Female six week old NOD/LIJ mice were sublethally irradiated and transplanted with ESC-derived HSC. To induce differentiation, R1 ESC were cultured methylcellulose-based medium supplemented with SCF, IL-3 and IL-6. An enriched c-kit+ ESC-derived cell population was injected intra bone marrow (IBM) or IV. Mice were followed by blood glucose measurements and chimerism analyses until onset of diabetes or until 40 weeks after transplantation. Nine NOD mice were held as controls. Peripheral blood donor (H2b) versus recipient (H2Kd) chimerism was measured by flow cytometry.

Nine out of 10 mice the from IBM group and 5 out of 8 from IV group did not become hyperglycemic in contrast to control group where 8 out of 9 mice were euthanized because of diabetes (Graph). The level of chimerism achieved after transplantation was 9.1% ± 6.71% in the IBM group and 2.5% ± 2.78% in the IV group. Histological examination showed that most of islets were replaced by lymphocytic infiltration or fibrous tissue in controls (even in case of a mouse without clinical evidence of diabetes). In 78% (14/18) of animals from ESC-derived hematopoiesis, remission was confirmed by histology revealing the absence of insulitis and normal immunohistochemical staining of islet cells for insulin. Prevention of diabetes / insulitis was predicted by the percentage ESC-derived hematopoietic chimerism. All mice with > 5% ESC-derived chimerism remained free of diabetes and insulitis. High concentration of IFNγ was detected only in culture containing GAS 65 and splenocytes from NOD control mouse. Proliferative response of splenocytes derived from ESCT-NOD chimeric mice were diminished toward recipient and host lymphocytes compared sustained response to third party antigens.

It is possible to establish mixed allogeneic hematopoietic chimerism in sublethally irradiated NOD mice without subsequent GVHD by using ESC-derived hematopoietic stem cell transplantation. Mixed allogeneic chimerism achieved with ESCT prevents diabetes in NOD mice when performed before onset of diabetes.

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**Paediatric issues 1**

**O124**

Megatherapy/SCT activity in paediatric solid tumours in Europe

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6757 patients (pts) were registered in the paediatric-adolsecent age group (< 18 years) from 256 centres and 35 countries. In 6530 pts minimal data for analysis was available. Survival rates refer to 5-year rates (OS, EFS). Neuroblastomas: 2903 pts are registered, 60% are male, median age: 3.8 yrs. The OS [EFS] is 37% [33%] with a median observation period (MOP) of 8.5 yrs. The OS for pts (n=2353) in first remission is 38% (CR1/VGPR/PR/SD/PRD) and 28% for relapse (CR2/RR) pts (n=248). The OS rates in compliance with the status at MGT are 42% for CR1 (1071 pts), 36% for PRD (1175 pts), 23% for SD (52 pts), 45% for CR2 (109 pts), 18% for SR (94 pts) and 5% for RR (23 pts) and 6% for UR (19 pts). The Busulfan-Melphalan MGT approach produced statistically superior OS rates during first remission: OS 48% vs. OS 36% with other regimens (p<0.001) and is currently under randomised investigation in the European HR-NBL-1/ESIOP study. Ewing tumours: 1175 pts up to 18 yrs., 55% are male, median age: 12.7 yrs. The OS (EFS) rates are 40% [36%]; MOP: 6.7 yrs. The OS for 756 first remission pts is 46% and 30% for 290 relapse pts. The OS rates according to status at MGT are 53% for CR1/VGPR (471 pts), 38% for PR (237 pts), 10% for SD/PR (48 pts), 44% for CR2 (144pts), 15% for SR (87pts) and 18% for RR (36 pts) and 0% for UR (18 pts, 16 deaths). Busulfan-Melphalan containing regimens resulted in statistically superior survival in first remission pts (OS 59% vs. OS 36% 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. Wilms Tumours: 249 pts, 44% are males, median age: 6.1 yrs. and MOP 8.3yrs. The OS [EFS] rates at 5 years are 51% [47%]. The OS was for 71 first remission pts 56% in 153 relapse patients. Currently CEM MGT is evaluated prospectively in defined relapse patients within the SIOP 2001 Nephroblastoma trial. Potential candidates for new prospective studies in 2004 are: Soft tissue sarcomas: 737 pts, 54% are male, median age: 9.0 yrs. , MOP: 8.5 yrs. The OS [EFS] rates are 29% [24%]. The OS rate of 448 first remission pts is 30% [26%] and 25% [21%] for 211 relapse patients. Retinoblastomas: 77 pts, 56% are males, median age: 3.7 yrs. and MOP: 9.7yrs. The OS [EFS] rate at 5 years is 62% [57%]. OS in 36 first remission pts was 76% and 50% for 37 relapse pts.

