competing-risks framework. The aim of this study was to give two different examples using these two methodologies: 1) the estimation of the crude probability of using Kaplan Meier curves or cumulative incidence curves; 2) the differences of the results of multivariate analyses using these two methods.

Patients and Methods: Two different populations were studied: First, to evaluate the probabilities of relapse, all patients allografted from a mismatch unrelated donor (MUD, n=116) and reported to the EBMT acute leukemia registry were studied. Second, we analysed 212 patients transplanted with hematological and non malignant diseases from a single centre in order to detect the influence of CD34 cell dose on neutrophils recovery. All covariates with a p value less than 0.1 in univariate analysis were entered in the multivariate analyses performed using 1) KM, Log rank test and Cox models 2) CI estimations and univariate and multivariate Cox-like regression models for the subdistribution hazard.

Results: For the first example on 116 patients allografted in CR1 from a MUD, the 5 year probabilities of relapse were 27% using CI curves and 37% using KM curves. On the second example, the comparison by KM curves by the Log rank test did not show significant influence of CD34 cell dose (>3 106/Kg) on neutrophil recovery (p=0.15). In contrast, the comparison of CI curves showed an association of CD34 cell dose with engraftment (p<0.1), which was included in the final multivariate model and became significantly associated with neutrophil recovery (p=0.04).

Conclusions: We underline here that the use of the conventional statistical method of Kaplan Meier could lead to biased estimates of probabilities after HSCT, and that the use of cumulative incidence curves would be the appropriate technique to model the competing risks after transplant.
Standards of Care

N893
Symptom measurement using a visual analogue scale (VAS) on a hematology / SCT ward
L. Lurati, P. Schiener (Basel, CH)

Background: In patient care continuous evaluations of the patient's clinical and personal well-being are made by nurses, doctors and other cooperating services. This view is one-sided, as it does not measure the patient's feelings and experiences. For the self-evaluation of symptom distress of all patients the 10 cm long visual analogue scale (VAS) is systematically and routinely used to measure pain, nausea, fear/stress and, more rarely, itchiness.

VAS is an internationally well recognized instrument that is frequently used together with other instruments to measure the patient's subjective experiences.

Goal: The topic of the submitted study evaluates benefit for and acceptance of the use of VAS in daily care at the Hematology / Oncology Isolation Ward, for both patients and the care team.

Method: This topic is being explored by 4 strategies:
- Survey on opinions, use and evaluations done by the oncology nurses,
- Survey on opinions, use and selfassessment by patients.
- Review of the documentation from the care team, the medical weekly report on the frequency of occurrence and exactitude of the collection and application of VAS results.
- Observation of the care team's report with regard to frequency of the use of the of the VAS measures.

Results: The results show a high acceptance and frequent use of the VAS within the care team. The majority of the patients use VAS only on request; nevertheless they think that it is useful. Patients as well as the team realize problems in the evaluation of fear and stress, but both clearly insist in continuous use of VAS. The potential of improvements for care practice and interdisciplinary cooperation is being described.

Further questions for research: Our results focus mostly on nurses and patients. Concerning physicians and other services we do not have sufficient data for statistical analysis yet. A separate evaluation of the results of other teammembers is necessary.

Prospects: The use of VAS in other fields seem valuable and necessary.

N894
Toxic epidermal necrolysis after sibling peripheral blood stem cell transplantation
D. Lerner, A. Caspary, A. Becker, B. Johann (Idar-Oberstein, D)

Toxic epidermal necrolysis (TEN) is characterized by bullae that arise on widespread areas of erythema and then slough resulting in large areas of denuded skin. The most common cause of TEN are bacterial and viral infections. In addition, various drugs and severe graft-versus-host disease (GvHD) also can induce severe TEN. The treatment is very difficult and needs carefully nursing in those patients.

We report here a 22-years old female patient who developed TEN at day +12 after a sibling allogeneic peripheral blood stem cell transplantation (PBSCCT) for aplastic anemia. She received a preparative regime consisting of cyclophosphamide and anti lymphocyte globulin according to the IBMTR protocol for severe aplastic anemia. The donor was the patient's sister. GvHD prophylaxis consisted of cyclosporine A and methotrexate (MTX) given as a short course on days +1, +3 and +6. On day +8 after PBSCT a renal and respiratory failure developed demanding continuous hemofiltration (CVVH) and a mechanical ventilation.

The aim of this report is to present the guidelines of our clinic in a case of TEN. The nursing management contained special skin care including soya-bean-bodywash, high fat contained ointment, tincture of iodine, patient positioning on sterile metalline sheets and the use of special equipment such as softcare matrace (KCI-Mediscus RIK fluid overlay). During the period of mechanical ventilation and CVVH, two nurses were necessary in order to take care of the patient. TEN is photo-documented before and during the period of hospitalization. The conclusion. TEN is a severe complication leading to the high mortality after PBSCT. However, the carefully treatment of the skin lesion is a challenge for the nurse staff and needs further investigation.

N895
Issues concerning the transfer of haematology patients to intensive care
H. Vos, G. Trout (London, UK)

There currently exists a gap in research, pertaining to the nursing transfer of Bone Marrow Transplant (BMT) patients to an Intensive Care Unit. At University College London Hospital, staff reported dissatisfaction at times when transferring BMT patients to ICU. Problems occurred during the handover of patients and affected the continuity of specific haematology nursing care.

A subsequent literature review explored possible reasons for these problems. The review highlighted misconceptions that ICU and Haematology nurses may hold regarding BMT admissions to ICU, and the possible detrimental effect to patient care if communication between the two specialities is flawed.

On the basis of these staff reports and themes in the literature, a standard of practice was developed, proposed and piloted regarding the transfer and care of BMT patients in ICU. This included joint teaching sessions across the two specialities, nurse attendance for haematology ward rounds in ICU and a guide to transferring a patient to ICU. The aim was to increase staff awareness and provide support for nurses from both specialities.

Once implemented, an audit cycle was set up to formally evaluate the standard and generate new ideas. A questionnaire survey was given out to 30 nurses working on the BMT unit. In the results, staff reported an increased awareness of the issues concerning the transfer of patients to ICU. The results also suggested that communication between the two specialities had increased and that improvement in transfer of patients had occurred. The standard was subsequently refined and updated and introduced as new unit policy.

N896
From intuition towards systematic observation
C. Korf, G. Suryadi (Rotterdam, NL)

The condition of the haematology-oncology patient may deteriorate in a short period of time, which can lead to an acute life-threatening situation. Often the nurse is the first one to notice and should communicate it quickly and adequately with the responsible doctor. A traumatology derived method has been adapted to suit...
the haematology setting. This is done in order to improve the observation and the inter- and multidisciplinary communication.

The training department of the University Hospital Rotterdam, NL, in close co-operation with the haematology departments has started this process and implemented the method.

The adbce-method is a method that enables the nurse to observe the vital functions of the patient systematically. It gives the nurse tools to make an inventory of the problems and to communicate its results.

The method is split in two phases:

primary research:
A=airway
B=breathing
C=circulation
D=disability (awareness level)

The second phase is to stabilize the vital functions before moving on to the second phase:
E=exposure/environment (head-to-toe check)

The method is taught in the Haematology/Oncology course at the University Hospital Rotterdam and implemented in the wards through clinical lessons and exercised periodically.

This method has proved to be a valuable instrument within traumatology and intensive care units. Despite the fact that the method has been introduced into haematology nursing recently, the first experiences with it have been positive. The ten nurses who have been educated to use the method are more aware of their actions where patients in life threatening conditions are involved.

The adbce-method provides the haematology nurse a tool to observe the condition of the patient quickly and systematically. It helps to give relevant and objective information, which improves the inter- and multidisciplinary communication.

Information and Education

N897
Promoting partnership in care: setting up a nurse-led Hickman line clinic in a transplant unit

V. Ragoonanan (London, UK)

Background: In keeping with the unit philosophy of promoting health and encouraging patients to be partners in all aspects of care, a weekly clinic was set up by nursing staff to promote patient education enabling them to care for their own line.

Thereby:
- Increasing patients' independence.
- Promoting self-care.
- Minimising the need for nursing intervention during treatment and post discharge.

Aims of the clinic:
- Promote patients' health and independence during and after treatment by:
  - Teaching patients and carers, the correct technique in line dressing.
  - Teaching the correct method in flushing lumens.
  - Enabling safe self-administration of intravenous drugs.
  - Monitoring for signs of infection and mechanical complications.

Methods: A weekly clinic lasting one hour is lead by nursing staff who demonstrate the correct care of the line using a model. All patients and their carers are encouraged to attend the clinic on admission. Posters displayed throughout the unit including patients’ rooms help to explain the benefits of self care. A video specifically recorded by nursing staff is shown to patients in their own rooms to help remind them of the teaching techniques hown in the clinic.

Findings: Over 80 patients and their carers have attended the clinic since it commenced, of those who attended at least 50% were self caring of their lines during treatment or discharge. A number of patients were able to administer intravenous antibiotics and take bloods from their line thereby enabling them to spend more time at home.

Patients have recognised the benefits of being able to care for their lines including:
- Less time waiting for nurses.
- Increased confidence in caring for their line.
- Standardisation of care.
- Increased knowledge enabling them to direct health care professionals.
- More time spent with their families at home.

Ward based and community nursing staff are able to dedicate less time to caring for central lines.

Conclusion: The weekly clinic while educating patients also enables patients to meet and share their experiences with others going through similar treatment and to discuss concerns in caring for their lines. Findings to date show that patients have an increased sense of being involved in this aspect of their care. An ongoing audit will hopefully show the continuing benefits of this nurse lead initiative.

N898
An exploration of nurses' experience of undertaking an informal telephone triage role on a hematology unit

M. Tadman (Oxford, UK)

Many haematology patients spend time at home between courses of chemotherapy. These patients are vulnerable to life-threatening infection and must have access to advice on how to respond to side effects of treatment. This advice is often attained by telephone from their haematology unit.

Nurses who take on the role of telephone support and triage are involved in deciding whether patients should be admitted to the unit or referred to other health care professionals. They must be able to carry out accurate assessment of patients from verbal information gathered from a telephone conversation.

There is little research into this role in oncology or haematology settings. A qualitative, exploratory study was designed to gain an understanding of the nature of telephone triage within a haematology setting. Six nurses from a regional haematology and bone marrow transplant centre were selected to participate in the study. Data was gathered using semi-structured interviews and analysed using a phenomenological approach described by Benner (1994).

Two main interrelated themes emerged from the data. First, patient safety was the main concern of participants undertaking telephone triage. Second, participants adopted cautious decision making strategies that allowed them to remain within their comfort zone, a perception of feeling safe about decision making. The comfort zone is a complex interplay of personal and organisational factors. Knowing the caller, high levels of experience and support and a propensity to take risk, all increase the size of this comfort zone.

A lack of clear guidelines and formal education directed at patient safety was the main concern of participants undertaking telephone triage. Second, participants adopted cautious decision making strategies that allowed them to remain within their comfort zone, a perception of feeling safe about decision making. The comfort zone is a complex interplay of personal and organisational factors. Knowing the caller, high levels of experience and support and a propensity to take risk, all increase the size of this comfort zone.

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Recommendations are made to clarify telephone triage through standard setting and preparation and support for those undertaking the role. Further research is required including the nature and safety of decision making and the effectiveness of tailored education and decision making tools.

The development of an interactive CD-ROM for information and education to support patient undergoing stem cell transplantation
A. Mank, F. Kresin, J. v.d. Lelie, M. Weterman, S. Molenaar (Amsterdam, NL)

Objective: Information and education in contemporary health care are essential for patients undergoing a complex treatment such as stem-cell transplantation (SCT). There are many different ways to give information about diagnosis, treatment, chemotherapy, side-effects, etc.: 1) personal contact with the physician, SCT-coordinator and wardnurses; 2) written information consisting of leaflets, books and other written material and the Internet; 3) information and experiences from (ex-)patients. The process of providing this information is often unstructured, unclear and incomplete. This can be confusing and may increase the patients' distress. Above all, most patient information is not directly oriented towards personal needs that change throughout the treatment period. Aim of this project is to improve the provision of information to patients, by means of the development of an interactive CDROM with information on SCT.

Methods: As an interactive device is being used, patients and their relatives, can decide for themselves in which that they are interested in. This will be achieved by a menu structure with underlying submenus. The main menu consists of the following sections: a) general explanation about cancer; b) the hospital; c) treatment options; d) SCT; e) side effects; f) follow up. Certain parts of the information on the CDROM are presented by fellow-patients who themselves have experienced a SCT.

The CDROM presents a combination of video, pictures, text and sound. It is suitable for touch-screen configuration and traditional use with a mouse. The CDROM has several other advantages, such as a high capacity and low production, duplication and distribution costs. When it is necessary to adjust the informational content, it is relatively easy to develop updates. The development of the CDROM will be structured by a steering committee in which an audio-visual consultant, a physician, a content, it is relatively easy to develop updates.

At present the user interface is ready. A demo version will be presented at the meeting.

Life stories narrated by people who have had leukaemia
L. Persson, I. Rahm Hallberg (Kristianstad, Lund, S)

Eighteen people, in remission from acute leukaemia or highly malignant lymphoma, were asked to narrate their lived experience of falling ill, of being under treatment and of life following this event. The transcribed texts were analysed from a phenomenological-hermeneutic perspective, expanded by their medical and social history as related in interviews. The analysis revealed three themes: Believed in life, fought for it and came through stronger; Life goes on, adapted and found a balance in the new life; Life was over, felt out of control and lost belief in life. The two first themes reflected a meaning to the experience that had given new insight or strength. The third theme reflected not having assimilated the experience and evaluated the situation with bitterness. To help people retell their experiences may be one way of processing the experience of the disease and treatment and to regain a balance in life.

Pediatric bone marrow transplant: can the length and effect of hospitalization be predicted?
S. Howard (London, UK)

The diagnosis of severe combined immunodeficiency (SCID) generally results in a long term hospital stay encompassing a variety of procedures and interventions which, in turn, have a dramatic psychosocial impact on both the child and their carers. Until recently the only curative treatment option has been a bone marrow transplant with a predicted stay through this procedure of about two months. Shah was admitted to the immunology unit in August 1999, aged eight months. A diagnosis of SCID was made, but the added complication of BCG infection limited the treatment options. Shah spent two months of his stay in Paris, undergoing gene therapy, which proved to be unsuccessful. He then underwent a bone marrow transplant from a matched unrelated donor and was finally discharged home in December 2000, sixteen months following his admission.
Shah and his family are from an ethnic minority group and his extended hospital stay caused added stress to the family unit. Shah's mother was resident throughout his stay. Shah was formally assessed in April 1999 by the Hospital Development and Disability Service (HODDS) and recommendations were given to aid his developmental progress. This was not performed earlier due to Shah's condition and post assessment he received input from physiotherapy and made encouraging progress. Input was also provided from the music therapist, play specialist, nursing staff and his family, resulting in improvement both in his motor and social skills and he enjoyed contact from a variety of different people. His mother needed support throughout his stay and she obtained this from the volunteer service provided by the hospital. She also received support from the social worker, psychologist, parents of other children and her own family.

Through this presentation, I hope to illustrate the extensive development and support network we are fortunate to be able to provide to our children and their families during an unpredictably long hospital stay. We hope to alleviate some anxieties and help the children to develop on an equal level to their peer group.

N903

Delayed 'back to school' after pediatric stem cell transplantation: The role of the educational society


With the increase of success in stem cell transplantation (SCT) the quality of life (QoL) is becoming more important. The return to school is a very important step both physically and emotionally. It is a way to make a new start and to leave the past behind. Delayed attendance to school is one of the educational problems seen after pediatric SCT. We have studied the problems of the patients and the families in delayed "back to school". This time in order to see the problem from the scope the educational society of our children we have evaluated the teachers and the students. We have given a questionnaire to 100 teachers and 200 students from different schools in different social status. The questionnaire consisted of the questions about the cancer, SCT, classmate who had SCT. From 200 students (120F, 80 M, median age 10) 30 (15%) defined cancer as contagious and do not believe in cure after SCT; 135 students (67.5%) have no idea about SCT; 40 (20%) feel unwell of being in the same class with a child who had SCT; 58 children (29%) thinks that the children who had SCT should go to the special schools; 40 students do not want to invite them to their parties. From 100 teachers (60F, 40 M) 17 feels irritated to have a student who had SCT, but 64% think the SCT patients should be educated in normal schools. 24% want to be educated in special schools; 12% have no idea; 65% of the teachers think cancer as a treatable disease; 4% do not believe in cure; 51% are not sure. The first thing that comes to the minds of the teachers is death in 61%, 15% defined cancer as contagious and do not believe in cure; 31% are not sure. The first thing that comes to the minds of the teachers is death in 61%, 15% defined cancer as contagious and do not believe in cure.

In conclusion, cancer=death belief, irritation of having a child who had SCT in class of the teachers and the students, non-acceptance, make "back to school" more difficult for our patients. Education of the society must be also the major goal of our efforts in adaptation programmes. To offer every child a comprehensive programme after SCT we should study hard to obtain and guarantee true cure with the highest possible QoL.

N904

Siblings: the forgotten ones

S. Simms, N. Hewitt (London, UK)

Due to significant advances in medicine, many childhood life-threatening diseases are now viewed as chronic rather than acute terminal ones. Improvements in survival have enormous implications in terms of psychosocial support for families (Evans and Kelly 1995). Whilst the introduction of family centred care has recognised this, the impact on siblings has often been neglected. Studies performed by Dominic (1993) and Faulkner et al (1995) have highlighted that siblings are unique individuals with special needs, who require a great deal of support. On the Bone Marrow Transplant Unit at Great Ormond Street Hospital following research performed by Lwin (1996-1998), a previous psychologist discussed with various members of the multidisciplinary team and through analysing issues raised in weekly psychosocial meetings, it was apparent that siblings of children undergoing bone marrow transplant may benefit from a support group, where siblings would be given the opportunity to visit the ward environment, meet some of the staff and experience some of the play activities that their brothers/sisters enjoyed. In addition, it would enable these children to discuss their feelings and allow them time to ask questions about their brothers/sisters diagnosis, prognosis and treatment. To enable the support group to proceed, parental consent was gained for those children involved and two nurses working on the unit at the time, with the help from the play specialist took on the responsibility of organising the activities. Support groups have been provided for siblings during school holidays, involving activities in the ward playroom and visits to a variety of sites in London. All the groups have appeared to interact well, despite the fact that the children have not met before. After distributing evaluation forms to all the children who attended the groups and analysing the comments, the group appears to serve as a valuable way in enabling the siblings to cope with the situation they find themselves in, during the period of hospitalisation.

References


Impact of new Therapies on Nursing Care

N906

The introduction of thalidomide (T) in BMT programs for patients with multiple myeloma (MM) : encouraging results and a challenge for nursing team

I. Dagan, R. Ovadia, D. Taou, L. Bar (Tel Hashomer, IL)

The potential of T as an active agent against MM and its tolerability, present an important breakthrough in the management of this devastating disease. The lack of myelosuppression makes T an ideal potential drug to be used in patients with poor bone marrow reserve or in combination with chemotherapy regimens. We studied the use of T in conjunction with BMT in order to evaluate both the risk of cumulative side effects of high dose chemotherapy and T, as well as the potential clinical value of the combination.

Method: Patients that had the combination of T and BMT were interviewed, examined and answered a questionnaire every month during the therapy. The quality of life, daily activity and main side effects were monitored, as well as the clinical outcome of the therapy.