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**O125**

Haematopoietic stem cell transplantation in childhood: report from the Paediatric Diseases Working Party of the European Bone Marrow Transplantation Group

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During the last 35 years, the number of SCTs in children who have been registered in the EBMT data base has tremendously increased, and the number of patients who were reported between May 1996 and 2002 is similar to the total number reported over the previous 28 years. The aim of this study is to provide the full picture regarding all children given SCTs between 1970 and 2002, and who were reported to the EBMT registry. In the studied period 31,713 SCTs (18,803 allogeneic and 12,910 autologous) in patients < 18 years of age were registered by 420 centres belonging to 29 European and 14 extra-European countries; 110 of the 420 centres performed paediatric transplantation alone. The number reported over the previous 28 years.

The OS [EFS] is 37% [33%] with a median observation period (MOP) of 8.5 yrs. The OS for pts (n=2353) in first remission is 38% (CR1/VGPR/PR/SD/PRD) and 28% for relapse (CR2/RR) pts (n=248). The OS rates in compliance with the status at MGT are 42% for CR1 (1071 pts), 36% for PRD (1175 pts), 23% for SD (52 pts), 45% for CR2 (109 pts), 18% for SR (94 pts) and 5% for RR (23 pts) and 6% for UR (19 pts). The Busulfan-Melphalan MGT approach produced statistically superior OS rates during first remission: OS 48% vs. OS 36% with other regimens (p<0.001) and is currently under randomised investigation in the European HR-NBL-1/ESIOP study. Ewing tumours: 1175 pts up to 18 yrs., 55% are male, median age: 12.7 yrs. The OS (EFS) rates are 40% [36%]; MOP: 6.7 yrs. The OS for 756 first remission pts is 46% and 30% for 290 relapse pts. The OS rates according to status at MGT are 53% for CR1/VGPR (471 pts), 38% for PR (237 pts), 10% for SD/PR (48 pts), 44% for CR2 (144pts), 15% for SR (87pts) and 18% for RR (36 pts) and 0% for UR (18 pts, 16 deaths). Busulfan-Melphalan containing regimens resulted in statistically superior survival in first remission pts (OS 59% vs. OS 36% 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. Wilms Tumours: 249 pts, 44% are males, median age: 6.1 yrs. and MOP 8.3yrs. The OS [EFS] rates at 5 years are 51% [47%]. The OS was for 71 first remission pts 56% in 153 relapse patients. Currently CEM MGT is evaluated prospectively in defined relapse patients within the SIOP 2001 Nephroblastoma trial. Potential candidates for new prospective studies in 2004 are: Soft tissue sarcomas: 737 pts, 54% are male, median age: 9.0 yrs. , MOP: 8.5 yrs. The OS [EFS] rates are 29% [24%]. The OS rate of 448 first remission pts is 30% [26%] and 25% [21%] for 211 relapse patients. Retinoblastomas: 77 pts, 56% are males, median age: 3.7 yrs. and MOP: 9.7yrs. The OS [EFS] rate at 5 years is 62% [57%]. OS in 36 first remission pts was 76% and 50% for 37 relapse pts.
of allografts between 1999 - 2002. The use of peripheral blood stem cells (PBSC) significantly increased, and represents 30% of the allogeneic SCTs and 85% of the autologous SCTs performed between 1999 and 2002. With regards to the underlying diagnosis, the number of children given allogeneic stem cell transplantation for ALL, AML, CML remained stable, while the number of patients given allogeneic stem cell transplantation for MDS and lymphomas increased. Moreover, the number of patients given autologous SCT for AL decreased, while the number of patients given autologous SCT for solid tumours increased.