Results: Nineteen patients with relapse of MM after ABMT were treated. Following the T therapy 5 of the patients underwent submyeloablative allogeneic BMT and 2 patients had a second ABMT. The conditioning regimen included Melphalan in combination with Fludarabine. The main side effects of T were: constipation, neurological manifestations, fatigue and mood disorders as described. In none of the patients T mediated toxicity.
which eliminate the possibility of secondary BMT. While, patients who had a previous therapy with VAD combination manifested severe neurological complications that obliged cessation of T therapy in 4/12 patients.

Conclusions: The use of T in combination with BMT is feasible. T therapy before second transplantation may be advantageous. The limiting factor of T therapy seems to be neurological complications which are more frequent in patients that previously received Vincristine therapy rather than ABMT. A tight nursing follow up and support is mandatory for patients receiving T in conjunction with BMT.

N907

Stem cell transplant in multiple sclerosis: a new challenge for transplant nurses
C. Burnett (Montreal, CAN)

As we move through the twenty-first century, nurses in transplantation will be presented with new and important challenges. In this paper, a new Canadian study in transplantation will be described. This study, based on the assumption that Multiple Sclerosis is an autoimmune disease, looks at the use of immunoablaotive therapy, followed by stem cell transplantation to induce remission and/or cure in a poor prognosis population of MS patients.

The collaborative effort between neurological and transplant nurses will be described. The learning objectives and competency outcomes of an intensive multiple sclerosis learning programme for transplant nurses will be outlined. Critical to the planning of care for this new population was the development of a care map, covering pre-, during and post transplant care. The contents of the map will be presented.

In conclusion, a summary of the potential new clinical, ethical and nursing research considerations will be put forward.

N908

Could this be the end of total body irradiation as we know it?
N. McKeag, J. Bomford (London, UK)

Bone marrow transplants (BMT) using chemotherapy and more often than not total body irradiation (TBI) has been the treatment of choice for patients with a haematological malignancy. However for some patients with multiply relapsed leukaemia or blastic chronic myeloid leukaemia there is a high relapse rate. It is known that high doses of TBI can increase long-term survival but this also tends to carry a high mortality/morbidity rate. One way of trying to increase the amount of treatment, thereby increasing the response but decreasing side effects is to try to target the treatment to specific areas. This unit has been using an anti-CD45 monoclonal antibody radiolabelled with Yttrium-90 (Y-90).

Previous studies using radiolabelled antiCD45 have shown that it targets the bone marrow and spleen. Unlike TBI this then can potentially deliver larger doses of radiation to the bone marrow, spleen and lymph nodes, sparing the lungs, liver and gastric mucosa, leading to a better long-term disease free survival, and a reduced transplant related mortality.

To date nine patients have been entered into this study which is dose escalated to find the best dose of radiation to use in conjunction with a traditional conditioning regimen of Busulphan/Cyclophosphamide. We are comparing the cases of these nine patients with comparable patients having a standard Cyclophosphamide/TBI conditioning regimen. The patients in the study arm have Indium at D-19, Y-90 at D-14 and Busulphan D-7 to D-4 and cyclophosphamide D-3 and D-2 with marrow returned D0.


Only 2 patients in each group are alive at least one year post transplant.

Comparisons have been made between

1. The number of days hospitalised, the average length of stay being 40.9 days (range 28-55 d) in the Y-9 0 arm, compared to 61.8 days in the Cy/TBI arm (range 35-120d), P>0.06.
2. Days spent neutropenic.
3. The degree of mucositis experienced.
4. Graft versus host disease (GVHD)
5. The need for nutritional requirements.

We will also be discussing the nursing issues related to the care of patients undergoing a trial procedure, and a new form of therapy.

N909

Percutaneous gastrostomy (PEG) feeding in patients undergoing allogenic bone marrow/peripheral blood stem cell transplantation. A feasibility study
R. Chidley (Cambridge, UK)

Weight loss and acute malnutrition are common in allogenic bone marrow transplant patients as a result of the side effects from total body irradiation, chemotherapy and anti-graft versus host therapy. These patients also develop severe mucositis making it impossible for them to achieve adequate nutritional intake by mouth. It is routine practice to provide nutritional support to this patient group whilst in hospital, though not following discharge when patients are still at risk of weight loss and malnutrition due to nausea, prolonged changes to oral mucosa, poor appetite and intolerance for food. In our hospital we have used radiolabelled Yttrium-90 to target the bone marrow with the aim of reducing transplant related mortality.

Currently, TPN is the usual method of nutritional support with NG or NJ feeding being used as alternatives. TPN has been associated with increased infection risks, disturbed liver function, abnormal glucose metabolism and gut atrophy. NG/NJ feeding maintains gut integrity and carries a reduced risk of infection but is often poorly tolerated by patients.

Enteral feeding via percutaneous gastrostomy tube would avoid the cosmetic and nasopharyngeal irritant effects of an NG/NJ tube as well as some of the problems associated with TPN such as metabolic disturbances and liver dysfunction. This alternative, while theoretically attractive has not previously been investigated in the setting of bone marrow transplantation. A Medline search covering the last seven years yields no reference to PEG insertion in relation to bone marrow transplants. However, in the few larger studies of PEG feeding in other circumstances (e.g. other malignancies) there is agreement that PEG feeding is potentially safe and effective method for improving nutritional status.

It is clear that alternative nutritional strategies to TPN are under-researched and that PEG feeding is potentially safe and effective alternative. This study, whilst not directly comparing TPN and PEG feeding, is intended to determine whether it is feasible to feed this patient group safely using a PEG tube as an effective way to provide adequate nutritional support and maintain nutritional status in a cost-effective manner.
patients; other late complications related of its use are infections, deep venous thrombosis, dislocation. To maintain patency of the system and prevent occlusions, is recommended a monthly or more frequently flushing. With the aim to reduce the cancer patients' discomfort during follow-up and insertion procedure, we evaluated a three monthly flushing schedule on 20 breast cancer patients in complete remission after high dose chemotherapy with peripheral blood stem cell (PBSC) support. The PAC flushing with heparinized saline (10 to 100 IU/ml) was performed during oncologic follow-up visits at 3.6 and 12 months after PBSC transplant.

Retrospective audit was carried out on 60 flushing procedures: no thrombosis, no infections, no dislocation were observed. Partial occlusion (only for the sampling of venous blood) occurred only in 2 cases (3%), both during last follow-up control (+12 months). Conclusions: Basing upon these very preliminary results, we planned for a prospective multicenter study on about 200 cancer patients in follow-up after high dose chemotherapy to confirm the safety and the efficacy of using this flushing schedule.

N913 / P954
An audit of Hickman Line infections in hematology patients
M. Devaney, C. Smith (Edinburgh, UK)

A Hickman line is an essential tool in the treatment of hematology patients. It is important that the lines remain under surveillance for infections as part of an on-going process. The purpose of this audit was to establish the current line infection rate, the length of time the line remained in and reasons for line removal in relation to infection.

Hickman lines that were placed in haematology patients between May 2000 - Aug 2001 were reviewed from a database. As the database on the database was limited, the audit was completed retrospectively by reviewing the patient's case notes. A total of 78 lines were reviewed.

Infections were reported in 86% of lines (n=67). These were grouped into clinical infections, exit site infections and tunnel infections. The clinical infection was defined as the patient being pyrexial with a suspected line infection (n= 36). An exit site infection was defined as inflamed skin around the exit site of the Hickman line (n=63). Defining a tunnel infection as inflammation and pain tracking up the chest area where the line was tunneled under the skin (n=9), 11 lines did not have any infections reported. Positive microbial results were reported in 56% of all the infections reported. 76% of all these infections had occurred within the first 100 days of the line insertion.

The neutrophil count at the time of line insertion was examined in relation to the first infection reported. 23 clinical infections were reported as the first infection. 30% had a neutrophil count of 1.0x10^9/l. 34 exit site infections were noted as the first infection. 29% had a neutrophil count of 1.0x10^9/l. There were 9 tunnel infections noted, but only 1 patient had a low neutrophil count at the time of insertion.

The rate of line removal due to infection had increased by 15% since the previous audit. This problem is being addressed and at present the trend on the infection rate is stable. Continued surveillance is vital to monitor line infections. The data will be continuing to be collated to highlight any infection problems at an early stage.

N914 / P959
Patient's involvement in the "at home" management of the long-term central venous access
S. Gini, F. Mazzufero, M. Canepa, A. Chioni (Pisa, Ancona, Genoa, I)

Constant use of the central venous catheters (CVC) made protocols for use and maintenance absolutely necessary to improve the management of the CVC and to conform procedures in the hospital and out of the hospital. Nurses have a fundamental role in the management of the CVC and in the education and information of the patient involved and his family.

S257
Psycho-social Issues

N915
Risk factors that change the communications within the relationship between patient, family and nurse
G. Bisaccia, E. Cervetto, M. Cotrozzi, F. Garibaldi, M. Leimer, C. Picasso, M. Canepa (Genoa, I)

Our department is involved in autologous and allogenic bone marrow and cord blood transplantation in children from 0 to 18 years old. Since 1985 681 HSCT, 79 unrelated donor, 134 related donor, 7 cord blood and 461 autologous HSCT have been performed in 564 pediatric patients.

In the pediatric field the communication involves the whole family, the child is the subject of the relationship.

Approaching the patient depends on his age:
- Early childhood and pre-school age: Communication using games and the family as a filter.
- School and puberal age and adolescents: relationship with the patient and the family, and difficulties due to understanding the disease and problems arising from living in a closed and restricted reality.

Our analysis of the communicative process outlines the risk factors that may change the relationship during the different phases of transplant.

Transplant phases and risk factors:

1. Preparatory phase: Preparatory talk with the doctor, sometimes together with the ward nurse – Admission.
   Risk factors: Absence of the nurse during the preparatory talk, parental confusion regarding professional roles nurse-doctor, generating information demand regarding scientific notions, false confirmation, inconsistencies.

2. Acute phase: Toxicity, complications and pain
   Risk factors: lack of parents' cooperation in the nursing process, the nurse perceived as disturbing figure, parents lack of respect of department rules due to a strong sense of guilt towards their child, anxiety and frustration related to the feeling of helplessness in the face of the child's pain.

BMT Failure
Death: Interruption of the communicative process because of the loss, our sense of guilt for the uselessness of HSCT, deluded expectations.

Target: Clear and efficient and therapeutical communication, mainly oriented towards listening feed-back, encouragement, availability, reflection and verification. The following operative means would be necessary for us to obtain this goal: work re-organization, more efficient subdivision of roles and tasks within the structure, training of the nurse staff.

N916
Who cares? Optimization of psychosocial and spiritual care for patients on a hematology ward
P. Horsting, M. de Hullu, M. Jorna, J. Raemaekers (Nijmegen, NL)

Background: Patients on a haematology ward are mostly being treated for a life-threatening disease. Contacts with patients indicate that patients experience in particular different psychosocial and spiritual problems, for which not always, action is taken. Also related disciplines like social work, psychology, psychiatry, et. are not efficient consulted. The staff of the ward concluded this as incomplete care because the patient doesn't receive the optimale care that he/she requires. A multidisciplinary project to improve the quality of psychosocial and spiritual care has started.

Aim: The patient obtains the (physical), psychosocial and spiritual care he/she needs during admission.

Method: To assess psychosocial and spiritual problems of patients and to define the problems for which disciplines are consulted next steps were defined:
1) Each discipline has described the problems they face in patients contacts.
2) A survey was done (n=25) by using a questionnaire (steggerda, 1997) in which 52 items were redefined about a) problems patients experience during admission, b) how patients act on problems and c) which disciplines or persons are contacted to support problems. An ordinal scale was used from "I do not have this problem" to "I suffer a lot of this problem". Also questions were asked about patient information about the different disciplines. In comparing the results of step 1 and 2 a policy could be defined.

Results and conclusions: Of the 52 items, 22 were selected. 12 items were found:
- What to do immediately and after CVC complications
- Activation of a telephone number to solve patient's problems at home

Conclusions: The project of involving the patient in the management of the CVC at home should obtain effectiveness in terms of less hospital care and efficiency in order to obtain and to maintain these results:
- Satisfaction of the patient
- Co-operation with the patient and his family
- Absence of complications
- Keeping the CVC until the patient needs

N917
Clinical supervision in the hemato/oncology setting
P.J. Morris, C. Walker (London, UK)

Nursing staff working in areas such as haematology (including BMT) and oncology are often highly stressed with excessive work pressures and high incidence of anxiety, depression and burnout. Because of this, the Cancer Services Directorate of a large,
London Trust was chosen as the site for a pilot study of clinical supervision.

"Clinical supervision refers to the activity of examining one’s practice with a more experienced and skilled professional in a formal relationship. The aim is to develop and extend knowledge and skills, to monitor quality of care and to gain understanding and support.”

Jean Faugier, 1993.

The overall aim was to utilise clinical supervision as a means of implementing staff support throughout the Trust and improving standards of care. Following discussions with nurse managers in the planning stages it was decided due to differences in staffing levels and counselling experience in the Cancer Services Directorate on both sites that 2 alternative strategies of clinical supervision could be implemented and tested:

Hospital A: Group Supervision
Hospital B: 1:1 Supervision

The initial meetings and teaching sessions for the commencement of the clinical supervision pilot study in cancer services began at the end of the year 2000 via an external facilitator. The commencement of the supervision of the first potential clinical supervisors commenced in September 2001. The implementation of clinical supervision in this way is a time-consuming and relatively expensive process.

The quickest and most cost-effective method of providing clinical supervision to the greatest number of individuals is by implementing group supervision. However, this is not without its difficulties. The commencement of group supervision requires the potential supervisor to: have experience of being supervised themselves (preferably in a group), have a great deal of confidence and to possess counselling and group management skills. It also relies on the confidentiality of the whole group.

The clinical supervision process in Cancer Services has just begun. Initial responses are favourable and it is possible to make certain recommendations. Its effectiveness in the Cancer Services Directorate and its optimum method of implementation throughout the rest of the Trust has yet to be confirmed.

Ambulatory Care

N918

Evidence-based nursing for supporting cancer patients coping process

H. Salovaara, E. Lauraeus-Käkelä, R. Wirén, T. Lehti, S. Lauri
(Turku, FIN)

Drawing on crisis, stress and coping theories, researchers and clinical nursing staff at Turku University Central Hospital set out to design a theoretical model of psychosocial support for cancer patients including the transplant patients. The model identified the stages of the crisis that the patient work through, the questions that patients have as well as the expected outcomes of the nursing interventions. This model has provided the basis for our work to draft a set of evidence based guidelines for supporting cancer patients coping process using the action research method. These guidelines consist of systematically developed statements that help the nurse and the patient to make informed decisions about the best possible way of caring the patients condition. The application of these guidelines is based on decision-making concerning the patient’s nursing problems and is continued to provide direction to the planning, implementation and evaluation of nursing care.

Our starting point has been the nurses’ experimental knowledge. At joint workshops the nurses presented the patients’ behavior traits and guidelines they had produced to the themes under discussion, which were then discussed at team meetings. For the next meeting the researches drew up summaries, and the discussion was continued until consensus of opinions was reached. Then the produced guidelines were put to the practical test in all units, and after that research evidence was produced to support them.

The first step is to identify patients mental status. It is important to establish what stage the patient has reached in the stress coping process: the stage of shock, reaction or working-tough the crisis. On the basis of nurses’ experiences and the research evidence available, we developed an instrument for the assessment of patient’s mental status. In the shock stage the aim in nursing is to get the patient to understand the new situation and to get the cooperation going between the nursing staff and patient. In the reaction stage the aim is to get the patient to adapt to the situation and accept it. In the working-tough stage the aim is to support the patient to rediscover a sense of well-being.

The development of evidence-based nursing presents a major challenge for nursing that we must now take up. It provides us with a new opportunity to demonstrate the significance of the nursing knowledge base to improving the quality of transplant patients care.

N919

Care of bone marrow donors: an original experience in Lyon, France

B. Bodet, P. Crova, E. Michel, I. Loubier, C. Portugues, M. Aubague, J. Etienne (Lyon, F)

Bone marrow allografts are currently done at the Lyon teaching hospital where there is an organ procurement coordination unit (OPCU). At the Lyon Edouard Herriot Hospital, bone marrows from donors living in Lyon are harvested. In association with the hematology unit and the French Blood Organisation, the OPCU has started a program to take care of the unrelated donors (UD) in March 1999. Related donors (RD) were included in the program in January 2001. Since the beginning of this program, 22 UD and 20 RD have been managed by the coordinators, who supplied every administrative formalities, and stay with the donors all along the process. At the « capacity day » and « harvesting day », donors were accompanied to the different appointments by a coordinator. A 17-items questionnary was sent to 31 donors (16RD, 15UD).

The goal of this study is to evaluate the satisfaction level in each part of the process. Satisfaction was quoted on a scale from A (very satisfying) to E (unstatisfying).

Feedback rate was 77.4% (global) 68.5% (RD) 86.6% (UD)

Data showed:

<table>
<thead>
<tr>
<th>Global notation</th>
<th>A: 81% B:14% C:4% E: 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>UD group</td>
<td>A: 86% B:12% C: 2%</td>
</tr>
<tr>
<td>RD group</td>
<td>A: 76% B:16% C: 6% E: 2%</td>
</tr>
</tbody>
</table>

Forty-seven percent of the UD were also blood donors ; 77% of the UD were male, 23% female Every UD said they would have lived this experience quite differently without this program: 30.8% would have hesitated to continue and 18.8% would have stopped the procurement procedure.

This program improves the donors’ quality of life during bone marrow procurement procedure and partly contributes to avoid UD motivation failure. Stem cell donors will be included next year.

N920

Ambulatory autologous peripheral blood stem cell (PBSC) transplantation: a single center experience in 79 patients

E. Benamo, L. Caymaris, C. Dulac for the Unité de Transplantation et de Thérapie Cellulaire (UTTC) - Institut Paoli-Calmettes - Marseille - France

Autologous PBSC transplantation is now recognized as the treatment of choice for different hematological malignancies, especially multiple myeloma and lymphomas. Although the use of PBSC in this setting resulted in easier graft collection, quicker
engraftment kinetics and economic advantages, the impact on the quality of life due to the necessity of hospitalization still represents an important issue. In the present study, we undertook a prospective analysis comparing conventional (patient hospitalized in a private room during the whole procedure) autologous PBSC transplantation to an ambulatory setting (if a permanent accompanying family member was available, the patient was discharged immediately after PBSC infusion and readmitted in case of complications). Patients included in the ambulatory arm, were systematically seen at the day-hospital 3 times per week. After informed consent, 79 voluntary multiple myeloma and lymphoma patients were included in this study. 37 patients were in the "ambulatory" arm (study group), and 42 were treated in a conventional setting (control group). For patients receiving the ambulatory transplant, the medical and nursing team realized a homogeneous educational plan focusing on infectious and hemorrhagic complications. The patients were also educated to detect clinical signs of anemia and to anticipate painful complications such as mucositis. The comparison between these 2 groups found no significant differences in terms of number of days of febrile episodes (Study group: 6; control group: 7) and the number of days of antibiotic therapy (Study group: 8; control group: 7). Time to reach an ANC > 0.5 x10^9/L and the number of red blood cells and platelet transfusions were comparable in both groups. Pain treatment especially morphine was also comparable in both groups (Study group: a mean of 40 mg/24H during 6 days; control group: a mean of 45 mg/24H during 7 days). These results confirm that autologous PBSC transplantation is feasible in an ambulatory setting without additional secondary or harmful effects for the patient. In addition to the economical analysis, we are now undertaking a detailed analysis of quality of life and anxiety parameters that appear to be improved in patients receiving ambulatory autologous PBSC transplantation. Conclusion: The programme received highly positive feedback from patients caregivers and nursing staff. Patients feel much more comfortable than with a conventional "ward" transplant and it is satisfying for caregivers to be more involved in the treatment. Home care for patients receiving ASCT is feasible and cost effective.

N922

A Pilot study to identify the physical and psychological outcomes in patients undergoing Autologous Peripheral Blood Stem Cell Transplant (APBSCT) in the in-patient and out-patient settings

S. Aston, S. Sheppard, A. Allsop, T. Bellamy, A. Metcalfe (Birmingham, UK)

APBSCT are used to treat a variety of malignant haematological disorders and other cancers (Neitzert et al 1998). In the United Kingdom (UK) this type of transplant has historically been delivered as an in-patient procedure due to the policy for isolation during the neutropenic stage of their treatment. Birmingham Heartlands Hospital has now established an out-patient service for this type of procedure, which is proving beneficial to the patients and their carers.

Objective: A cohort study has been undertaken, comparing the in-patient and out-patient settings with regard to physical and psychological outcomes throughout the duration of the transplant to ascertain whether there is a benefit for out-patient care.

Method: The methods used for this study employ both quantitative and qualitative approaches to enhance the validity of the research. The quantitative data includes a collection of basic demographic information, a daily collation of physical assessments relating to the transplant process, such as blood counts, mucositis score, nutrition score and additional therapies such as antibiotics and analgesia. The psychological status of the patient has been assessed using the Hospital Anxiety and Depression Score (Zigmond & Snaith 1983) and a qualitative questionnaire.

Results: The two groups (in-patients/out-patients) were compared to establish whether patients receiving APBSCT in the community setting have less physical and psychological problems compared to those who receive all their care in the hospital setting. While this may seem intuitively obvious, it requires validation. The study will be completed in January 2002 and the results comparing the two groups will be presented.

Supportive Care

N923

Implementing a nausea assessment tool on a bone marrow/stem cell transplant unit

H. Goad (Cambridge, UK)

Nausea and vomiting can be a very distressing experience for bone marrow and stem cell transplantation patients. Treatments such as high dose chemotherapy and total body irradiation are well documented as causing nausea and vomiting. Other side effects such as mucositis also compound this problem and result in increasing patients' levels of anxiety and distress. Intractable nausea and vomiting, which can be common in transplant patients, has a large impact on their quality of life and plays a significant role in the malnutrition of patients.

Nurses play an important role in the treatment and control of nausea and vomiting, through assessment, close monitoring and evaluation of the patients' experience and effectiveness of anti-emetics used.

Nausea like pain is very subjective. Pain assessment tools are regularly used, but nausea assessment tools seem not to have been adopted as readily, apart from the use in trials.
Clinic nutrition monitoring and nutritional assessment of patients receiving hematopoietic blood stem cell transplantation

X. Wang, Y. Li, P. Wei (Guangzhou, CHN)

Objective: Hematopoietic blood Stem Cell Transplantation (HSBCT) is used largely in treatment of hematologic malignancies, including leukemias and lymphomas, non malignancies disorder and solid tumors. Severe catabolic stress and increased basal energy expenditure have been documented in HSBCT recipients. At the same time, gastrointestinal toxicities limit nutrient intake. Anorexia, nausea, vomiting, and mucositis due to conditioning regimens (chemotherapy and/or total body irradiation), septic complications, and graft-versus-host-disease (GVHD), almost invariably result in reduced food intake and protein-energy malnutrition(PEM). Nutrition is therefore an important issue in the management of all HSBCT patients. But current nutrition supporting plan was relying on estimation mostly. The purpose of this prospective study was to monitor the accurate amount of calories and protein which HSBCT recipients receives via oral and parenteral nutrition route, to assess patients nutrition status based on this nutrition plan and then alter formula to meet HSBCT recipients changing nutrition requirements.

Measurements: We study 10 HSBCT recipients during days 0 +15.1.The provided energy from dietary and parenteral nutrition was investigated. 2.The provided protein intake and whole body protein metabolism was assessed by nitrogen excretion, nitrogen balance. 3.Nutritional states was evaluated pre-transplantation and at day +15, day +12, days +12, days +15 by anthropometric(body weight, triceps skinfold thickness, and arm muscle circumference) and biochemical(seum levels of total Protein, albumin, transferrin, prealbumin, indices). Meanwhile, we use Subjective Global Assessment(SGA) to assess nutrition state of HSBCT recipients.

Results: 1.Patients had a large individual variation in the energy intake From dietary and parenteral nutrition, the provision of dietary intake calories at 30% 18% basal energy expenditure(BEE), the provision of PN support at 68% 19% BEE.HSBCT recipients received total calories only 100% 21% BEE. 2.The Supply of Protein for 10 patients were 0.7kg-1ABW day-1. nitrogen balance was negative in all 10 recipients, with protein-energy malnutrition(PEM). 3. The main results are that 28 patients show fever in the first week, same results for catheter infection during days +0 to +9 in the 72,9%. About nutrition side-effects anorexia was present in 45,8%, 87,2% of them need parenteral support. Vomits were presents mostly in days 0 to +4 in 63,8% patients. Fluid retention in 78,8% and diarrhoea in 89,7% during days 0 to +2, constipation annotations in 70,3% related with non-mobility and morphine drugs. Mucositis was registered in 93,8% (days 0 to +3), with pain in days +0 to +9, with morphine requirements. 59,6% patients with anxious problems non psychiatric serious pathology. About mobility problems 10,6% interventions. Cutaneous EICH in 6 patients and 2 Hepatic EICH.

Conclusions: In the nursing management of the side-effects in allogenic post-infusion process the efficiency of the nursing interventions are reflected in well organised and designed registers, but are not equally balanced on some patient`s needs and others. Anxiety or psychological needs and mobility or sleeping problems are the ones which are less registered in controversial with patients demanded on this areas. This study propose to analysis priorities in nursing interventions, and to protocol other patient needs to be able to co-ordinate and multidisciplinary team work.

N925

Nurses’ annotations on patients’ needs in allogenic transplant

L. Aguilera, A. Lobo, M. Prieto, F. Viccaíno (Barcelona, E)

Introduction: The importance and concept on patient's needs are validated from nurses annotations and registers, but sometimes reality and what we register are not in concordance. Patients experience important side effects during high dose chemotherapy and post-infusion in haematological malignancies. We believe that the nursing registers must reflect all patient's needs and complications in the best way as possible in order to establish nursing care interventions. Purpose: The main area we wish to focus is to analyse nurses records and how they make priority in acute problems during post-infusion period. Methods: Retrospective systematic revision on nursing annotations in 48 clinical histories, with variant statistic analysis on the following variables: Fever(/>= 38a C), mouth problems such: (mucositis, xerostomy and gingival-bleeding ). Problems in elimination as diarrhoea, constipation and fluid-retention Problems in Nutrition as (anorexia, vomits, nausea and parenteral nutrition).Conclusions: The aim of the project is to review existing nausea assessment tools and adapt them to the requirement of the unit. This will provide a pratical and "user friendly" scale that can be used on patients undergoing bone marrow/stem cell transplantation. This tool will provide a visual pattern of the patient's level of nausea and compare that according to the anti-emetic used. This in turn will give nurses and doctors a guide to the effectiveness of the drugs used at that time. To reinforce the assessment tool will be a teaching package for nurses. This will include the physiology of nausea and vomiting and the mechanisms of different anti-emetics used. This will provide a practical guide for nurses on which anti-emetic may be used in different situations. The overall aim of the project is to provide high quality, efficient and cost-effective care for transplant patients experiencing nausea and vomiting. The reduction of the distressing side effects of treatment and providing a better quality of life is paramount.

N924

Problems encountered during IFN-alfa therapy following autologous stem cell transplantation: a preliminary report

S. Kav, Y. Koc, E. Kansu (Ankara, TR)

The aim of the study was to assess tolerability of IFN-alfa treatment during the post-transplant period following high dose chemotherapy and autologous hematopoietic stem cell (AHSC) support in patients with relapsed or high-risk lymphoma or multiple myeloma. Since May 2000, a total of 29 patients with lymphoma or myeloma underwent high dose sequential chemotherapy and AHSC support at the Hacettepe University Institute of Oncology. Of these patients, 3 died due to transplant related causes. Out of 26 patients surviving the transplant procedure, 22 went through the day +100 evaluation tests. All were found to be in remission by CT
scans, bone marrow biopsies and M-protein levels for patients with myeloma, and gallium scans for patients with lymphoma. Patients were eligible for post transplant IFN-therapy for treating possible minimal residual disease if they are in complete remission and their platelet counts are above 100,000 on after day +100. One patient died due to sepsis on day +106. Of 21 patients beyond day +100 who were evaluated for IFN therapy, 7 could not receive because of thrombocytopenia. We were able to place 14 patients on IFN-alfa therapy following AHSC transplantation. The reasons barring IFN therapy other than transplant related mortality and thrombocytopenia were; relapse (3 patients), cardiomyopathy (1 patient), and socioeconomic problems (1 patient). The planned duration of post-transplant IFN-therapy was 18 months (iIFN dose of 1-1.5 MU/tiw). Dose was escalated to 3 and 5 MU/tiw every two weeks if tolerated.

All patients were followed by a nurse specialist for initial instructions and education including self injection, side effects, their management and treatment plans. Majority of patients were residing outside the city, requiring information on CBC follow-ups every other week and contact with physician or the nurse. Patients were followed monthly by their physician. Patients receiving IFN therapy required very close follow-up due to severity of the hematologic side effects and intolerance induced by limited bone marrow function. Only 4 out of 14 were able to reach the planned final dose of 5 MU tiw. Compliance of patients to IFN therapy can be enhanced by carefully programmed follow-ups conducted by specialized nurses.

N927

Vancomycin resistant enterococci (VRE): a worldwide problem

VRE has become a major challenge to nurses in the performance of their duties in the Bone Marrow Transplant setting over the past five years. The importance of VRE resides in its ability to spread easily, its resistance to practically all antibiotics and to many disinfection processes.

Sporadic infection has occurred in St. James Hospital, since the first isolate in 1994, but was not a major challenge until September 2000 when a significant outbreak resulted in the closure of the unit.

A number of contributory factors to the cause of the outbreak were identified, resulting in a major review of the standards and practices employed by the multi-disciplinary team (MDT), in order to facilitate the reconstruction and redevelopment of the service provided.

This review was facilitated by the collection of information through:
- Interview and focus groups,
- MDT meetings,
- Benchmarking exercises
- Expert consultation
- Literature Review.

Data collected resulted in the following:
- Reconstruction of the unit.
- Redevelopment of Nursing services.
- Reconfiguration of service location.
- Extensive educational drive for all members of the MDT.
- Redevelopment of the cleaning services within the Hospital.
- Microbiological screening.

Conclusion: In summary, the implementation of the above changes continue to provide many challenges, however since the re-opening of the unit in February 2001, although continuing to obtain positive faecal cultures there has been no evidence of cross infection demonstrating the effectiveness of a cohesive multidisciplinary approach in the management of a VRE outbreak in a Bone Marrow Transplant unit.

N928

A programme for discharging a patient after BMT
L. Shvarts, J. Sasson (Ramat Beit Shemesh, Mevasert Zion, IL)

Approximately fourteen patients are treated in the bone marrow transplant unit every month. The average hospitalization of patients is roughly thirty days. The transplant entails isolation and aggressive treatments as well as a prolonged recovery process for both patient and family. Many questions arise during the hospitalization process and recovery period at home.

Up until now, the unit did not have a structured instruction program toward discharge from hospitalization. In light of the matter’s importance, the unit’s nursing staff decided to promote and develop such a program, which provides patients and their families with oral instruction as well as with a booklet that concentrates all of the essential information within it.

A questionnaire was conducted prior to intervention in order to ascertain patients’ level of contentment with the existing instruction. The anonymous questionnaire was given to eighteen patients during the course of three months (in 2000). The questionnaire’s results emphasized the need to put out a training booklet detailing subjects that are essential to the patients.

Following a summary of the questionnaire’s results, it was decided to compose instruction booklets. The unit’s staff was trained with regards to the patients’ discharge procedure and distribution of the booklet. Another questionnaire was conducted, which examined patients’ satisfaction level with the instruction.

The questionnaire was given to twenty-two patients during the course of two months (2001). Results pointed to a notable improvement in patients’ satisfaction level with the training given.

The following are examples of some of the questions present in the questionnaire – results from 2001 compared with those of 2000.

- Did you receive satisfactory information in the following subjects?
  - Follow up clinics and tests 2000 2001
  - Closeness with spouse 20% 100%
  - Social benefits 22% 77%
  - Medication 59% 82%
  - Central vein infusion treatment 50% 91%

In light of its publication and success, the booklet was translated into Arabic, Russian and English and was put on the Israeli bone marrow transplants website.

Quality of Life

N929

Chronic graft versus host disease of the vulva and vagina
D. Baker, C. Boothroyd, S. Durrant, R. Western (Brisbane, AUS)

Chronic Graft Versus Host Disease of the Vulva and Vagina (CGVHDV) is a reported condition (Corson SL et al 1982) which has received little attention in recent times. It is not clear whether this is due to a low prevalence of CGVHDV or reluctance on the part of patients and clinical staff to discuss such matters.

Clinical observation suggests that vaginal stenosis is preceded by vulvodynia and/or vaginal pain, and that early intervention with immunosuppressive therapy may modify the early course of CGVHDV.

We have conducted a survey on 60 long term (>12 months) female survivors of allo-BMT in order to establish the incidence of systemic HRT use, topical estrogen use, coital activity, and vulval pain/ulceration which may be related to CGVHDV.

Of 40 evaluable responses it is apparent that 75% of women use some form of oestrogen replacement, 62.5% are sexually active and of those 76% have significant dyspareunia, genital ulceration is present in 12.5% (and biopsy proven CGVHD in 5%).
We conclude that there is a high prevalence of sexual morbidity in women post allo-BMT and that at least 42.5% have symptoms which may be attributed to CGVHDVV.

N930

Long-term survival with GvHD; a patient’s view
A. Postma for the Patient Support Group

I'd like to share with you my history of myelodysplasia, with the emphasis on the Graft versus Host Disease and my role as a contact on the field of GVHD for the Dutch patient support group for people who had a bone marrow or stem cell transplant.

In 1993 after a long period of extreme tiredness, myelodysplasia was detected. The same year I received a T-cell depleted bone marrow transplant with the bone marrow of one of my four brothers. That was like a rebirth to me, a second life started.

Everything seemed to be okay, but within a year myelodysplasia returned. At first a specialist told me nothing could be done. Two weeks later I heard an experimental treatment with a suppletion of donor T-cells was offered. As the treatment for myelodysplasia was still experimental, it was not known how many T-cells were necessary. To be sure they gave me a lot of them. Six weeks after that the GVHD started. I received a high dosis of corticosteroids. Because I was intolerant of corticosteroid in that dose, I received a combination of cyclosporine, corticosteroid and azathioprine.

Nine years later I heard an experimental treatment with a suppletion of donor T-cells was offered. As the treatment for myelodysplasia was still experimental, it was not known how many T-cells were necessary. To be sure they gave me a lot of them. Six weeks after that the GVHD started. I received a high dosis of corticosteroids. Because I was intolerant of corticosteroid in that dose, I received a combination of cyclosporine, corticosteroid and azathioprine.

Nine years after transplant, my liver and my muscles are troubling me most. I have many handicaps in daily life. I have to arrange things in a different way. Sometimes it is still difficult to accept that I can’t do the things I did before the BMT.

I am always optimistic, I was never desperate, except when the specialist told me nothing can be done. Information of specialists was not always correct and effective.

In my presentation I like to tell about the implications of the GVHD on social and working life of myself and other members of the patient support group.

Health care is for me restricted to a few people. My family doctor can’t give me the medical aid I need, because he doesn’t understand what happened to me and what the implications are for medical treatment. The hematologue had become more important for all medical advise. I did use alternate treatment, and I believe this made me recover so well. It’s a pity that many specialists are not open to alterate therapy in addition to normal treatment.

Finally I’ll focus on the role of nurses and other persons involved in supporting care and what they did to relief the periods in hospital.

N931

Prospective assessment of quality of life (QOL) after allogeneic stem cell transplantation
S. Kondo, K. Kikuchi, T. Yashtima, M. Nishihira, I. Inaba, T. Mori, R. Watanabe, S. Okamoto (Tokyo, JP)

With an increasing number of long-term survivors after allogeneic stem cell transplantation (SCT), there have been growing interests in conducting research on their QOL. This study prospectively examined the QOL of a relatively large group of survivors after SCT. A total of 106 patients with malignant and non-malignant hematologic disorders who underwent SCT at Keio University Hospital from 1995 to January 2001 and survived without previous relapse more than 6 months were included in this study. QOL was assessed by Medical Outcome Survey-Short Form 36 (SF-36) starting at 6 months after transplantation and every 6 months thereafter. At 6 months after posttransplant, the scores of both physical and emotional domains (physical functioning PF, role physical RP, general health perception GH, social functioning SF, and role emotional RE) were significantly lower compared with those of general population. At one year, those scores remained low; however, all domains came back to the average of age-adjusted general population six months later. Of interest, two emotional domains (RE, mental health MH) exceeded the average two years after transplantation. Two physical domains (PF and SF) also exceeded the average at three years after transplantation. The presence of extensive chronic GVHD, availability of caregivers, and supports available for returning job seem to have a significant influence on posttransplant QOL. We conclude that the time since transplant has a significant influence on QOL after SCT, and those who had survived beyond 3 years are indistinguishable from normal population and significantly better in certain domains of QOL. These data may also assist transplant teams in developing strategies to support individual recipient in post-SCT period.

N932

Quality of life of the Hong Kong adult BMT survivors
J. Tai (Hong Kong, HK)

Background: In Hong Kong (H.K.), 5-year disease-free survival after bone marrow transplantation (BMT) was around 60%. However these indicators only contribute knowledge of survival after BMT but have not provided a comprehensive understanding of how this procedure affects the recipients’ quality of life (QOL).

Objectives of the study: (a) Understand the meaning of QOL to the local BMT survivors, (b) Identify the effect as well as the positive and negative impact of BMT in the local BMT survivors, and (c) Explore their lived experience after BMT and the perceived quality.

Methodology: Qualitative phenomenological design was adopted and 12 survivors were invited in the semi-structured interview. Content analysis was used to analyze the data, and informants’ check was used to validate the identified categories and themes.

Results: QOL was meant in terms of being satisfied (67%), being normal (50%), being healthy (42%), being sustained (42%), being free (33%), being functional (33%), being independently (25%), and being together (25%). However their perception was not as expected for there were more negative impacts of BMT identified.

Nevertheless 92% of the survivors were still satisfied with their lives after transplant. The reason was that they have underwent a process of adjustment and adaptation which were illustrated by the categories of confronting and enduring, seeking and connecting, struggling and balancing, and transcending and replenishing. This phenomenon enlighten an existed relationship between treatment and effects and this required the survivors’ perception of illness and its treatment, expectation of self, and appraisal of risk or harm. Another distinguished phenomenon was that the local survivors apart usual coping strategies, used also culture specific ones to promote their well-being such as intake of herbs or tonics, practiced qiqong, thought positively, letting go etc. Overall their lived experiences after BMT was a process to “rebirth”.

Conclusion: The study results captured the local survivors' meaning of QOL, the effect of BMT, their lived experience after BMT and the perceived quality. Knowledge generated not only shed light on the understanding that the impact of the BMT experience permeates all aspects of one’s life, but also serves as a basis for the health care professionals especially nurses to initiate interventions to improve their QOL.