We conclude that major changes have occurred during the last few years. Knowledge of this data provides a basis for better definition of transplant indications, for decision making in health care planning, and for patient counselling.

O126
Allogenic stem cell transplantation for children with advanced MDS: results from the EWOG-MDS study group employing a pre-transplant preparative regimen with busulfan, cyclophosphamide and melphanal

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MDS in children is a rare disorder characterized by dysplasia and defined genetic abnormalities. In most patients (pts) MDS arises without known predisposing conditions (primary MDS). Here, we report the results of 56 males and 32 females with advanced primary MDS enrolled in the prospective EWOG-MDS trial 97. Data were analysed according to the most advanced Fab-type prior to SCT: 33 pts were classified as RAEB, 42 as RAEBt and 13 as MDR-AML. Median age at diagnosis was 9.5 yrs (0.1-17.6) and median time from diagnosis of advanced MDS to SCT 4 mo (0.5-31). Cytogenetics revealed monosomy 7 in 31 pts, trisomy 8 in 4, a complex karyotype in 9 and other abnormalities in 13; karyotype was normal in 29 pts and unknown in 2. 30 pts had received AML-like therapy prior to SCT. All pts were given an unmanipulated graft after conditioning with busulfan 16 mg/kg, cyclophosphamide 120 mg/kg and melphanal 140 mg/m².

Source of stem cells was bone marrow in 60 pts, peripheral blood in 25, cord blood in 2 and unknown in one. 38 pts were transplanted from an HLA-identical related or unrelated donor (UD), 22 pts from an HLA-1 or -2-antigen disparate unrelated donor (UD), 5 pts from an HLA-identical or 1-antigen disparate unrelated donor (UD). GVHD prophylaxis consisted of CSA alone for MFD, whereas recipients of a UD graft generally received CSA, methotrexate and anti-lymphocyte globulin. Two pts suffered graft failure. The cumulative incidence of grade II-IV acute GVHD and chronic GVHD was 40% (SE 5%) and 24% (SE 5%), respectively. 18 pts suffered transplant-related mortality (TRM), the cumulative incidence of TRM in pts grafted from a MFD or UD being 13 and 26%, respectively (p=0.03).

Presence of acute GVHD II-IV (p<0.01), spleen size at SCT >1 cm below the costal margin (p=0.03) and age >12 years (p=0.04) predicted an increased risk of TRM. 17 patients relapsed at a median of 12 mo after SCT (1-107). The 5-year probability of leukemia recurrence was 23%, with no difference between MFD and UD transplants. While the highest FAB type prior to SCT predicted relapse with a cumulative incidence rate increasing from RAEB (13%, SE 10%) to RAEBt (21%, SE 9%) and MDR-AML (58%, SE 18%) (p=0.02), the use of intensive chemotherapy prior to SCT or blast percentage at SCT did not. With a median observation time after SCT of 35 months (6-107) the EFS at 5-years was 68% (SE 9%) and 46% (SE 10%) for pts given SCT from a MFD or UD, respectively (p=0.03). These results indicate that a large proportion of pts with advanced MDS can be rescued by SCT.