N933

The role of the nurse in decision making about whether or not patient’s treatment ought to be continued
G. Dijker van Schie, A. v.d. Akker, R. Kok, T. de Vries (Leiden, NL)

Introduction: The haematology and bone marrow transplant unit, Hebe, is staffed by specialist nurses. Intensive care of haematology patients is carried out in a uniform way and according to protocol. Less clear is the role of the nurse in decision making about treatment of seriously ill patients with little chance of recovery, in which the interests of the patient are of prime importance. This is an aspect which lies within the domain of nursing ethics, defined by Arend as “the consideration
of responsible action while carrying out the professional duties of a nurse”.

The situation at present: In principle, a patient will have the ultimate say in decisions concerning his life and therefore also about his treatment. On the basis of clear and comprehensible information he can come to a decision (informed consent). There are situations in which the patient is unable to play a part in the decision-making process about treatment. The doctor may offer alternative or further treatment. The nurse, who has been closely involved in day-to-day care for the patient and who therefore knows him well, is in some cases in possession of information which would lead to a different decision being made. This information is not taken sufficiently into consideration in the decision-making process. It is evident that, based on the professional nursing code, professional profile, job description and ethics, a nurse has a specific role to play when decisions have to be made about treatment.

The following points are essential if the nurse is to play a sufficiently important role in decisions about treatment:
The nurse must be aware of his or her role and responsibilities on the basis of job description, professional code and ethics;
The nurse must possess communication skills which will enable her to take an equal part in discussions with the doctor;
The nurse must document fully all information concerning the feelings and explicit wishes of the patient;
The doctor must be aware that the specific knowledge and information possessed by the nurse are of great importance in the decision-making process.

Based on legal, organisational and ethical arguments, a plan has been agreed that a teaching video should be developed specifically to aid health-care professionals to address sexuality issues. The questionnaire contained quantitative and qualitative questions ranging from 2 -15 years, completed a questionnaire.

Factors identified as hindering discussion included:
- Lack of time
- Lack of knowledge

Findings: Nurses recognised their role in supporting clients with cancer facing sexuality/fertility concerns. In both studies the findings were similar.

Sexuality

N935

Do barriers still exist in communicating sexuality issues with patients?

M. Brown (London, UK)

Sexuality has long been recognised as an important aspect of quality of life (Ferrel, 1995). Hughes (1996) goes further, adding “…it is an integral part of the healing process”. Nursing research has indicated the importance of nurses having an in-depth understanding of issues related to sexuality. In particular, the need to have the skills, competence and confidence to address patients’ concerns or access to a subject specialist.

Following an EBMT joint session in Hamburg on sexuality, it was agreed that a teaching video should be developed specifically designed to aid health-care professionals to address sexuality issues with patients. To ensure core issues of this teaching aid were covered it was decided that a pilot study be conducted to identify those concerns most relevant to nurses. Using convenience sampling 30 haematology course nurses, working in varied clinical settings, from medical/oncology-haematology wards to specialist BMT units with experience in haematology ranging from 2-15 years, completed a questionnaire. The questionnaire contained quantitative and qualitative questions in areas such as: definition of sexuality, optimum methods of approaching the subject and identification of barriers and methods to improve communication.

All the nurses studied highlighted the need for training as critical, particularly in areas around fertility options and interpersonal dynamics. Factors identified as hindering discussion included: cultural issues, age, experience, lack of time and knowledge. The development of an educational video for addressing such concerns in addressing sexuality issues was deemed essential to assist nurses in particular to address this traditionally sensitive subject.

N936

Listening to patients’ concerns regarding sexuality and fertility on a BMT unit

A. Stokes, E. Hogbin (London, High Wycombe, Bucks, UK)

Background: It was reported that a growing population of patients were voicing questions and concerns regarding sexuality in post treatment clinics. Reasons for this were investigated through local studies (Quinn & Kelly 2000), and an extensive literature review. It was evident that health care professionals (HCP) found difficulty in addressing patients and partners sexuality and fertility concerns (Guthrie 1999, Williams et al 1986). This audit was part of an ongoing practice review to find out the patients’ perspective in the light of the above studies.

Aim: Accepting this premise, that staff find it difficult to talk with patients about sexuality and fertility concerns, the aim was to investigate by audit the patients’ perspective.

Methods: A questionnaire was distributed to 40 patients, 20 of whom were new to the Unit and 20 patients approached in the Out-patient setting who had completed treatment.

Findings: The findings show that patients were not receiving consistent and accurate information. At times they were unaware of the effect their disease and treatment were having on their sexuality. It was also found that patients and their partners were keen to have more information and support in order to understand sexuality changes related to disease and treatment. These views mirrored the comments of the studies above.

Practice Development: A booklet was designed, to be distributed to patients within the Unit.

*To ensure patients are aware of HCP support in addressing sexuality and fertility concerns.
*To offer practical advice during and after treatment.
*To guide and help patients and their partners to access further help and support.
*To help nurses in broaching the subject.

Conclusion: An audit cycle was implemented to prove the findings from studies and literature reviews were accurate. The results highlighted the need for a form of reference for patients; a booklet seemed the ideal solution. The next stage of the cycle will be to re-audit the usefulness of the booklet in six months.

N937

Promoting sexual health in cancer care

B. Quinn (London, UK)

Background: Clinical practice developments were initiated following two studies among nurses, fertility staff and doctors in a London teaching hospital, addressing the nurses’ role in supporting clients with cancer facing sexuality/fertility concerns.

Studies: The first study based on a Medical Oncology Unit focussed on finding out what was available to clients. Who addressed sexuality/fertility issues? And particularly nurses’ experiences and suggestions. Findings were received through unstructured interviews and written comments.

The second study took place a year later and was carried out on a Bone Marrow Transplant Unit. This study involved nurses answering a questionnaire about their role in addressing clients’ sexual and fertility needs. Medical staff where asked to describe their clinical practice. In both studies the findings were similar.

Findings: Nurses recognised their role in supporting clients and partners with sexual/fertility concerns. Medical and fertility clinic staff believed nurses had an important role to play. Nurses were requested to address sexuality/fertility concerns but often avoided the issue.

The reasons cited were:

-Lack of experience.
- Not knowing what support was available.
- Lack of time and privacy.
- Patients’ and nurses’ embarrassment.
- Fear of making mistakes.
- Cultural, gender and age differences.
- Myths/prejudices/social rules.
- ‘Opening up more than you can handle’.

The findings reflect the findings found in other studies (Wilson & Williams 1988, Smith 1989, Webb 1993, Hughes 1996).

The following practice developments were initiated:
- Clinical guidelines to guide and support practice.
- Study days for all relevant hospital personnel.
- Regular Unit based teaching sessions.
- ‘Link Nurse’ for sexuality/fertility concerns on each ward.
- Closer liaison between nursing, medical and fertility staff.
- Pamphlet to offer practical guidance for clients/partners.

Conclusion: Following the above studies and practice developments, the sexual and fertility concerns of clients and partners has an increased profile across the hospital. An ongoing review of clients’/partners’ views, show that while practice has improved patient care, further developments are needed to help staff to address these issues appropriately, accurately and sensitively.

Poster Session Nurses Group

P939
Oral care in patients receiving hemopoietic stem cell transplantation
Y. Li, P. Wei (GuangZhou, CHN)

Objectives: Oral mucositis is very vulnerable in patients receiving hemopoietic stem cell transplantation. The oral care play an important role in daily care for patients in cleaning room. Systemic oral care strategy was applied in patients receiving hemopoietic stem cell transplantation to see if the oral mucositis can be controlled to the minimum extent.

Methods: 31 patient aged 13 to 42 were included, in whom 19 receiving bone marrow transplant and 12 receiving peripheral blood stem cell. The onset, severity, duration and pathogen of oral mucositis were documented and the severity was graded according to WHO criteria. Special attention was paid in different stages of peri-transplantation period. In brief, the odontolith was cleaned out completely and the primary oral disease was treated before entering the cleaning room. In the cleaning room, the patients were encouraged to drink more water than usual, the frequency of gargle increase from 6 per day previously to 1 per hour in daytime and every 4 hours at night in this studying period. The solutions for gargle included normal saline, vitamin B2, natrium hydrocarbonate, 1-3% hydrogen peroxid, nystafungin, while the two groups.

Results: The results indicated that the oral mucositis occurred in 25 patients (80.6%), and the affected sites, in descending sequence were bucca (52%), tongue (40%), lips (36%), gingival (28%), and palatum (20%). The severity of the oral mucositis was grade 0 in 6 (19.4%), grade 1 in 17 (54.8%), grade II in 4 (12.9%), grade III in 3 (9.7%), grade IV in 1 (3.1%). Culture of pathogen showed all of the microorganisms were habitual bacteria. All patients recovered from this complication in an average of 6 days (range 4 to 12 days), and none of the patients developed general infection with the oral origin.

Conclusion: Oral mucositis is a common complication in patients with hemopoietic stem cell transplantation. With systemic oral care, although the incidence is still very high, the severity and duration oral mucositis tends to be reduced, which is certainly in favor of the success of transplantation.

P940
Infection rates in patients using laminar air flow room vs portable isolator after allogeneic stem cell transplantation
S.J Kim, Y.H Choi, M.K Hong (Seoul, KOR)

Background: Laminar air flow (LAF) rooms are widely used to prevent infection after allogeneic stem cell transplantation. However, they are quite expensive and limited in supply and portable isolator are proposed as an alternative to LAF rooms.

Patients: Infection rates were compared between 18 patients transplanted in LAF rooms and another 18 patients transplanted in portable isolators. All the patients were given allogeneic stem cell transplantation from HLA-matched sibling donors for hematologic malignancies.

Methods: Nursing flow sheets based on WHO infection guidelines were used. Ciprofloxacin, fluconazole and sulfamethoxazole trimethoprim were given for infection prophylaxis.

Results: Demographic and clinical characteristics such as age, gender, diagnosis, HLA match, conditioning regimens and occurrence rates of GVHD were not different between the two groups. There were no differences in the total duration of admission, LAF room or portable isolator stay, duration of fever or amount of administered antibiotics between the two groups. Isolates of pathogenic micro-organism were also similar between the two groups. Only duration of G-CSF use was longer in patients using the portable isolator (p=0.04).

Conclusion: There were no differences in infection rates and severity in patients using LAF rooms or portable isolators for allogeneic stem cell transplantation.

P941
Bone marrow transplantation for sickle-cell syndromes dramatically improves patients’ quality of life

Patients with sickle beta-thalassemia are heterozygous for HbS and beta-thalassemia and have a variable clinical phenotype. Most of them require regular transfusions or exchange transfusions to treat anemia and prevent sickling phenomena and their clinical manifestations: painful crises, seizures or strokes, acute chest syndrome, osteonecrosis. Despite proper treatment, severely affected patients suffer from many of those adverse events, some of which leave permanent sequelae, compromising patient life quality and expectancy. In our Unit, two children with severe sickle-beta thalassemia have been treated with BMT. They were both 14 year old, transfusion dependent and very frequently hospitalized because of painful and hemolytic crises, osteonecroses, acute chest syndrome, infections, seizures and spleen sequestration crises. One of them had also Hodgkin’s lymphoma in 1st CR. Upon their arrival at the Unit both children were anemic, jaundiced, underweight with thalassemic facial changes. One of them was limping due to osteomyelitis of the knee. Both failed to attend school regularly and they were anxious, depressed and frustrated.

The patients had an HLA identical Bone Marrow graft from a sibling donor. Post transplantation course was uneventful and they restored a normal donor-type hemopoiesis. Now, 8 an 16 months post BMT, both children are in excellent clinical condition with no GVHD. Their physical appearance and nutritional status is significantly improved. They go to school and have a normal social life. Additionally they feel safe, healthy and optimistic for their future.

Bone marrow transplantation for sickle-cell syndromes, in addition to offer cure to some genetic diseases can dramatically improve the quality of life of the most severely affected patients.
The quality of life for patients after hematopoietic stem cell transplantation
B. Song, J. Bok, K. Park, K. Kim, E. Hong, Y. Bae, Y. Ro

Objectives: 3,820 patients underwent hematopoietic stem cell transplantation (HSCT) from 1983 to 2001 in Korea with increasing cases and survival rate. At the same time, there is an increasing interest in quality of life (QOL) after HSCT patients because toxicities of conditioning regimens and complications after HSCT such as GVHD, pneumonia, or infection affect negatively QOL after HSCT patients. For these reasons we studied QOL as an outcome variable in post-HSCT patients.

Subjects and methods: The subjects for this study were 208 patients who had experienced HSCT at Catholic Medical University Youido St Mary's hospital Hematopoietic stem cell transplantation center in Korea from December 2000 to February 2001. QOL was measured with modified the City of Hope Medical Center’s Quality of Life tool for bone marrow transplant survivors. Data were analyzed with SAS program for descriptive statistics, t-test, ANOVA, and Duncans multiple range test.

Results: The mean of QOL score was 369.9. The highest QOL score was observed in Physical well-being, followed by Spiritual well-being, Psychosocial well-being, and Social concern. The study showed higher score of QOL in those who younger than 30, those who more than 1 year after HSCT, and those who received HSCT younger than 30. Major variables explaining 8.25% affecting QOL after HSCT were age, religion, diagnosis, and gender. Physical well being is higher in male patients. Psychosocial well being is higher in those who have hobby, and spiritual well being is higher in male and those who have religion.

Conclusions: We speculated QOL after HSCT patients and concluded that post-HSCT QOL score was high. We have to focus on post-HSCT patient’s spiritual well being, psychosocial well being and social concern because the score of physical well being was considerably high but other QOL scores were relatively low. The early HSCT is important in order to improve post-HSCT QOL because those who receiving HSCT younger had higher post-HSCT QOL score.

Nonmyeloablative stem cell transplantation in the outpatient setting
B. Smithers, M. Adamson, M. Murphy, O. Gilligan, M. Garg

Non-myeloablative stem cell transplantation (SCT) offers new hope to high risk patients who are not eligible for conventional BMT because of age, co-morbid conditions and previous transplant procedures. It is a new form of treatment that is less myeloablative than a standard allogeneic transplant and therefore it can be carried out in the outpatient setting. However the large number of eligible patients and increased pre and post transplant support has implications for nursing practice in this setting with regard to development of new patient guidelines, protocols and changing working practices.

This paper describes the nursing experience for non-myeloablative BMT in the outpatient setting from a single centre. Over a three year period from 1998 to 2000, 15 mini SCT were carried out, 5 of which were matched unrelated donor transplants and the rest were matched sibling transplants. The haematological conditions treated included acute myeloid leukaemia, chronic myeloid leukaemia, myelodysplasia and multiple myeloma. All patients were conditioned using Fludarabine, Busulphan and Campath, apart from the patients with myeloma who received Melphalan instead of Busulphan. Two transplants were undertaken entirely in the outpatient setting, with 5 patients requiring short hospital admission due to the adverse effects and toxicity of Campath. G-CSF was given post transplant to accelerate granulocyte recovery. The period of neutropenia was therefore short but much care and support were required to manage the immunosuppressive effects of the treatment as an outpatient. In order to facilitate this procedure four key nursing areas were highlighted - patient information, monitoring, teaching and patient support. We have shown that by identifying and addressing these issues this procedure can be carried out successfully in the outpatient setting.

Nursing care workload and nonmyeloablative conditioning regimen for bone marrow transplantation
J. Claissse, K. Tabani, C. Chadelat, E. Gluckman

Background: the practice of bone marrow transplantation (BMT) is evolving with the use of non-myeloablative conditioning regimens (NMCR). Here, we discuss the necessary evolution of nursing care practices.

Methods: we describe the case of a patient undergoing a genoidentical BMT for multiple myeloma after a NMCR. After a short and uncomplicated initial hospitalization, the patient had to be readmitted several times due to either graft versus host disease or infectious complications. We evaluated the nursing care workload during those periods, based on a previously described score. We compared this score to the one evaluated in a previous study of a genoidentical BMT after a classical conditioning regimen.

Results: after the NMCR BMT, the mean score reached 2.77 as compared to 3.05 for the BMT after a classical conditioning regimen. Although the values seem to be similar, the distribution was different depending upon the conditioning regimen. The initial score was very low after NMCR, increasing dramatically during more frequent rehospitalizations for severe complications.

Conclusion: evolution of BMT practice using NMCR induces a modification in the management of the nursing care. The availability of structures adapted to frequent rehospitalizations is of a crucial necessity to achieve adequate nursing care for those patients.

Benchmarking in discharge planning from pediatric BMT units
K. Newton, J. Stone for the Paediatric BMT Nurses Group, PONF

Discharging children from a paediatric bone marrow transplant unit is a complex process for both health care professionals and families. Discussions amongst nursing staff working in units around the United Kingdom indicated that differing discharge information was being given to families, depending on the unit that they had been cared for during their transplant. However, the same fundamental information was being given to all families across the UK.

In order to try to identify the best practice in discharge planning and information giving, it was decided to benchmark discharge planning from paediatric bone marrow transplant units in the UK. It was hoped that the benchmarks would provide a framework to build upon and a tool to determine progress within and across the units (Pantall, 2001). It was felt that benchmarks would also allow current nursing practice and roles to be explored and areas for future development in the units would be highlighted, potentially improving the care for the patient and the child and family. Using a collaborative approach nurses working across the UK have produced two benchmarks; the co-ordination of the discharge process from the paediatric BMT unit by an identified team member and the specific written BMT discharge information available. These two benchmarks are currently being used throughout the paediatric bone marrow transplant units in the UK.
outlining the process of creating such benchmarks, exploring practical issues in bringing about national changes to clinical practice and will evaluate the effectiveness of the benchmarks themselves in the assessment and development of nursing practice. Reference: Pantall, J (2001) Benchmarking in healthcare. NT Research 6 (2), 568-580.

P946

Prophylactic nebulised liposomal amphotericin-B in pediatric stem cell transplantation

Pulmonary fungal infection is a serious opportunistic infection, occurs in immunosuppressed patients who had stem cell transplantation (SCT). The treatment of pulmonary fungal infection is difficult, time consuming and very expensive. Amphotericin B is an important drug in the treatment of serious pulmonary infections but its use is limited because of renal toxicity. The route of entry for many pulmonary fungal infections is by inhalation. Prophylactic aerosol treatment with liposomal AmB were effective and without side effects. We started this study in order to evaluate the efficacy of amphotericin-B inhalation in the prevention of pulmonary fungal infections. Between February 1998 and August 2001, 46 pediatric patients (24MLD, 6 ALL, 4FAA, 2SAA, 2NHL, 3 Neuroblastoma, 2MDS, 2thalassemia, 2 metabolic dis) with a median age of 11.5 years received amphotericin-B inhalation prophylactically (Group I) Medic AID Porta-Neb compressor and ventstream set is used for 5 mg liposomal amphotericin-B (diluted with 3 ml distilled water) inhalation for 15 minutes and 2 times in a day. In this way amphotericin-B particles of 2-5 micron size is dispersed to the smallest airways and alveolus. Another 15 pediatric patients treated with similar chemotherapy protocols with a median age of 10.5 years didn’t receive prophilactic amphotericin-B inhalation (Group II). 2/46 patients in Group I had pulmonary fungal infection while 10/15 patients in Group II had pulmonary fungal infection. One patient in each group died with pulmonary infection (Acremonium). Patients were treated with intravenous amphotericin-B in the suspect of fungal infection (severe mucositis, oesophagitis, febrile neutropenia, continuing fever, pulmonary infection resistant to treatment). Patients in group I were administered amphotericin-B inhalation for 52 (17-250) days and intravenously for 40 (10-120) days. In group II, seven patients who developed fungal infection received amphotericin-B intravenously for 80 (16-180) days and also amphotericin-B inhalation for 90 (16-180) days. Group I patients were neutropenic with a median of 20 (10-64) days while Group II patients had a neutropenic median period of 27 (10-60) days. As a result we can say that for the prevention of pulmonary fungal infections, amphotericin-B inhalation administration is an effective prophylactic therapy.