O127
Results of stem cell transplantation in paediatric patients with chronic myeloid leukaemia

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Hematopoietic stem cell transplantation (SCT) remains the only proven option for cure of young patients (pts) with chronic myeloid leukemia (CML) - a disease which is rare in childhood and adolescence. From Dec. 1995 to Nov. 2004 pts younger than 19 years (median age: 11 yrs) with Philadelphia-chromosome positive CML (n=200; 100 boys, 100 girls) were treated by hydroxyurea +/- interferon and scheduled for SCT from an HLA-matched family donor within 6 months after diagnosis (Dx) and from an unrelated donor within 12 months. Treatment was performed within the multicenter GPOH-trial “CML-paed”.

85% of the pts were diagnosed in chronic phase (CP), 6 pts (3%) died from disease without SCT with a median interval from Dx to death of 6.5 months (range 0.5 – 12 mos) and 18 pts are still searching for a donor. 176 pts underwent SCT (n=50 HLA-matched related; n=71 HLA-matched unrelated (MUD); n=19 HLA-mismatched related; n=36 HLA mismatched unrelated) in CP (n=142), in accelerated phase (AP, n=9), in blast crisis (BC, n=9), or in 2. CP (n=14). Probability of overall survival (OS) was 75% if SCT was performed < 7 mo after Dx (in 133 pts) and 60% (n=100 pts) if pts were transplanted later, however, this difference was statistically not significant. Conditioning regimens included either total body irradiation (n=82) or busulfan (n=80) resulting in no statistically different impact on OS. Five year OS was 82% for SCT from HLA-matched related and 55% for HLA-MUD-SCT reflecting a higher transplant-related mortality for the latter (p=0.0017). SCT from HLA-mismatched unrelated and HLA-mismatched unrelated donors, OS was 56% and 50%, respectively. 14 out of 176 pts (8 %) relapsed following SCT after a mean interval of 11 mos (range 1 – 137 mos) and 7 of them so far have died of CML. Outcome was inferior if SCT was performed in advanced stages of CML (OS of all pts in CP: 67%; in AP: 55%, in BC: 21%, respectively). During the last decade the 3-year OS after SCT from HLA-matched unrelated donors improved gradually from 45 % before the year 1994, to 53 % in the period 1995 to 1999 and to 62 % after 2000, respectively. This large series of pts from a controlled trial shows an excellent OS of 82% for pediatric pts with CML undergoing SCT from matched sibling donors and constantly improving results during the last decade in the setting of MUD-SCT.

O128
Haematopoietic stem cell transplantation for Shwachman-Diamond disease: a retrospective survey from EBMT registry

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Shwachman-Diamond disease is a rare autosomal disease of infancy characterized by pancreatic insufficiency, short stature and bone marrow dysfunction. About 20% of patients develop
aplastic anemia and 20-33% evolve to myelodysplastic syndrome and/or acute leukemia. Limited data exist about the use of SCT in these patients. In this study we describe the clinical characteristics and outcome of 21 patients registered in the EBMT file who underwent stem cell transplantation for bone marrow failure. 13; MDS/AML, 4; unknown, 4. The patients were transplanted in 14 centers and only 4 centers performed more than one patient. They were 15 F and 6 M. The median age at diagnosis and SCT were 0.6 (range 0-22.9) and 10 years (range 4-38), respectively. The type of donor was unrelated, sibling and parent in 18, 3 and 1 case, respectively. The source of stem cell was bone marrow in 19 and peripheral blood in 2 patients. Conditioning regimen was based on BMT (+ Cy or L-PHAM) in 3 patients and on chemo (Bu 7, other combinations 9; not specified 2) in the remaining 18 patients. Ciclosporin + short MTX was mainly used as prophylaxis of GVHD. The patients who received the graft from an unrelated donor were given also ATG or Campath1G. The collection of SCT data regarding the median TNC infused, time to PMN and PLT engraftment, acute and chronic GVHD are still ongoing. Overall, 7 patients died after a median time from SCT of 70 days, range 20-175: 3 for ARDS/MOF, 2 for infection, 1 for acute GVHD; in 1 patients the cause of death was not specified. The 100-TRM was 29.6% (C.I. 9.7-49.6). After a median follow-up of 4.4 years (range 51 days-16.2 years), the overall survival was 65.3% (range 44.5-86.1). According to known diagnosis before SCT, 1 of 4 patients affected by MDS/AML (25%) and 11 of 13 patients affected by SAA (76.4%) are alive, p = 0.053. This study showed that SCT allows the correction of bone marrow dysfunction in almost 2/3 of Shwachman-Diamond patients and that the results, for SAA, are superimposable to acquired SAA. Further improvement of cure rate may require a strict monitoring of these patients for early diagnosis of MDS or leukemic transformation and early SCT.

O129
Intravenous busulphan given once a day
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In a previous study [Shaw, PJ et al.(2004) Bone Marrow Transplantation 34: 197 – 205], we found that oral Busulphan (BU) given on 4 days as single daily dose of 150 mg/m², in combination with cyclophosphamide 120 mg/kg, was safe and effective in children with acute leukemia and MDS. Area-under-the-concentration-versus-time-curve (AUC) achieved by 27 children was (mean ± SD) 22.8 ± 7.5µg/mL.h. This study provided us with a normal AUC range for BU of 22–37µg/mL.h. We now report our experience with intravenous BU, administered as a 2 h infusion to 10 children aged 1-18 years with both malignant (n = 5) and genetic (n = 5) diseases. Patients: BMT conditioning was Fludarabine/BU/CY (n=4), BU/CY (n=1), BU/Melphalan (n=2) and BU/Fludarabine/Melphalan (n=1). Pharmacokinetic analysis was performed as previously described [see reference above]. Results: In all patients, the decline in BU concentrations after the dose was log-linear, fitting a single compartment model. The first two children had 120 mg/m² BU and achieved AUC values towards the low end of our range, of 20.3 and 23.6µg/mL.h. The dose was therefore escalated to 130 mg/m² for the next 8 children. Exposure to BU for 7 of the children was (mean ± SD) 23.0 ± 5.8 µg/mL.h, suggesting that the 130 mg/m² intravenous dose tends to provide slightly lower exposure to BU than the 150 mg/m² oral dose for the majority of children. The 8th child receiving the 130 mg/m² BU dose was an outlier, achieving a very high AUC of 50.3 µg/mL.h. His dose was then reduced to 74 mg/m² on day 3 of BU and his AUC, at 31.5 µg/mL.h, was then within our normal range. This child, with SCID, had low BU clearance (1.1 L/h) due to slow elimination of BU (elimination half life was 3.7 h-1). The other 9 children had BU clearance values of (mean ± SD) 5.4 ± 2.0 and elimination half-life values of 2.5 ± 0.4 h-1. Conclusions: Our preliminary experience with the intravenous single daily dose of 130 mg/m² suggests this may provide an acceptable exposure for most patients. As it is towards the low end of our normal range this may not be adequate for patients with genetic disease. The one patient with extremely high exposure suggests that BU levels do need to be monitored until we have better predictors of patients who may have slow or fast clearance with intravenous BU. The advantage of the intravenous formulation and a single daily dose is that this may be readily achieved with a limited sampling model.

O130
Blood and marrow transplantation. Depression in oncological paediatric patients and their siblings
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The oncological disease is involved, in a dramatic way, the family of the paediatric patient around to extreme of life and death. The transplant is one of the moments of greater difficulty, above all when the donor is one of the siblings-sisters; in this case, the prevailing feelings in the familiar are loaded with saving omnipotent or, to the contrary, depressive expectations. Objectives: This study places the objective to quantify the depressive difficulties of the siblings of forehead to the donation - transplant, trying to differentiate the feelings of the patients from those of the siblings. Methods: We have selected a champion of 16 patients with acute lymphoblastic leukaemia, suitable to the transplant from brother/sister, of family units in which an other brother or sister appears at least (not donor). We have subordinate to psychometrical appraisal is the group of the patients that one of the siblings, donor and not, with the Children Depression Inventory. The appraisal has been carried out on the patients: to the diagnosis, one week before the transplant and within a month from it; to the siblings donors: one week before and within a month from the transplant: to the non-donors siblings within a month from the transplant. Results: Patients’ depressive feelings accompany experience without to cause disturbs in individual and social sphere: there are no significative differences in studied moments: diagnosis, pre and post transplantation. Sibling’s donors express a less depressive feeling than not donors. The appraisal pre-transplant, with one difference, regarding the patient, statistically meaningful. Between not donors siblings, sisters show one greater depressive difficulty. Conclusions: The psychometric evaluation confirms The clinical observations regarding to: a greater defensive order in the oncological patients who, seldom, show depressive syndromes; a depressive reaction of the donor siblings to transplantation that stretches to disappear little within time; a depressive tendency in the not donor sisters. These considerations confirm our attention to the group of the donor siblings: they express, in solitude, a psychological suffering of depressive type that risks to complicate familiar course after transplantation.