P947

The role of the bone marrow transplant nurse co-ordinator: a multi-center study
S. Crook for the UK Pediatric BMT Nurses Group

Bone Marrow transplantation is a highly specialised area of paediatric nursing as it is a complex and intensive treatment with significant short and long-term implications. The process places enormous demands upon the child and family and part of the role of the BMTC must extend to providing support to families to enable them to successfully care for themselves and their child.

The role of the BMT Co-ordinator has developed and evolved over a number of years requiring a nurse with specialist skills, the ability to understand and respond to the technological changes in treatment, whilst providing emotional support and advice. In addition, many nurses also contribute to the wider nursing agenda.

As this role has developed in different ways over the years a working party was established within the UK Paediatric BMT Nurses Group to undertake an evaluative study.

The Working Party aimed to map the disparate nature of the role, identify the core elements of the post and gain insight into the needs of parents of BMT patients in relation to the service provided by the nurse co-ordinators.

In order to meet the first objective BMT Nurse co-ordinators from 6 of centres agreed to complete a diary, outlining all activities undertaken during a working week. A questionnaire for parents was designed and piloted. Once finalised 10 questionnaires were sent to each of the BMT Nurses in the 6 participating centres for distribution. Each nurse gained permission to approach those parents with children being treated their centres, who agreed to participate in the study.

Questions posed related to parental experience and expectation of the BMT process and the role of the BMT co-ordinator in relation to information and support.

The aim of this paper is to share the findings and experiences of undertaking a multi-centre study of this kind.

P948

“Time out” during isolation for children in the BMT unit
S. Geck, R. Sauer, B. Kremens, O. Basu (Essen, D)

We would like to present different ways to alleviate a child’s life in isolation when facing a bone marrow transplantation. In our transplantation center the children spend an average time of six to ten weeks confined to a LAF unit struggling between life and death. During this time they are isolated and live on few square meters. Their social contacts are restricted, apart from the hospital staff only parents are allowed to visit the young patients. A child’s imagination allows a certain amount of freedom which we want to support and enlarge by different arrangements. In our ward we have therefore established several facilities to make the time of loneliness and fear more enjoyable and to allow the children to stay in touch with the world outside of the hospital:
- animation by clowns of the hospital, once/week
- motivation by games and craftwork with an educator, 3x/week
- daily lessons with school teachers
- in half of the rooms computer with internet, web cam and microphone to communicate with friends and home school and to work with educational programs; qualified personnel gives technical instruction
- entertainment by hospital staff (reading tales, talking about the child’s problems)
- TV and video in each room with a collection of movies suitable for children

This variety of opportunities requires a close cooperation between different occupations and an individual relation to the patient. Thus, children can make new experiences and pass by the time in the BMT unit more quickly. The continuing contact with the world outside and the possibility to keep up with the development of their healthy friends and peers should help the children to reintegrate into normal life after transplantation.

P949

Putting partnership into practice
G. Bott (Pendlebury, Manchester, UK)

Metachromatic leukodystrophy (MLD) is a rare progressive neurodegenerative disease. The late infantile form is usually recognised in the second year of life and is fatal in early childhood. There is no specific treatment for MLD. Bone marrow transplantation has been introduced as a therapeutic option in pre-symptomatic patients with the late infantile form of MLD. Presently it is unknown whether BMT in this stage ameliorates the course of the disease. The uncertainty of the outcome for these patients produces a significant amount of anxiety for the parents.
of these children undergoing BMT and it is often the nurse who provides the emotional support and education to help the family. Laura was diagnosed with late infantile MLD six weeks after birth. Testing occurred due to a family history of MLD. At the time of her diagnosis, Laura’s older sibling was exhibiting clinical manifestations typical of stage III in the disease process. Laura’s sibling was cared for at home by her parents, in conjunction with a community nurse.

Laura’s parents were well informed about MLD and had a sound knowledge of research and literature relating to the disease. Indeed, following her admission for BMT after HLH testing, it became apparent that they were better informed about MLD than the nursing staff on the BMTU. However, lack of parental knowledge regarding BMT and Laura’s impending future also needed to be addressed.

Sharing of information between the two parties was established. Due to active participation in the care of their older child, Laura’s parents expressed a need to be involved actively with her care. This presentation will demonstrate the close partnership which developed between nursing staff and Laura’s parents throughout her bone marrow transplant leading to a review of current practice. As a result of this unusually close working relationship, it became evident that a family-centred nursing model was not in place on the BMTU. Consequently, a nurses working party was established to develop a model which focuses on working in partnership with parents in caring for their child.

P950
Job description of the SCT-assistant in pediatric hematology/oncology
S. Wider, U. Konrny, A. Peters, B. Strahm, C.M. Niemeyer
(Freiburg, D)

In August 1999 a new position for a SCT-assistant was created in the Department of Pediatric Hematology and Oncology. The SCT service consists of 15 beds including 2 LAF rooms. About 20 allogeneic SCTs are being performed per year. The purpose of this new position was to assure the quality of SCT in the field of nursing and organization. The requirements for the position were at least two years of job experience in the field of SCT. One task of the SCT-assistant is to instruct patients and their parents with regards to hygiene and nutrition during SCT. In addition, families are regularly addressed for questions. Specifically, when changes in the patient’s care have to be implemented the SCT-assistant communicates all necessary details. For other pediatric oncology nurses seminars are being conducted on a regular basis. Special training by the SCT-assistant is given to nurses, when they enter the field of SCT for the first time. Furthermore, nursing standards are developed and constantly screened for revisions. In the working field of the physician, the SCT-assistant takes over the organization of diagnostic tests. Daily routine procedures such as blood drawing and sending off diagnostic specimen are being conducted. In order to guarantee the follow-up of discharged patients appointments in the SCT day clinic and out-patient department are made. Two years after introduction of the pilot-program of a SCT-assistant, this position has been proved to be a major support for the area of SCT. With organizing and coordinating tasks being held by the SCT-coordinator, continuity in patient care is guaranteed and the interaction of different care takers has been improved, thereby assuring the quality of our work.

P951
“G-CSF alone” is a mobilization therapy as effective as “CTX 4 gr/m2 plus G-CSF” in lymphoma patients not heavily pretreated - A prospective study

Comparison of PBSC mobilization with “G-CSF alone” and “Cyclophosphamide + G-CSF” has not given in Lymphoma patients univocal results. For this reason we conducted a prospective study in 40 patients affected with Hodgkin’s or Non-Hodgkin’s Lymphomas. Patients were divided in two groups: 20 pts were mobilized using G-CSF alone (10 mg/Kg/die subcutaneously from day +1 to the end of harvesting) and 20 pts were mobilized using CTX (4 g/m2 day +1) + G-CSF (10 mg/Kg/die subcutaneously from day +5 to the end of harvesting). Results were analyzed stratifying patients according to number of previous lines of chemotherapy received (< o = 2 lines vs >2 lines).

In the whole population of patients and in the stratum of patients not heavily pretreated the group mobilized with G-CSF alone obtained in peripheral blood a mean CD34+ cells peak not different than the group mobilized with CTX + G-CSF (p = 0.6) while in patients heavily pretreated the group mobilized with CTX + G-CSF obtained a CD34+ peak significantly higher than the group mobilized with G-CSF alone (p = 0.04).

The percent of patients that reached a CD34+ peak superior of 20 x 10^6/L and therefore underwent harvesting of PBSC in these two treatment groups (CTX + G-CSF and G-CSF alone) was not different when results were analyzed in the whole population of patients and also when only the stratum of not heavily pretreated patients was considered. However the percent of successful mobilization was higher in patients who received “CTX + G-CSF” when only heavily pretreated patients were considered (p = 0.03).

A multivariate analysis showed that age (p = 0.02) and mobilization with CTX + G-CSF (p = 0.04) were important factors in order to obtain a PB CD34+ peak superior to the mean.

Our study shows that in patients affected with Lymphomas and not heavily pretreated “G-CSF alone” has the same mobilizing capacity of “CTX + G-CSF”. Considering the lower costs and lower morbidity of G-CSF alone, this treatment represents an useful choice for PBSC mobilization in Lymphomas.

P952
PBSC harvest, via Quinton type central venous access, results in improved CD34+ yield with greater patient comfort
E. Rom, O. Daniel (Tel Aviv, IL)

One of the central problems in facilitating peripheral blood stem cells (PBSC) collection is that of adequate access. This problem is compounded in the cancer population due to heavy chemotherapy treatment pre-collection, resulting in more debilitated patients with decreased peripheral venous access.

Methods: PBST collection was performed via central venous catheters (CVC) in 49 patients, using Cobe Spectra AutoPBSC Cell Separator. Management and data collection was supervised by a team consisting of an anesthesiologist, a specialized pheresis technician and a coordinating nurse who together followed the patient through the process and monitored results and complications.

Results: We performed 73 procedures in 49 patients, 24 with lymphoma, 17 with Multiple Myeloma, 5 with AML and 3 with other malignancies. Average patient age was 49 years (range 20-60). The median number of collections per patient was 1.7 (range 1-4), with flow rate of 120 ml/min (range 90-130). The total blood volume processed was 4 TBV with median duration of pheresis of 3 hours and 25 min. The average number of mononuclear cells harvested was 6.5 x 10^6/kg (range 1.5-17.5 x 10^6/kg), and that of CD34+ cells was 6 x 10^6/kg (range 0.7-33.5 x 10^6/kg). There were no significant complications except one mislocation of
the CVC, and two patients who had local oozing at the insertion site.

Conclusion: Our experience suggests that use of the CVC access leads to excellent stem cell collection product with minimal complications, and more rapid pheresis time, providing an environment of decreased stress and a more positive pre-transplant experience.

P953

Preparation, collection and utilization of peripheral blood stem cells for children in the department of oncology and hematology, in a Finnish university hospital
K.-M. Lähteenoja, M. Ekholm, I. Piirto, K. Liljeström (Helsinki, FIN)

We have collected for 66 children with body weight between 9 and 77 kilos peripheral blood stem cells (PBSC). PBSC are collected by apheresis generally after high dose cyclophosphamide followed by human colony stimulating factor.

Thorough information about the procedure is given to the child and parents prior to the collection of PBSC. The collection is made with central venous catheter and on occasions with peripheral venous catheters. Sedation or anaesthesia is provided when needed.

PBSC are collected with continuous flow blood separator (Cobe Spectra). Collection time is approximately two to three hours on one to two consecutive days. The aim is to collect 20 x 10^6 CD34 cells/kg.

The procedure is controlled by manual adaptation of blood rates to improve the quality of nucleated cells. A selection of CD34 cells is made with the use of Cell Pro and later Cline Macs (Miltenyi). PBSC are cryopreserved in liquid nitrogen usually in two separate bags. The aim is to cryopreserve for each patient a sufficient amount of CD34 cells for two occasions and a small number of cells for stem cell culture.

After myeloablative therapy, patient is reinfused with PBSC and treated in a heparinfiltered room with positive air pressure until hematopoietic recovery. During the reinfusion of PBSC patient is receiving standard IV fluids in her other central venous catheter.

The use of stem cell rescue is a useful alternative to bone marrow transplantation for children as the hematopoietic recovery occurs two to three days earlier than with bone marrow transplantation and both the volumes of the transplant and DMSO are smaller.

All patients had hematopoietic recovery 7-10 days post PBSC reinfusion.

P955

Campath by subcutaneous injection instead of ATG infusion as part of reduced intensity conditioning for allogeneic transplantation - from a nurse’s point of view
U. Frödin, G. Julisson (Linköping, S)

It’s well known that ATG (antithymoglobulin) gives side effect when given as an intravenous infusion. The common side effects are fever, shivering, nausea, vomiting and headache, despite premedication with steroids, paracetamol and clemastin. The side effects usually start on the first day of ATG administration, and are very distressing for the patient.

For the nurse it takes a lot of time, because she must stay with the patient under the infusion.

For some nurses it can also be stressful to give the ATG infusion, because of the patient’s expected reaction. In our clinic we now use a protocol for allogenic stem cells transplantation with an adjusted conditioning, focusing on reducing side effects but with a retained antitumor effect. One part of the study is to evaluate the acute toxicity from subcutaneous injections of Campath. The subcutaneous route of administration may reduce toxicity and simplify administration. Campath is available at the concentration of 10 mg/mL, and our schedule includes 30 mg daily for 3 days. One ml solution is given at each injection site. The nurse gives premedication with betamethasone and clemastin to the patient before the injections, and then she gives Campath subcutaneously on three different sites on the abdomen. The nurse checks the blood pressure, pulse, and skin reaction during and after the injections. From September 2001 through November 2001 we have included 8 patients into this protocol. Until now the results show that subcutaneous Campath is easier to administer and it saves time, as compared to ATG infusions.

Our conclusion is that subcutaneous Campath injection is to prefer on short term, because of the lack of clinically significant side effects, and the easy handling for the nurses.

P956

Research nurse’s role in the treatment of advanced pancreas cancer with metastasis
S. Gini, S. Barbieri, M. Canepa for the EBMT Italian Nurses Group

Pancreas cancer is the 5th death cause in oncology. Most of the patient affected by the advanced stage of this cancer can’t undergo any surgical operation and there isn’t an efficacious one on old treatment.

Some clinical trials stated that Gemcitabine could have positive effects in those patients, so a trial started: the protocol is Gemcitabine (intravenous prolonged infusion)+ stem cells rescue + G-CSF administration.

The aims of this trial are:
- to evaluate the effectiveness of the treatment (the number of partial/complete answers)
- to evaluate the maximum dose of Gemcitabine in association with peripheral stem cells rescue and administration of G-CSF.

Even if research nurse is a member of the trial team and works together with main investigator, chemist, pharmacology expert, data manager, surgeon etc., his/her role is very important for a good execution of a trial.

In this case research nurse works in three main phases:
- Management (includes evaluation of the protocol, contacts with chemist, knowledge of appropriate administration of the drugs, involvement of the clinical staff)
- Information (correct information to the patient to get a real informed consent, and to ensure the protection of patient’s privacy and rights)
- Execution (the whole treatment must follow the research protocol, side effects or toxicity symptoms have to be reported)

The research nurse work has a positive effect on research quality. He/she must carefully integrate the requirement of trial and specific needs of the patient in the trial.

P957

Continuous parenteral infusion of anti viral agents in the home environment - A feasible and cost-effective alternative to hospitalization
F. Belal, V. Frauche, S. Angevin, V. Piquet (Villejuif, F)

Purpose: Medico economic considerations together with the lack of a national network for domiciliary care, led us to reconsider cost effective alternatives to hospitalisation for haematology patients with out compromising continuity, security and overall quality of care. A structured collaboration between the unit and freelance domiciliary nurses has evolved, enabling the continuous infusion treatment of anti viral agents in post transplantation patients, in the home environment.

Method: In 2000 a collaboration was initiated between the hospital haematology unit and freelance domiciliary nurses working in the suburbs of Paris and surrounding provinces. The different actors and their roles in this collaboration were:
1. The haematology medical and nursing unit: writing of procedures, protocols, treatment plans: selection criteria for suitable patients, telephone follow up of patients.
3. Home Care Support Services: provision of material, ambulatory pumps, supervision of nurse training in manipulation of material.
4. Hospital Ambulatory Day unit/Emergency Dept: indirectly concerned: provision of 24h medical and nursing back up in the event of problems.
5. Hospital pharmacy: Suitability, stability of agents for continuous infusion, type of material.
7. Local laboratory for blood analyses.

The first treatment course is administered in hospital and the domiciliary nurse comes in to meet the patient and the unit domiciliary nurse team, the patient nursing/medical file and treatment care plan is common to all participants, thus facilitating access to information between the different members of the team.

Results: After a feasibility study of 1 year, nurses have performed over 300 treatments including initial chemotherapy without significant incidents or accidents. We extend our collaboration to include the follow up of: patients in aplasia after chemotherapy for peripheral stem cell mobilisation: biological and clinical follow up post autologous transplantation (Day 1 to 5) after stem cell perfusion + Melphalan; parenteral nutrition from Day 16 onwards if necessary and 2 patients will also be established to improve pain control in the home environment. An evaluation of patients' and nurses' satisfaction is now underway.

Conclusion: The continuous infusion of anti-viral agents in the home by freelance domiciliary nurses is a feasible alternative to domiciliary nursing and the ward, thus improving the continuity of care. Overall the scheme has been very successful and enables us to provide a more integrated service.

P950

Implementation of a rotational post between a ward and a day unit on a hematology and bone marrow transplant unit

E. Bruce (Cambridge, UK)

The haematology and bone marrow transplant unit at Addenbrookes consists of a 14 bedded ward and a day unit in the same geographical area. Continuing care is provided for patients in between courses of high dose chemotherapy, and the role of the day unit is therefore functionally different from follow up care provided by the haematology out patients clinic.

The day unit previously operated as an independent unit from the ward with 2 staff employed solely for the day unit. This provided a number of advantages: district hospitals knew specifically who to liaise with and close relationships were built between patients and staff, enabling individualised, holistic care to be provided.

However there were also several disadvantages. Ward staff covered the unit during holidays and sickness and were often unfamiliar with specific procedures and how the unit operated, and patients became reliant on specific staff members.

In order to address these shortfalls a rotational post was introduced. The unit now has a permanent co-ordinator with an E grade staff nurse from the ward rotating into the unit every 8 weeks.

This has provided several benefits. The permanent co-ordinator provides continuity and stability within the unit, acts as a resource for colleagues, and ensures good communication with surrounding hospitals is maintained.

Opportunities for the ward rotating staff are plentiful. Management skills are developed through working more autonomously and working as part of a wider team; practical skills such as venepuncture and cannulation are gained and consolidated; a broader overview of out patient care is gained through attending clinics; and there are opportunities to observe and participate in care and procedures uncommon to inpatients e.g. apheresis, outpatient chemotherapy protocols etc.

More importantly, our patients benefit from meeting ward staff either before admission or after discharge and also from the improved communication that now exists between the day unit and the ward, thus improving the continuity of care.

P960

Mini-transplantation at Helsinki University Central Hospital

R. Keskimäki, S. Haavisto, M. Pyykkö (Helsinki, FIN)

Mini-transplantation means stem cell transplantation (SCT) with reduced intensity conditioning but on the other hand a very intensive immune suppression treatment afterwards. This kind of conditioning (Fludarabine 25 mg/m2 i.v. daily during three days and one 2 Gy total body irradiation) does not need hospitalization of the patient but there must be a well organised out-patient system in the transplantation clinic to care the patients. Before transplantation the patient visits the doctor of the out-patient clinic for a medical examination. The doctor of the out-patient clinic evaluates the patients condition and if it is good enough SCT can be carried out as out-patient treatment. A week before the SCT the patient visits again the doctor and the nurse and instructions particularly about the importance of taking fluids are given. During days -4 - -2 to SCT Fludarabine is infused, blood tests are taken and instructions about medication are repeated. In the morning of SCT-day the patient gets first TBI and then is the graft infused and after a follow-up of one day the patient leaves the out-patient unit. If the distance between home and hospital is short enough it is possible to undergo the treatment living at home or otherwise the patient will stay at the apartment-hotel. At the beginning of year 2001 two flats with bathroom were renovated for our unit at the apartment-hotel for patients undergoing mini-transplantation. The flats are well equipped. During office hours the out-patient clinic has the responsibility of the patient and during the weekends, nights and in case of emergency the patient contacts the staff of the haematological ward. Therefore it is very important to share exact information on the patient between these two units. At the beginning the patient visits our clinic twice a week. After the first month they visit once a week for two months and then if everything is okay the visits are less frequently. Therefore only have experiences from 12 out-patient mini-transplantation during one year it has become clear that the patients prefer out-patient treatment to hospitalization.