S21
O131 Outcome of children undergoing allogeneic transplants using unmanipulated grafts from unrelated donors allele matched or mismatched in HLA - two centre experience

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Matched sibling HSCT is the efficient treatment for many malignant and non-malignant diseases. Unfortunately only 15-20% of patients have HLA-matched siblings. For the remaining patients an alternative donor is required. Finding fully matched unrelated donor (MUD) may be difficult or too time-consuming. We compared outcomes of patients transplanted from matched versus mismatched (MM) unrelated donors (UD). All patients where it was possible to obtain prospectively or retrospectively POR-SSP high resolution typing for HLA alleles A*, B*, Cw*, DRB1* and DQB1* were enrolled. Single allele mismatch at A*, B* or DRB1* in GVH direction was acceptable with preference to have the best match at Cw* and DQB1*.

Other patient/donor characteristics were also considered. Until IX/04 141 evaluable patients (0,3-20,5; med. 10,6 yo) were transplanted (152x) from UD in Prague (79; since 1997) and Wroclaw (62; since 2000) using unmanipulated BM in 80 (57%) or PBSC in 61, for malignant (n=123, 87%) or non-malignant disease. Following first HSCT patients received GVHD prophylaxis with cyclosporine (n=140), MTX (n=134), steroids (n=3), MMF (n=2); rATG (n=135) or Campath 1H (n=2). Conditioning regimens varied according to disease. 63 patients (45%) were transplanted using fully MUD, in further 78 patients there were 121 allele disparities (A-10, B-26, DRB1*-4, DQB1*-4, Cw*-65). 45 patients donors were mismatched at one allele only: Cw*(n=27), DQB1*(n=4), A* (n=5), B*(n=8), DRB1*(n=1), other patients were engrafted from donors mismatched at two (n=23) or three alleles (n=10). Primary engraftment was achieved in 136/139 (98%) evaluable patients, 11 patients were re-transplanted: for relapse (n=1); JMML or graft rejection/failure (n=10; 1x). Overall survival (OS) is 65% with median follow-up 1.2 years (2-80 months), 50 patients died in median 107 days post-transplant: 12/123 (10%) died due to relapse, total 38 (27%) died due to transplant related complications (TRM) with TRM D+100 14%. 25 patients (18%) suffered from severe acute GVHD gr.III-IV, 29 patients experienced extensive cGVHD, further 2 only after DLI (in total 27% out of evaluable 113). OS according to match in HLA is: MUD: 63%, one MM (DQ or Cw): 58%, one MM (A, B, DRB1): 57%, two MM: 74%, three MM: 80%. For those patients without a fully MUD even HSCT may be feasible alternative to haploidentical family donors.