P961

Development of a nurse-led pre-transplant clinic

K. Midgley, R. Copland (Leeds, UK)

Nurses have taken a leading role in important areas of transplant research including care procedures, quality of life and survivorship issues (Whedon & Wujick, 1997). The special needs of patients and families are well documented, but research suggests that patients are often dissatisfied with communication often not understanding or forgetting what they have been told and written information continues to be produced in language too difficult for its intended audience (Ley, 1982; Teasdale, 1993). Thus the challenge remains for nurses to provide information for their patients and their families that they need without overwhelming them or scaring them in the process (Morra, 1998). In the recent UK Government papers Making a Difference (1999) and The NHS Plan (2001) key areas have been identified for innovation and reform. One such area is the identification of ways of working for nurses to improve patient care, standards and quality and includes the management of patient caseloads and running of clinics. Recent innovative practice has seen the development of nurse led clinics in various areas of practice, thus a business plan was formulated to introduce a nurse led pre-transplant clinic to facilitate practice and improves patients' experience of transplantation. The poster describes the implementation and evaluation of the pilot study of this clinic.
P962

How can we guarantee that patients get the correct information on care during stem cell transplantation?

H. Ikäheimo, N. Pietilä, T. Seppänen-Savela (Helsinki, FIN)

Nurses have closer relationship with patients than other healthcare professionals and this puts them in a unique position to assess patients’ needs for education and information. Continuous relationship with patients enable nurses to establish a partnership situation where a patient and a nurse together can plan the coming care. In our haematological ward patients are treated with autologous and allogeneic stem cell transplantation (SCT). These treatments are demanding with many special features. It is important for a patient to be very well involved and informed of the coming treatment. We developed in our ward the guidance program for autologous SCT patients. It is self-limiting as patients are not given the need for the nurse. We believed that certain patient information needs had not been produced by nurses and covered what the nurses felt the patient information needs. Historically, patient information had been produced by nurses and covered what the nurses felt the patient information needs. We believed that certain patient information needs were not being adequately addressed using this approach but needed to establish what these were.

The audit design was a retrospective, anonymous questionnaire over a one month period. Questionnaires were given to 20 patients that had undergone at least one course of high dose chemotherapy and subsequently experienced side effects. The questionnaire comprised of closed-ended questions to make the findings easier to interpret. There was also a section for comments. According to the results of the audit it would appear that our pre chemotherapy information was flawless! The patients did not cite any omissions in the information they were given and did not suggest any areas for improvement. This led us to question our method of data collection. We presented our lack of findings to the Research and Development Group within the unit for comment. A unanimous conclusion that this is how not to conduct a patient centred audit was made for the following reasons:

- The audit design was self-limiting as patients were not given enough scope to express their real needs.
- There may have been reluctance on the part of the patient to criticise care whilst they were still undergoing treatment, fearing that what they say may have had adverse effects on future care.
- The questionnaire was non-specific and needed to ask more direct questions, possibly covering one side effect at a time and using more open-ended questions.
- The fact that the chemotherapy team conducted the audit themselves may also have put patients off being honest in fear of affecting rapport with the team.

The lack of usable results indicates that data collection methods for this style of audit need to be carefully considered. Poor data collection methods can invalidate a study as useful data cannot be obtained. Any further audit should consider:

- An informal interview approach rather than a questionnaire.
- A sample of patients that have completed their chemotherapy so that no perceived threat to care is posed.

P964

Impact of full time research nursing team on the utility of the questionnaire method of prospective data collection

D. Heming (Sutton, UK)

The Royal Marsden Hospital NHS Trust, Leukaemia and Myeloma Unit receives 100-150 new referrals each year. It was found that compliance with Clinical Trials in this patient group is 86%, highlighted following an audit in 2000 within the Leukaemia/Myeloma Unit (Fletcher 2000). The aim was to evaluate if the availability of a dedicated nursing research team affected patient compliance with prospective data collection.

The evaluation was based on questionnaire data collected for a GCP standard Phase II trial (Ethics no 1871), to compare the quality of life on PEG Intron and Intron A in myeloma patients. This will be measured by the EORTC QLQ-C30 and QLQ-MY24 questionnaires.

Patients completed questionnaires unaided to remove investigator bias. A total of 60 patients will be recruited and trial period is 24 weeks for each patient. This report is based on the first 30 patients who had completed the 24-week study period by November 2001. Questionnaires were administered at initiation of study, 12 weeks and 24 weeks. At this point 87 questionnaires have been administered (3 withdrawn pre 12 week crossover requiring 2 not 3).

87 questionnaires were returned (100%). Of a total of 4698 answers, 102 were unanswered (98% compliance). Completion time 2-30 mins (median 7.28 mins).

The minimum and the maximum time showed no difference in the number of unanswered questions (all questions answered for both times). The least answered question receiving 65 responses of not applicable related to hair loss. As the average time for Interferon therapy for this group is equal to 3.38 years, this question would be irrelevant to the majority.

By having a full time skilled research team compliance with returned questionnaires can be 100%. This is a positive evaluation in clinical trials, especially where quality of life is the primary endpoint. However there is still an aspect of missing data due to unanswered questions.

This report confirms the importance of patient education to maximise the collection of data.

P965

Information and communication at the isolation ward of an university hospital: the Basel experience

C. Pino Molina (Basel, CH)

Present situation: Information is of central importance not only to the patient but to the whole care team at the Isolation Ward. When an illness is diagnosed, patients and relatives are inundated with information. They feel overwhelmed, confused, lose interest and become mistrustful. This prevents them from digesting the information constructively.

Aim: We assume that well informed patients and relatives are more cooperative, less anxious and uncertain and that they feel taken seriously. They therefore generally feel better. A continuous flow of information is vital to the care team.

Methods: The poster displays many details about the patient work that are readily available to the team. It shows the professional methods with which the flow of information is organised and maintained.

Results: Information flowing between patient and care team:
- Daily talks between patient and team member, during care and treatment, during medical visits and examinations.
- Patients and relatives should systematically be provided with relevant information.
- Our information should be systematic, easily understandable, to-the-point, up-to-date and complete.
- For this purpose information brochures were compiled at entry and discharge. The brochure "Wegweiser (Guide)" is in modules and can be put together for each patient individually. Upon discharge a detailed brochure dealing with essential questions is presented to patients. Both brochures were compiled from various disciplines and evaluated with patients and relatives.
- Existing methods of collecting and distributing information are applied systematically. These comprise a first care talk, daily talks, patient's medical history and talks before, during and after the hospital stay as well as psychosomatic (and spiritual) talks. These discussions create a flow of information to and from the patient.
- Good information, even bad news, is indispensable when coming to terms with and overcoming an illness and improves the life quality of both patients and relatives.
- Diverse information brochures are directly available. Information flow within the care team:
  - Relevant data on the patient must be collected and placed at the disposal of the whole care team.
  - Daily care and medical reports and long, weekly, interdisciplinary team reports should help to maintain the information flow. With regard to the latter, observations and assessments on each patient are exchanged between nursing staff, doctors, psychologist and pastor. The topic "contact to relatives" is discussed for each patient.
  - Team reports serve to provide an expert consensus if problems arise with unstable data.

How to construct a new BMT ward

B. Birkenau, W. Kail, P. Hoffelner, C. Peters (Vienna, A)

Within the past years the improvement of the supportive therapy and the introduction of less toxic conditioning has led to a considerable extension of the indication for stem cell transplantation. To be able to account for a rising demand in hospital beds, it was decided in 1999 to build a new transplantation ward. The new ward was to be built on the roof of the old one. For reasons of time and space, it was not possible to close the old ward while the construction work for the new ward was going on; work had to be continued during the entire time of the construction work.

Together with the co-operative management, the architect and the builders a list of hygiene measures were worked out and written down to help ensure optimal protection for our patients, their relatives and our staff during the construction works. Although a number of precautions against dust and noise were taken, such as the construction of dust shelters and a tunnel, the stress generated by noise and the problems produced by dust were enormous during the summer of 2000. The carrying out of works which produced extreme noise were arranged to be limited to certain times of the day.

However, even despite accurate planning and supervision, a number of unplanned, and partly grotesque incidents occurred, which could only be managed by efforts of all people involved.

The construction works for the new ward were finished in June 2001, after only 16 months. Moving from the old to the new ward was an extreme burden for all nurses involved, since a few final works had to be carried out while the rooms were already prepared for patients to move in. Our first patient to use one of the new sterile compartments moved in on the 25th of June 2001. From the nurses' point of view this new ward is the best prerequisite for up-to-date care of patients receiving stem cell transplantation. The physical closeness to the new oncological intensive care unit, and the new bridge with glass roofing that connects the new ward to the oncological units allow a perfect cooperation between the individual wards.

Unfortunately, the nurses' increased work load caused by the additional beds in the new ward has not been taken into account yet. Nevertheless, the optimal employment of all resources available, as well as structural changes in the ward's daily routine made it possible for the nurses to start work at the new ward at full capacity immediately after its opening.

Oral care guidelines

A. Molcanova, D. Zappova, P. Cetkovsky (Prague, CZ)

Backgrounds: Oropharyngeal mucositis is one of the major side effects of the chemo/radio therapy. This painful complication leads to inadequate oral intake with early administration of systemic opioids. Damage to the mucous membranes permits invasion by endogenous microflora and subsequent local and systemic infection in most patients. As the therapy of the mucositis is not possible, prophylaxis with routine systemic oral care is crucial in order to prevent or to mitigate incidence and severity of conditioning-induced (primary) changes as well as infectious (secondary) complications. Oral care is a basic nursing responsibility and so we present here our new "Oral care guidelines".

1. Brushing:
   a) patients should brush teeth using appropriate soft tooth brush after every meal and at bedtime (ultrasoft toothbrush should be used during neutropenic and thrombocytopenic period and when evidence of irritation is present)
   b) fluoride containing toothpaste is preferred.

2. Rinsing:
   a) patients should rinse mouth every hour during day with either saline or mixture of saline and sodium bicarbonate (these solutions should be alternated)
   b) mouth rinses containing irritants (e.g. alcohol, hydrogen peroxide, chlorhexidine, etc) should generally be avoided.

3. Denture Care:
   a) denture can be soaked in an antiseptic solution (especially during night)
   b) denture should be used during aplasia only while eating
   c) patients should avoid wearing dentures during period of mucositis.

4. If mucositis is diagnosed and when brushing is not possible
   a) debris on teeth and tongue should be removed using saline and bicarbonate soaked gauze (toothettes are ineffective)
   b) rinsing of mouth with saline/bicarbonate solution should be continued.
   c) topical or usually systemic analgesis is necessary according to institutional protocol.

5. Products that can discolor teeth and tongue should be avoided.

6. In the case of secondary complications: topical antifungal agents could be used simultaneously with systemic administration of antimycotics, other secondary infections are topically treated with oral hygiene only (without local application of antibiotics, etc).

7. Oral assessment, oral dysfuction/dyscomfort score and oral care given should be documented at nurses part of patient’s documentation.

Conclusions: Systematic assessment and prophylaxis with routine systemic oral care according to strict guidelines play a pivotal role in the care about this cohort of patient.

Who cares for aftercare?

M. de Groot, S. Harmens, M. de Wit (Leiden, NL)

During the prolonged admission for children undergoing BMT relatively much attention is paid to the delivery of psychosocial care to the children and parents. Two nurses are assigned as mentors who regularly have evaluation sessions to assess the experiences of child and parents during the admission and assess if and how issues require changing.

What however occurs after discharge?
A task-group "Aftercare" was formed which was aimed at assessing whether the care provided to children and parents after discharge from the hospital was adequately organized, whether there was a need for more/different care and if the hospital was in a position to contribute. Prior to this a policy of phoning parents in the first week after discharge, which was experienced as very positive.

In collaboration with the Social Services a small scale study was carried out amongst a group of 20 parents in which, by means of an enquiry based on interviews with parents, the possible need for more/different care was reviewed. This pilot study has as yet not been concluded. Parents interviewed so far have indicated that care provided up to now, is sufficient.

Many studies have been conducted into the provision of care for parents of deceased children. For these, amongst others, support group meetings have been organized. Parents have indicated they are very satisfied with these arrangements.

P969
How are you? Quality of life among Danish allogeneic BMT patients
H. Laursen, A. Berthelsen, H. Borup, J. Holder, T. Lanther, C. Ruus, M. Sander (Copenhagen, DK)

The purpose of this study is to investigate how allogeneic BMT patients describe their overall quality of life (QoL). Methods: A post enquete survey was given to 224 BMT survivors, all in remission and without secondary malignancies. The questionnaire was modified form Bardell et. al.’s (BMT1998, suppl2p.68-71) investigation and adjusted to Danish conditions.

Results: This survey is an ongoing study and the results are preliminary. 138 of 224 possible questionnaries was answered(62%). Patients consisted of 51% female and 49% male, aged 18-63 yr. (mean:38 y). The patients was transplanted from 1980-2001, mean observation time of 8 y. from transplant. More than 90% was transplanted for a malignant disease. At the moment 72% of the patients are working or undergoing education. Patients indicated that their QoL was: very well 41%, well 38%, average 18%, poor 2% and very poor 2%. The patients were asked if they thought they had fewer, similar or more problems compared to a non-transplant person of the same age and sex. App. 60% answered that they had the same problems as others. In relation to physical appearance 28%, sleep 33%, illness 38%, insurance 54% and leisure activities 35% indicated more problems than others. In relation to siblings 24%, parents 20% and freinds 19% reported fewer problems. We asked the patients to rate their QoL according to the Karnofsky score, leaving out the lowest class categories. Overall 72% stated that they were able to live a normal life, but 34% had some difficulties, 15% answered that they were unable to live a normal life and needed some assistance or professional help. The patients were asked to state their greatest joys in life and the answers was categorized into issues. "Relationship with family and friends" 64%, "breeing well, healty and leukemia-free" 27%, others 9%. Regarding major concerns in life the patients answered: "health/relaps" 45%, "family" 26%, others 29%.

Conclusion: Overall 75% of the patients appears to be able to live a normal life with few complaints. It appears that app. 25% of the patients continue to have problems many years post transplant, but <5% indicated that they have a poor QoL. Very few patients mentioned GvHD or any other postransplant complications as their major concerns.

P970
Sexuality: A neglected aspect of care in hematological nursing
L. Brennan, N. O Sullivan (Dublin, IRL)

Recent studies of cancer survivors indicate that one of the most negative influences on social well being is the change in sexuality that health professionals fail to address (Hughes 1996, Anderson 1998, Frost et al 1997). By virtue of the intimate nature of the nurse-patient relationship, many nurses are ideally placed to discuss sexuality with their patients. Yet, in practice sexuality is not given the attention it deserves. The purpose of this presentation is to explore why this neglect has occurred

P971
Adherence of cyclosporin A to central line catheters results in increased blood levels long after switching to oral therapy in pediatric patients
A. Peles Bortz, R. Gez, J. Stein, I. Yaniv (Petach Tikva, IL)

The adherence of Cyclosporin A (CsA) to indwelling catheters can lead to misreading of blood CsA levels during monitoring. To determine if this factor continues to be important after the patient is switched to oral CsA, we measured blood CsA levels in samples drawn from the line previously used for CsA infusion and from a different line. The study population consisted of six children (age 1 month to 12 years) treated with CsA after bone marrow transplantation who were switched from the intravenous to the oral route. Evaluations were made 2 to 25 days after oral administration was initiated.

Mean CsA level was 354 ng/ml in blood drawn from the central (CsA) line and 174 ng/ml in blood drawn at the same time from the other line (p<0.001). The difference in CsA levels between two lines diminished with time, but even after 25 days of oral CsA treatment, it remained statistically significant. These results emphasize the need to always use the same line for CsA administration and to take blood samples from a different line even long after the patient is switched to oral CsA therapy.

P972
A comparative study examining the accuracy of Tempadot thermometers with mercury and electronic thermometers
J. Walker (Newcastle upon Tyne, UK)

Introduction: Accurate temperature measurement is a critical component of detecting infection in the child undergoing bone marrow transplant. As risks associated with mercury/glass thermometers become more widely documented, our unit decided to explore more suitable alternatives. On our unit mercury/glass thermometers are used for the children with immunological disorders and small handheld electronic thermometers are used for the children with haematological and oncological disorders. The axillary site is always used.

Methodology: 6 children took part in the study with ages ranging from 2 weeks to 8 years old. 286 Tempadot readings were taken, 172 mercury/glass readings and 114 electronic readings.

Results: 7 temperature readings were the same on both the Tempadots and the electronic thermometers. 15 temperature readings were the same on both the Tempadots and the mercury/glass thermometers. The Tempadot readings ranged from 2.2 degrees centigrade greater than to 2.1 degrees centigrade less than, when compared to the mercury and electronic readings. The overall average Tempadot reading was 0.32 degrees centigrade greater than, when compared to the mercury and electronic readings. 61% of Tempadot readings...
were between 0.5 degrees centigrade higher or lower than the mercury and electronic readings. Tempa-dot readings showed an extra 41 pyrexial episodes (37.5 degrees centigrade or above) and failed to show 11 pyrexial episodes.

Conclusion: On evaluation (questionnaires and group discussion), staff found the Tempa-dots easy and convenient to use, 84% of staff found the Tempa-dots easy to read and the elimination of the risk associated with mercury spilt a further bonus. The only disappointing disadvantage appeared to be the inaccuracy. The results demonstrate that this product is ineffective for our use on this particular unit. The search for a more suitable alternative to the glass/mercury thermometer continues.

P973
Implementing allogenenic BMT(allo BMT) on a pediatric intensive care unit
L. Hvidt (Copenhagen, DK)

When a child needs to be treated with allo BMT it presents a complicated nursing challenge and even more so, if the need arises to implement the procedure in a pediatric intensive care unit. Six major areas need special attention from the transplant team:

1. Ensure a safe and germfree environment for the child and parents during the period of cyclophosphamide.
2. Ensure that nursing staff and other health professionals understands the need for special precautions, when caring for a child undergoing allo BMT.
3. Decide which precautions should differ from those applying to other immum suppressed children in the unit, making sure that the necessary facilities are available.
4. Teaching parents and child hygienic precautions relating to allo BMT. That includes the need for special prepared food, accepted behaviour in the BMT room and precautions regarding personal belongings and toys for the child or parents wants to bring with into the BMT room.
5. Caring for the child and parents in a safe and germfree environment, and at the same time making sure that their emotional and social needs are fulfilled.
6. Ensure appropriate training for new staff members both theoretically and as bedside teaching. Ensure that older staff members keeps their knowledge up to date.

When allo BMT takes place in a pediatric intensive care unit, the nursing staff also cares for other very sick children with complexed healthproblems. As a result of that, it becomes very important that nursing staff are kept up to date on their nursing skills in relation to allo BMT. The only way to do that successfully is to have every nursing procedure written down. A flowchart on procedures were every day was prepared before the first patient was admitted to the unit. It becomes very important that the transplant team keeps evaluating the procedures and makes sure that adjustments are made in such a way that all relevant health professionals are kept informed. To ensure a safe environment for the allo BMT patient, also when the unit is busy and there is a nursing shortage, the flowchart on all daily nursing procedures becomes a life safer.

P975
Improving discharge planning on a bone marrow transplant ward
J. Foulston, J. Pearse (Cambridge, UK)
The BMT Unit at Addenbrookes is a 13 bedded ward catering for up to 8 transplant patients at any one time. It was felt that some patients on discharge following BMT were not receiving optimal support from community services. Investigation revealed that many staff members were not aware of the extent of the services available or how to contact the agencies providing support. This was despite a considerable amount of information being available. The problem arose because the information had not been organised into a single readily accessible source and staff did not have time to carry out lengthy searches. It also seemed that the existing discharge documentation was not always being used to the best advantage. To remedy this, examples of completed discharge documentation were brought together with sources of information on community support services in a “Discharge Planning Information File”. This file deals with matters such as how referrals to other District Hospitals should be carried out; the procedures to be followed when making referrals to District Nurses, Macmillian Nurses and local authority social services. It includes checklists for ensuring that outpatient clinic appointments have been made and patient transport requirements considered. Lists of contacts and a Directory of all District Nurses in England are also incorporated.

At present most of the detailed information contained in the file is relevant only to the Cambridge area with outline information included for other areas, although it is hoped to expand this at a later date.

It is also intended to appoint a Discharge Facilitator to the ward.

P976
Preceptorship in the HOPE Directorate, St James's Hospital
R. Wilkinson, C. Healy (Dublin, IRL)

Current trends in healthcare such as staff shortages can help create a rapid turnover of staff (Meng&Conti 85). Additionally the impending relocation of schools of nursing away from hospital sites within Ireland have helped to move the ideology of preceptorship programmes for newly employed and newly qualified nurses from the “nice to have” category into the “must have” category. Evidence of same is tangible as nurses indicate that the availability of such initiatives can influence the choosing of a job offer (Coates&Gormely 96).

Within the nursing profession preceptorship endeavours to help bridge the gap between education and practice (Usher et al 99). It also provides a fixed and limited amount of time in which a person usually a staff nurse acts as a tutor, role model, motivator, counsellor and supporter of the individual (Morrow 84). In doing so this approach offers one option for the integration of nurses into their new role (Westra & Graziano 92).

The role of preceptorship has been reported on at several levels. For example Crawford et.al. (2000) report on its use with baccalaureate student nurses, and Dusmohamed and Guscott(97) report on preceptorships use in instigating change initiatives within rural healthcare settings. Cummore(1999) in an anecdotal article discussing her feelings on taking up a new post claims that doing so can unearth of a myriad of emotions. These may range from excitement, as one starts a new stage in ones personal or practice competencies and ones personal integrity may be critically questioned. Staff on Denis Burkitt Unit, St. James's Hospital, a busy 17 beded Haematology / Bone Marrow Transplant Unit, recognised such anxieties and further more realized that these could be further compounded by the intensive nature of care that is practiced on the unit. A period of preceptorship was suggested by staff on the unit for new recruits.

In this poster presentation the author shall portray the local preceptorship initiative in The HOPE Directorate, St. James's Hospital including:

- The Preceptors Bill of Rights
- Suggested role description for the preceptor
- Objectives for the preceptee
- Benefits to the preceptor and preceptee
- Hints on giving feed back
- Suggested structure for preceptorship period
- The preceptorship documentation
- The realities of the situation
- Plans for the future.
The study was performed on two different stem-cell-transplantation designs. The central question of this study was: which of the two dressings is superior to protect the implantation site? Subquestions concerned the patient satisfaction with either one and which dressing should be preferred from the economic point of view. Data were collected in two German university hospitals. All patients had either a Hickman- or Groshong-catheter for a highly myeloablative therapy including SCT mainly for a hematological disease. The following 5 criteria: local infection, local allergic reaction, secretion of pus, leukocyte value in the peripheral blood and once a week a culture of the exit site was taken.

Data of 53 (out of 66) patients could be considered for statistical analysis. The mean time of implantation was 85 days. The results show significantly more advantages in favor of the hydrocolloid-dressing regarding local infection as well as allergic reactions. But we found a higher level of contaminations with bacteria under the hydrocolloid-dressing without an infection of the catheter itself. Based on this study we have changed the standard care for CVC at our institute and now we are using hydrocolloid dressings.

In this poster we would like to discuss advantages and disadvantages of both dressings based on data generated from our study.

A comparison was done between the Cutiplast® (7 x 5 cm) and the hydrocolloid-dressing Comfeel transparent plus® (7,5 x 5 cm). The positive effect of hydrocolloid-dressings on wound-healing is well known and evidence can be found in the literature. Whether this effect can also be observed in the care of central venous catheters (CVC), is neither fully understood nor published. Only a few observations from clinical practice show a positive influence on the rate of infection and therefore a longer time of implantation of the cathetersystems. A waste amount of nursing care for a patient in the neutropenia phase of the treatment aim at the prevention of infections. Taking this fact into account, we looked at the option of using hydrocolloid dressings for CVC’s for neutropenic patients as new standard-dressing.

An open, controlled, prospective, randomized, multicenter study design was chosen to compare two dressings for the care of CVC. The study was performed on two different stem-cell-transplantation (SCT) units in Germany during a period of almost two years.
The true incidence of opioid side effects experienced by patients receiving morphine during a BMT is unknown, but all experienced clinicians working in this area will encounter patients for whom nausea and vomiting or impairment of cognitive function are disabling adverse effects which fail to settle in apparent steady state or with a change in the rate of drug delivery. This patient group may be unable to tolerate oral medications because of treatment related mucositis, and, because of renal impairment, maybe unable to tolerate the dose escalation of diamorphine necessary for adequate analgesia. We therefore decided to look at the role of Fentanyl intravenous infusion as a suitable alternative.

Fentanyl is a synthetic phenyl piperidine derivative and a chemical congener of the reversed ester of meperidine. Its pharmacological effects are similar to morphine and meperidine but is 50-100 times as potent to morphine on a weight basis. (Calis et al, 1992). It's shorter duration of action, 30-60 minutes are a single intravenous dose of 100 mcgs (AHFS drug information 1994), means that continuous infusion is required in chronic pain management. There are no reports in the literature documenting improvement in the side effect profile when switching from morphine to Fentanyl and yet it is well tolerated. There are, however, anecdotal reports of IV Fentanyl being used successfully with this patient group as an alternative to morphine/diamorphine.

We therefore undertook a prospective audit of the use of IV Fentanyl as first line alternative parenteral strong opioid for patients undergoing BMT in our unit. This presentation will discuss the role of Fentanyl and our experience in this group of patients.

P982
Nursing implications of somnolence post allogeneic hematopoetic stem cell transplant (AHSCST) with total body irradiation (TBI) conditioning
J. Brennan (Sutton, UK)

The aim of this analysis was to evaluate the problem of post TBI somnolence and its implications for the nursing management. 74 patients (M:39, F:35;median age:38yr., range:14-56) who received AHSCST for haematological malignancies (Ac. Leuk:45, Chr.Leuk:9, Other:20) with TBI conditioning from 1999-2001 were evaluated for the incidence of TBI somnolence. Donor was HLA identical sibling (52), MUD (20) or family member (2) and CyA+Mtx was GVHD prophylaxis in all patients. Source of stem cells was BM (18) or PBSC (56) and 36 patients had good risk and 38 had poor risk disease. Dose of TBI was 9.5 Gy single fraction (n=53) or 12 Gy in 6 fractions (n=21). 15 patients received cranial top-up radiation. Post transplant 29 patients (39%) developed somnolence at a median of 58d (range:34-110d). Cumulative incidence of TBI somnolence was 56% (+7% SE) at 100 days. Incidence of somnolence was not related to patient sex (F:11/35,M:18/39, p=0.19), donor sex (F:11/35, M:18/39, p=0.19), dose or fractions of TBI conditioning from 1999-2001 were evaluated for the incidence of TBI somnolence. Donor was HLA identical sibling (52), MUD (20) or family member (2) and CyA+Mtx was GVHD prophylaxis in all patients. Source of stem cells was BM (18) or PBSC (56) and 36 patients had good risk and 38 had poor risk disease. Dose of TBI was 9.5 Gy single fraction (n=53) or 12 Gy in 6 fractions (n=21). 15 patients received cranial top-up radiation. Post transplant 29 patients (39%) developed somnolence at a median of 58d (range:34-110d). Cumulative incidence of TBI somnolence was 56% (+7% SE) at 100 days. Incidence of somnolence was not related to patient sex (F:11/35,M:18/39, p=0.19) donor sex (F:11/35, M:18/39, p=0.19), age , primary diagnosis (p=0.24), risk category (Good:16/36 Poor:13/25, p=0.37), source of stem cells (BM:4/18, PBSC:25/56, p=0.09), dose or fractions of TBI(9.5Gy: 21/52, 12 Gy: 8/21, p=0.9), cranial top-up (yes:8/15, no:21/59, p=0.21) or occurrence of AGVHD (yes:26/66, no:3/8, p=0.92). Incidence was significantly lower in female patients receiving stem cells from female donors (3/17 vs. 26/57, p=0.038). Patients with TBI somnolence had borderline significantly higher creatinine levels on day 58 post allograft (134 vs.108, p=0.047) possibly due to dehydration. In all patients somnolence recovered without any specific therapy apart from management of hydration status. In conclusion, somnolence post TBI is common. The risk seems to be lower in female patients receiving stem cells from female donors. Somnolence post TBI allograft is a self limiting process and its occurrence does not result in inferior survival. Proper nursing management and maintenance of hydration are important during this phase.

P983
Nausea and vomiting experienced by patients undergoing peripheral blood stem cell transplant (PBSCT) treatment
S. Wheatley (Bristol, UK)

Nausea and vomiting are two common and distressing side effects experienced by patients undergoing autograft (Grundy, 2000; Walsh et al, 1997). Poor management of these symptoms can lead to a range of both physical and psychological problems, not least malnutrition, dehydration, chemical imbalances and low morale (Grundy, 2000).

To achieve optimal management of nausea and vomiting a structured approach has been adopted by the Avon Haematology Unit. It involves both the use of an 'anti-emetic' protocol and involvement of a multi-disciplinary team approach.

This poster presentation shows the result of the recent audit undertaken to investigate compliance to the anti-emetic protocol and highlights the use of alternative therapies. Overall, compliance to the protocol was good, but other interventions were evident. The most frequently used 'non-protocol' drugs were Methotrineprazinene (Noziminan) and Cyclizine, in cases of persistent nausea and vomiting. Alternative therapies were also shown to be helpful, for example spiritual healing and crystal therapy. This suggests that a third line of anti-emetics would be useful as a holistic approach to nausea and vomiting may prove beneficial. Grundy, M et al (2000) 'Nursing in Haematological Oncology' London: Bailliere Tindall Walsh, M et al (1997) 'Watson's Clinical Nursing and Related Sciences' 5th Ed London: Bailliere Tindall

P984
Overview of the Swiss stem cell transplantation centers

Switzerland has SCT-programs since 1973. Since then the care for the Patient and his family develops rapidly and many efforts are undertaken to improve and consolidate good quality care. There are eight Stem Cell Centres in Switzerland, located in:

- Aarau,
- Basel,
- Bern,
- Bellinzona,
- Geneva,
- Lausanne,
- St. Gallen,
- Zürich.

Those eight centres are offering excellent modern nursing care and treatment facilities. All centres provide care for patients undergoing treatment for Leukaemia and other haematological malignancies. Bone Marrow (BMT) as well as peripheral Stem Cell transplantations (PSCT) are performed.

Children are transplanted in Zürich, Geneva and Basel. Analysing the questionnaires an analysis was made on actual handling and protocols of different nursing-acts for the SCT-patient and his family. The data shows clearly the differences in the Swiss SCT-Units. One of the main obstacles in Switzerland for the collaboration among the centres are the four languages that are spoken, namely:

- German,
- French,
- Italian,
- Retro-Romancan.

English is not spoken at all and only a few nurses read and write English fluently. Also different health care issues play a role in different centres, so cooperation between sites show some difficulties that we are working on at the very moment.
Conclusion: We think in addition to all the difficulties that the Swiss nurses face, it will be necessary to work together on national and international level, to share experience and various knowledge and expertise.

**P985**

**Advanced training courses for nurses in hematology, the development of a program for nursing education based on clinical practice**

*M.L. Ekestrom, J. Larsen, E. Hellstrom-Lindberg (Stockholm, S)*

A programme for advanced training courses in nursing care related to haematological disease and treatment has been initiated by nurses at the department of Haematology, Huddinge University Hospital, Stockholm, Sweden in co-operation with Karolinska Institutet. The aims of the programme are to give nurses a deeper knowledge in nursing care related to haematology, prepare them for a clinical career development and create links between clinical nursing, nursing education, nursing development work and nursing research. The programme started in spring 1999 and is divided into four different courses. The course participants attend the courses one at the time, and in preferred order. Each course is equivalent to five weeks of full-time studies.

2. Nursing care related to Chemotherapy
3. Palliative and Psychosocial Nursing care
4. Nursing care related to transfusion therapy and stem cell transplantation management.

Each course has been given once yearly and nurses from different parts of Sweden have attended. In order to facilitate participation we now use WEBB-based programme for distance-education. The evaluations from the programme show that the courses are much appreciated. Today we collaborate with the Swedish Association for Haematologic Nurses (HEMSIS) in an effort to develop advanced courses on Master of Science level - aiming towards a national education programme for clinical Nurse Specialist in haematological nursing.

**P986**

**Early mortality causes after hematopoietic stem cell transplantation in Iran**

*S. Khalilvand, M. Hosseinzadeh, F. Tahsili, M. Golipour, M. Hadadi (Tehran, IR)*

Introduction: Allogeneic and autologus BMT/ PBSC is curative for almost 50% of patients but these procedures especially allo –graft is associated with high risk of treatment related mortality. We have studied 510 pts who received transplant between 1989- 2001 in Shariati hospital of Tehran to identify and analyze the major causes of early mortality under 100 day after BMT. The nursing management plays an important role in preventing some of these adverse events.

Methods: Patients who received transplant were allo -graft 383 pts, auto graft 133 pts and syngeneic 4 pts. Conditioning was without TBI.

Results: 64 pts (12.5%) died before day 100 of BMT. The main cause of death in these pts were infection 11, GVHD 18, hepatic failure and VOD 4, ARDS 9, multi organ failure 2, TTP/HUS 3, drug toxicity 3, heart failure 2, GVHD and bleeding 5, renal failure 1, relapse of primary disease 6. Hence, it shows that 28.1% deaths were due to GVHD and 17.1% due to infection, 28.1% due to organ failure, 7% due to relapse of primary disease and finally 11% due to TTP/HUS and bleeding.

Conclusion: Nursing role is important in: early recognition of signs of infection, good fluid management, early identification of GVHD, prevention of bleeding and qualified nursing management will be complementary to medical management in preventing these life-threatening complications.

**P987**

**Nursing care in veno-occlusive disease**

*A. Moosavi, A. Nejati (Tehran, IR)*

Veno-Occlusive Disease (VOD) is defined as a clinical syndrome of liver enlargement and pain, fluid retention and weight gain, and elevated serum billirubin concentration that follows cytoreductive therapy, in the absence of other explanations for these signs and symptoms.

From June 1997 till August 2001, 230 patients (pts) (Thalassemia, AML, Fanconi anemia, CML, Aplastic anemia and Hodgkin) have undergone transplantation. For early diagnosis and prevention of VOD development, we measured waist circumference of the pts (from+1 to +20) twice a day and their weights 4 times a day (during hospitalization). Serum billirubin and liver function tests (LFT) of all the pts were checked two times a week. Prophylactic albumin was administered (if serum total protein <6 gm/dl) and prophylactic heparin was infused 100U/Kg/24hr for all of the pts. 31 out of the 230 pts (M/F: 15/16, mean age: 17.5 yrs, range: 2.5-53 yr) developed VOD. Malignant disease was (45.5%) and non-malignant disease was (54.8%). Sex of donors were matched with pts in (34.5%) and mismatched in (45.5%). Types of transplantation were allogeneic (93.5%) and autologus (6.5%). The sources of stem cell were bone marrow (9.6%) and peripheral blood (90.4%). HLA typing of the pts full matched (79.3%) and partially match (20.7%). Donors blood group were compatible in (69%), major incompatible (13.8%) and minor incompatible (17.2%). Pretransplantation LFT of the pts were normal in 87.1% and were upper limit of normal in 12.9%. Date of onset of VOD development was in the first three weeks (83.9%) and from +21 (16.1%). Acute GVHD developed in 20% of the pts (grade I: 24.1%, grade II: 20.7%, grade III: 3.4%, grade IV: 20.7%). Mortality rate was 23.3% and causes of death were CMV, GVHD, VOD, sepsis and ARDS respectively. Elevation of serum billirubin, SGOT, SGPT and alkaline phosphatase were detected in 87.1%, 41.9%, 71% and 12.9% of our pts respectively. Hepatomegaly, weight gain, increased waist circumference, ascites and edema were observed in 64.5%, 93.9%, 35.5%, 38.7% and 25.8% of VOD pts respectively. Albumin and diuretic were administered for treatment of these pts.

VOD is one of the most important hepatic complications of HSCT and early diagnosis of this syndrome can reduce the mortality rate. Differentiation of VOD from other hepatic complication such as hepatic GVHD, viral infection, cholestasis due to cyclosporin and TPN–induced liver injury is critical and sometimes difficult in these pts.

**P988**

**Small survey on research activities of nurses within EBMT in 1995-2000**

*E. Bystricka, S. Vokurka, V. Koza (Pilsen, CZ)*

Research is considered to be one of many other important activities of professional nurses. Experiences and observations gained in any research can help improving quality of nursing and general education of the staff. In an attempt to describe current status of research activities in transplant-medicine nursing we evaluated characteristics of presentations registered for EBMT- LFT Annual Meetings in 1996-2000. There were 480 presentations registered in that period there. It was possible to define several main topic groups: - Psychosocial topics, communications and quality of life: 153x (32%). – Management and standards of care: 121x (25%). - Side-effects care: 63x (13%). - CVC care and related problems: 41x (9%). -Infections: 36x (8%). - BM and PSC donorship and apheresis: 35x (7%). - Case reports and special topics: 31x (6%). There were 24 observations compared with control groups, 8 studies were prospective and randomized, we have not found any multicenter randomized prospective study. To evaluate situation in the Czech Republic we revised nurses research activities registered for our Annual Haematology Meetings in Olomouc in 1998-2000. There were 38 presentations.
registered there, with psychosocial and organization of care topics counting more than a half of all the presentations. There was organized only one comparable study. Conclusions: research activities of European and Czech nurses are in general quite rich. The most favourable presentations and research topics are those psychosocial and concerning standards and management of care. Although there are many interesting data presented, lack of larger and homogenous groups of patients could bee seen. Prospective randomized studies with significant data are, unfortunately, not common. As it is more than obvious that nurses are really keen on carrying out research, we think that close cooperation with physicians and other transplant centers could be helpful for the future. In an attempt to promote slightly weaker nurses’ research activities in our country, we have set a new randomized multicenter study on oral cavity prophylactic care in patients after high-dose chemotherapy. Of course, we are open to any international collaboration. Details concerning the study are presented separately in our next abstract.

P989
An educational support program (ESP) for patients with hematological malignancies and their relatives

B. Ingelsson, J. Winterling, E. Johansson, L. Wettergren, J. Sjöberg (Stockholm, S)

Introduction: Most patients diagnosed with haematological malignancies and their relatives experience a major change in life and may benefit from extended medical information and psychological as well as social support. For this purpose a six-week ESP was developed which has been offered to patients at our unit and relatives since 1996. The aim of the present work was to evaluate satisfaction with the ESP in the target group.

Method: Two nurses served as group leaders and participated during the sessions. The program consisted of both information and group discussions. The group dynamic process was used to make participants more open and supportive to each other. Five to seven patients with various haematological malignancies and in different disease states were included in each program. Every six months at least one separate program was held for patients and for relatives, respectively. A questionnaire to measure satisfaction with ESP was developed consisting of 15 items covering the areas included in the program. This was sent out one month after the program was finished.

Results: During the period 1996-2001, 61 patients and 68 relatives have participated and 90% answered the questionnaire. The results showed that the participants were positive to receiving support in a group constellation and both patients and relatives appreciated to meet other patients and their relatives in a similar situation. The majority of the participants were satisfied with the level of information given.

Conclusion: Patients and relatives participating in the ESP, were satisfied with the level of information given. The impact of the activity was measured using a questionnaire adapted from Woodcock (1996) which provided a comprehensive review of team performance. It was distributed prior to the activity to obtain a baseline for the team and then repeated two weeks after the exercise. When analysing the results it was clearly shown that the team building exercise helped the staff to work together as a cohesive group with an increase in:
- mutual respect
- workload sharing
- recognising the need for emotional support in colleagues

In conclusion it was felt that team building activities offer an excellent means of boosting morale on the ward, improving communication and promoting retention of staff.

P992
Gene therapy - the anxieties
T.A. Stanton (London, UK)

Gene therapy is a new and exciting treatment possibility for X-linked severe combined immunodeficiency (X-SCID). But how can we, the nurses, support families through this uncertain time? Parents are presented with an innovative but unfamiliar treatment and because of this they will have unique concerns.

During the summer of 2001 the second world-wide trial for gene therapy for X-SCID was given local and national ethical approval and we treated out first patient. This followed the success of the 1999 Paris trial (Cavazzana-Calvo et al 2000) where they have now treated seven X-SCID patients.

Our first trial patient was an eight-month-old baby who did not have a sibling match for bone marrow transplant and therefore fulfilled the criteria for our trial. This poster is presented as a case study and will explore the psychological issues of this alternative therapy for the family.


P993
First steps in developing a nurse led-tissue typing clinic
D. Campbell - Nurses

Background: I am a senior staff nurse who has worked as part of the haematology team for six and a half years, firstly as a staff nurse in the inpatient unit and then as bone marrow transplant coordinator. This was a new post created by the trust, which was filled with opportunity, and I was delighted to embark on the challenge. Allogeneic and autologous transplants had been carried out by the trust since 1980, hence the need for efficient and effective co-ordination.

Current Status: In order to establish the current status and identify areas of deficit relating to patient care, I carried out a Needs Analysis Assessment. Aspects of the service which needed immediate attention were:

1 Pathway of Care
2 Communication with Patients and Donors
3 Documentation
4 Recognition of Service Provided

Action Plan: I have engineered this action plan by taking the following steps.

1 Clear lines of communication have been established with:
   General Practitioners, Patients, Relatives, Medical Staff, Nursing Staff, Laboratory Staff.
2 Developed relevant documentation
3 Established formal counselling sessions with patients and donors.
4 Addressed the information needs of patients and donors.
Outcome: These measures have resulted in an improved structure, and a reduction in stress previously experienced by patients and donors. This clear pathway of care will result in an equitable efficient service that complies with best evidence. For this system to be sustainable, a fully functional nurse led clinic with supporting facilities needs to be established.

P994

Protective isolation and other measures for preventing infections in caring for patients with malignant blood diseases


Background: The risk of severe bacterial infection is significant one to three weeks post intensive chemotherapy. To prevent infections during the neutropenic, phase the patients are placed in protective isolation. Protective isolation may vary from having a single room to rigorous barrier nursing with an airlock and special ventilation. Routines pertaining to food, water, hygiene and oral care can vary between different hospitals. Patients in different phases of treatment who were treated in different hospital found those differences confusing and demotivating.

Objectives: The objective was to develop regionally uniform, simplified, and evidence based routines for haematological intensive care within the region of Uppsala/Örebro. The aim being to provide parents with the same protective isolation care independent of which hospital the patient was admitted to in the region.

Method: A working group consisting of one physician and one or two nurses from each hospital providing haematological care in the region was set up in February 2000. Seven hospitals in the region were included. Since Uppsala was the only hospital that provided bone marrow transplantation for children, allogeneic transplantation from unrelated donors, care associated with these procedures were not considered. The working group has compared routines and consulted experts. The working group had several meetings and telephone conferences.

Results: The results were divided into seven subgroups:
- indications for protective isolation of neutropenic patients,
- nursing routines for protective isolations,
- nutritional advice,
- oral care,
- care of central line (central venous catheter),
- psychological aspects
- advice given at discharge.

The results were presented at the regional meeting in February 2001 as a Care Programme.

The agreed routines and care programme are now used in all haematology wards in the region.

The care program will be reviewed in May 2001 in order to maintain an up to date programme.

P995

Improved nursing based on analysis of parent's experience

T. Zijlstra, S. van Dijk, M. Bierings, K. Brinkhoff, A. Langenberg, M. Kars, R. Maas (Utrecht, NL)

In the Wilhelmina Children's hospital part of the Nursing research is aimed at exploring the caring role of parents of children with leukaemia. The parent's role was analysed from diagnosis until one year later. The central theme of the parents' life in their own experience was: "To be there for your child".

In the course of the child's disease the parents changed their attitude. Their over time changing expectations for the Nursing staff also became apparent.

Based on this analysis we designed changes for our Nursing Practice and implemented them. Their aim is to support the parents better during the various phases of their child's disease. Main focus is on the transition back to home life. Based on our analysis parents can be better prepared to independently take care of their child at home.

P996

Symptom distress before and during stem-cell transplantation

J. Larsen, G. Nordström, P. Ljungman, A. Gardulf (Stockholm, S)

The aim of this study was to investigate how a group of Swedish patients with different malignant diseases perceived symptom distress at admission for stem-cell transplantation and during hospitalisation. Forty-three patients participated in the study. Data were collected by using the self-administrated Symptom Frequency, Intensity and Distress Questionnaire for SCT. The proportions of patients reporting symptoms varied over time; 83% the week before admission to the hospital (T0), 95% at the day before start of the conditioning treatment (T1), 100% at day of SCT (T2), 100% at the start of the protective isolation period (T3), 100% at the mid-point of the protective isolation period (T4), 98% at the time when the protective isolation period ceased (neutrophil count = 0.5 x 10^9/litre) (T5) and 85% at discharge from the hospital (T6). Seven percent of the patients reported >10 simultaneous symptoms at T0, 17% at T1, 45% at T2, 36% at T3, 54% at T4, 33% at T5 and 23% at T6. Tiredness, loss of appetite, mouth dryness, nausea, sleeping disturbances, diarrhoea and changes of taste were the reported dominating symptoms during the hospitalisation period. Loss of appetite, mouth dryness, nausea and sleeping disturbances were reported to be highly distressing when present.

The findings in our study indicate that it is important to distinguish between intensity and distress as this information in combination provides more information than intensity measurement alone. If symptom management in the SCT patients is to be realised then the patients’ perceptions of their symptoms need to be taken in consideration during the delivery of care. Tools such as the SFID-SCT could be useful for nurses and physicians in the clinical setting.

P997

The phenomenon of total pain in patients submitted to bone marrow transplantation in physical isolation

D. Pestana Ricardo, I. Costa Carvalho, R. Martins de Carvalho (Porto, P)

Total pain is a concept, created by C. Saunders, in 1967, for chronic pain of oncology origin. This concept is also accepted by O.M.S., and presupposes four components:
- Physical pain - related/ caused by treatment or cancer disease himself;
- Emotional pain - Manifested by anxiety, fear and depression;
- Social pain - caused by de family separation, loss of social and community paper, physical isolation;
- Spiritual paper - related with faith, fear of death, when patients put in question is own life.

Tends in attention the ambiguity of Total Pain, we decided to verify as the patients submitted to transplant interpret their pain, being the main objective to determine which the four divisions of total pain are more valued, in the several stages of internment, and if the treatment is effective. To reach this objective we elaborated a multiple choices questionnaire, with justification. We chose a pattern of 50 patients, between the 20 and 60 years, submitted to bone marrow transplantation in physical isolation regime, for Leukaemia, Lymphoma or Multiple Myeloma. At this moment we already did 20 questionnaires and we get some
primary conclusions, very close to the one that we think is the final conclusions.
So we concluded that the physical pain is the more referenced by ours patients as the main pain cause, in most of the cases caused by mucositis. In compensation this component is exacerbated mainly by the emotional pain, because for most of the patients the pain is related with the disease/treatment, increasing by the anxiety, the fear and the depression. The social pain is attributed to the isolation, and the loss of paper family being also quite valued. The spiritual pain is also referenced, mainly in the moments of increase of the physical pain for the fear of the death, when the patients question the meaning of their existence.

P998
Secondary malignancy post allogeneic stem cell transplant for chronic myeloid leukaemia
E. Dannie, R. Szydlo (London, UK)
Allogenic stem cell transplant (SCT) is the only hope of a cure in patients with CML. Success is largely attributable to a better selection of patients, appropriate conditioning regimens and supportive care. However the incorporation of irradiation in the conditioning regimen has been consistently identified as a major risk factor for the development of secondary malignancies post transplant.
This retrospective study analysed 608 patients with CML treated by allogeneic SCT at the Hammersmith Hospitals NHS Trust between 1979 and 2000. There were 30 children (age <16years). 566 patients (93.1%) received TBI as part of their conditioning. 19 new malignancies were diagnosed in 15 patients 12.2-189 months (median 87.1 months) post SCT.
Details as follows:

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Date of 1st transplant</th>
<th>Secondary malignancy</th>
<th>Date of 2nd malignancy</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.11.1987</td>
<td>Seminoma</td>
<td>13.2.1992</td>
<td>Alive</td>
</tr>
<tr>
<td>3</td>
<td>3.5.1985</td>
<td>Skin melanoma</td>
<td>5.1.1997</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>16.6.1985</td>
<td>Renal adenocarcinoma</td>
<td>29.5.2001</td>
<td>Dead</td>
</tr>
<tr>
<td>5</td>
<td>28.3.1986</td>
<td>Squamous cell Ca</td>
<td>11.11.1999</td>
<td>Alive</td>
</tr>
<tr>
<td>6</td>
<td>24.7.1987</td>
<td>Ca oesophagus</td>
<td>1.8.1995</td>
<td>Dead</td>
</tr>
<tr>
<td>7</td>
<td>30.10.1987</td>
<td>High grade NHL</td>
<td>2001</td>
<td>Alive</td>
</tr>
<tr>
<td>8</td>
<td>16.1.1989</td>
<td>Ca cervix</td>
<td>1990</td>
<td>Alive</td>
</tr>
<tr>
<td>9</td>
<td>22.10.1989</td>
<td>Acinar Ca</td>
<td>1998</td>
<td>Alive</td>
</tr>
<tr>
<td>12</td>
<td>10.9.1993</td>
<td>Lymphoma</td>
<td>15.5.1994</td>
<td>Dead</td>
</tr>
<tr>
<td>13</td>
<td>15.10.1993</td>
<td>High grade NHL</td>
<td>9.4.1996</td>
<td>Dead</td>
</tr>
<tr>
<td>15</td>
<td>16.3.1998</td>
<td>Squamous cell Ca</td>
<td>6.10.1998</td>
<td>Dead</td>
</tr>
</tbody>
</table>

The development of secondary malignancies after allo SCT was not associated with a number of factors including age at transplant, gender, disease status at transplant, conditioning regimens, T cell depletion, occurrence of CML or stem cell source. Conclusion: The risk of developing secondary malignancies post allo SCT for CML is relatively small but new tumours can occur at anytime post allo SCT. However some CML patients may have a predisposition to develop more than one malignancy. These observations emphasize the need for careful long-term follow-up after allogeneic SCT.

P999
The Cytomegalovirus (CMV) status of leukemic patients pre allogeneic transplant (Nov 2000- Nov 2001). Incidences of reactivation post transplant including conditioning regimes employed
G. Maynard-Wyatt, J. Bain, F. Dallas (Sutton, UK)
Background: Cytomegalovirus is a member of the Herpesvirus. It can occur in the GI tract, Lungs, Liver, Brain, Retina and other organs. CMV occurs most commonly in the Lungs of allogeneic Bone Marrow Transplant recipients greater than 30 days post transplant. It is present in body fluids such as Saliva, Tears, Semen, Cervical secretions, Urine, Blood and Blood products. The virus usually remains dormant in the body, but can reactivate and cause major problems for those people who are immuno-suppressed. The incidences of CMV reactivation varies with the type of conditioning regimen employed.
Treatment: Ganciclovir-5mgs/kg is related to aciclovir but with a wider in vitro activity against CMV. Also it has a suppressive effect on the bone marrow. It is given BD for 14 days, then OD for 7. If after 14 days there is no evidence of CMV antigen negative, then Foscarnet is now employed. Foscarnet 60mgs/kg TDS for 2-3 weeks then OD. This poster will note the CMV status of those patients receiving allogeneic transplants from November 2000-November 2001 pre transplant. Each persons conditioning regime and the incidences of reactivation of CMV. It will also state the treatment currently employed for CMV at one cancer centre and compare uniformity of treatment in other transplant centres.
Nursing issues: There are major nursing issues relating to CMV re-activation. Psychological status of the patient is of great concern. They have already spent many weeks in hospital due to their transplant, and now require re-admission for further treatment, which then has a bearing on their quality of life. Infecion, as we know brings with it fatigue, nutritional problems and possibly a fear of the disease returning. These patients have already been through aggressive but necessary treatment and believe that they should be putting their lives back together again rather than having a further stay in hospital.
Conclusion: CMV is a complex aspect of transplants. Through this poster, we want to portray the incidences of re-activation and compare with the different conditioning regimes employed. We will also address the nursing issues that these patients endure due to the re-activation.

P1000
Nursing requirements for the establishment of methods for the non-invasive ventilation (NIV) in a BMT-Unit
D. Lerner (Idar-Oberstein, D)
The use of non-invasive ventilation (NIV) has expanded rapidly in critical care units over the past decade. Recent studies indicate that the use of NIV can reduce the need for intubation and lower the mortality rate also in immunocompromised patients with respiratory failure. Several studies support the recommendation that NIV should be considered the ventilatory mode of choice for selected immunocompromised patients with respiratory failure. The timing of the initiation of noninvasive ventilation is very important. Studies show that the early implementation can prevent respiratory failure from progression; However, a variety of important questions should be answered, such as:
What are the complications resulting from NIV application? What are the benefits of NIV? What are the limitations of this method in the treatment? What about skilful and intensive nursing intervention to promote comfort and compliance to this method? How shall one train the nurse staff to deal with complications resulting from the NIV application? The implementation of NIV requires certain conditions. The most important factors are the prompt and precise education of the patient, the early implementation of NIV without delay, the absence of possible complications, the selection of the non-invasive ventilation (NIV) in a BMT-Unit.
A Standard operating procedure for the initiation of NIV is already established in our BMT unit.
P1001

15 years working party of stem-cell transplantation nurses in the Netherlands: From LBMT with LOVe(ST)
A. Mank, B. Horst (Amsterdam, NL)

Objective: In the Netherlands, nurses of stem cell transplantation centres have been united in a national working party since 1985. The working party grew from 6 to 16 participating centres. In 2001 the name of the working party has changed from "bone marrow transplant nurses working group (LBMT)" into "stem-cell transplant nurses working group (LOVeST)". At first the aims of the working party were to stimulate uniformity and exchange of experiences and networking. While new goals were added, it became more and more a professional working group. Nowadays, new goals are to stimulate and to participate in nursing research as well as the development of products to improve the quality of care.

Method: The working party is known at national and international level and contributes to conferences worldwide. There is also a tight co-operation with national and international EBMT working parties. It provides education to colleagues and publishes a newsletter twice a year. Once every two or three years an update of information about all stem cell centres is published. Topics are: a) transplant protocol; b) research; c) medication; d) decontamination; e) nutrition; f) infusion; g) personnel; h) research

The working party meets 4 times a year. In the morning there is a general meeting and in the afternoon attention will be paid to developing new products. Currently the subjects are: a) national survey on patient information; b) development of mouth care protocol; c) after care; d) leaflet 'stem cell donor'.

Much of this important work would not have been possible without the generous financial support of pharmaceutical companies (Main sponsors at present time: AMGEN Nederland B.V. and Gilead)

P1002

Does the administration of intravenous itraconazole increase the risk of blocking central venous catheters?
D. McGuigan, J. Hudson, D. Carrol, G. Butters, R. Nolan, J. Cornish (Bristol, UK)

Intravenous itraconazole is a useful agent in the prevention and treatment of fungal infections in immunocompromised patients. Evidence in our practice suggests that there is an increased tendency for lines to block following cumulative administration of intravenous itraconazole in Bone Marrow Transplant (BMT) patients. We have stopped its intravenous use which limits the options for prophylaxis and treatment of patients with established fungal infections following BMT.

The nursing staff in collaboration with the wider multi disciplinary team and Ortho Biotech Pharmaceutical Company decided to undertake a study observing all patients who had received intravenous itraconazole. The aim was to note the incidence of blocked lines, compliance with manufacturers guidelines, and risk factors associated with line blockage. There are three arms to the study:

1) Review of case notes from patients who had received intravenous itraconazole.
2) Laboratory research to identify risk factors associated with precipitation and hence line blockage, looking specifically at optical densities of solutions when added to itraconazole.
3) A questionnaire for staff using the guidelines issued by the pharmaceutical company to monitor practice related to the administration of intravenous itraconazole.

The case note review has confirmed a higher prevalence of line occlusion in patients receiving intravenous itraconazole.

The laboratory research has shown that precipitation occurs in a variety of aqueous solutions in contact with itraconazole most notably hepflush.

The questionnaire devised for staff has highlighted variation around the interpretation of the pharmaceutical guidelines and compliance.

Line occlusion is undoubtedly multifactorial and whilst it is inappropriate to highlight itraconazole as the sole contributor to line occlusion, concerns about its solubility in solution, particularly in combination with heparin make it a potential risk factor for line blockage.

The importance of invasive fungal infection as the leading cause of infectious death post BMT demonstrates the need for further research as intravenous itraconazole currently has a proven role in local and national paediatric and adult antifungal protocols.

P1003

ASCT - Proposals for nurses study research Edipo 2001
E. Bystricka, S. Vokurka, V. Pavlicova, M. Patorkova, J. Scudlova (Pilsen, Hradec Kralove, Prague, Olomouc, CZ)

Oral mucositis (OM), causing various complications, is one of the most significant nursing problems in patients treated with high-dose chemotherapy. In spite of the fact that OM is repeatedly a subject of researches and studies, there has been defined only a minimum of explicit and for daily practice useful recommendations so far. In view of not unambiguous, or controversial, Oral cavity prophylactic care in patients treated with high-dose chemotherapy with conclusions of many studies, it will be necessary to continue on revising nursing approaches in terms of prophylactic care of oral cavity in risk-patients. There should be an effort to introduce rather simple nursing procedures taking into consideration the fact that some solutions used locally for oral cavity rinses can keep on impairing the feeling of anorexia and nausea in patients. In an attempt to help define reasons of antimicrobial solution rinses in OM prophylaxis after ASCT, we have set a prospective, comparative, randomized, multicentric study. Within this research we would like to compare simple prophylactic nursing procedure using antimicrobial effects of the PVP-iodine solution (Betadine 1:100 diluted) with procedure using just mechanical effect of regular rinses of oral cavity with the help of normal saline solution. Patients with multiple myeloma or NHL treated by ASCT (conditioning HD-L-PAM 200 mg/m2 or BEAM) will be included in the study. Our research should help comparing the occurrence, course and gravity of OM and the occurrence of sepsicaemia, and fevers of unclear origin (FUO) in the monitored groups. There will be also verified the tolerance and evaluation of the nursing procedure by the patient himself/herself. For reason of homogeneity of the group, the final evaluation shall be carried out with respect to the diagnosis and chemotherapy of the patient. More research details and criteria will be available in our poster.