Supported by:UHKT/CEZ/MZ-0023736001;IGA MZ 8223

O132 In silico approach to identify GvL-relevant mHags by genome-wide SNP expression and subtraction profiling combined with HLA peptide binding and processing prediction

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Minor histocompatibility antigens (mHags) derived from protein variations encoded by single nucleotide polymorphisms (cSNPs) are capable of inducing allogeneic T cell responses in HLA-identical transplants. SNP-caused amino acid variations can result in a differential HLA peptide binding strength or in differential proteasomal processing and thus significantly influence the presentation on the cell surface. To identify potential mHag sources, which mediate graft versus leukemia reactions, an array-based genome-wide gene expression profiling in hematopoietic and non-hematopoietic cells was performed and followed by subtraction analysis to single out the hematopoietically expressed proteins. These selectively expressed genes were screened in silico for SNPs and allele frequencies. In frequency-relevant SNPs the derived amino acid sequences were checked for HLA peptide binding strength and proteasomal processing, for both constitutive and immunoproteasomes. Subtraction profiling showed that 388 genes were expressed selectively in both CML and CD34 stem cells but not in keratinocytes or colon epithelial cells, the latter two representing cell species of GVHD target organs. In 102 of 388 genes, 220 allele frequency typed SNPs were found. For 151 cSNPs in 82 genes, 310 mono- or biallelic epitopes restricted to the most frequent HLA class I alleles were concordantly predicted by two different HLA binding prediction algorithms. The predicted epitopes were analyzed for proteasomal processing by three different processing algorithms using stringent criteria: (i) correct C-terminal processing, (ii) no or no major epitope-internal cleavages. The constitutive processing algorithm identified 30 correctly processed epitopes of which 4 were not destroyed by any internal cleavages. The immunoproteasomal processing algorithm correctly predicted 40 epitopes of which 8 were not cut by any internal cleavages. Only 6 epitopes were determined to be processed by both types of proteasomes. Three epitopes were predicted not to be internally cleaved by the constitutive prediction algorithm. Of these three epitopes, two were predicted not to be cleaved by immunoproteasomal prediction. It is expected that genome-wide mRNA expression and SNP profiling combined with stringent HLA peptide binding and proteasomal processing prediction will be the most effective tool in identifying new mHag sources and provide a major basis for individualized treatment protocols in stem cell transplantation.

O133 CD8+ T-cell-mediated alloreactivity against renal-cell carcinoma in vitro: lessons learned from six different donor-recipient pairs

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Efforts aiming to improve the specificity and efficiency of allogeneic hematopoietic stem-cell transplantation (HSCT) in metastatic renal-cell carcinoma (RCC) will benefit from the identification of potential anti-tumor effector mechanisms and their corresponding target structures. We analyzed RCC-
directed immune responses in peripheral blood mononuclear cells (PBMC) of HLA-matched healthy sibling (1) and unrelated individuals (5) and compared these results with available autologous patient PBMC. While mixed lymphocyte/tumor-cell culture (MLTC) responders derived from allogeneic donors showed a robust antigen-dependent proliferation, a weak proliferative response was seen with autologous MLTC populations. By analysing the fine specificity of MLTC-derived clonal CTL the majority of allogeneic effectors recognized RCC, but not natural killer target K562. These CTL were restricted by various HLA-A, -B or -C molecules. We further isolated CTL clones that exhibit an extraordinary strong recognition of RCC and various epithelial tumor-cell lines. Antibody blocking experiments provided clear evidence that these CTL are restricted by a not yet defined HLA-Ib molecule and, simultaneously, by a NKG2D-dependent mechanism. Other rapidly proliferating CD3+ CD8+ CTL clones were obtained that showed a non-HLA-restricted reactivity against RCC and a minor but consistent reactivity against targets with low or absent HLA-class I expression (e.g. K562).

In conclusion, our results demonstrate that a broad panel of RCC-reactive CTL can be isolated from PBMC of HLA class I-matched healthy donors in vitro. Inter-individual heterogeneity included the underlying type of CTL response (i.e. restriction by HLA-Ia, HLA-Ib, or non-HLA-restricted) as well as its target structures (i.e. choice drugs for restricted tissue expression). Our observations might further reflect the superior ability to expand RCC-directed T cells from PBMC of allogeneic healthy donors compared to the autologous setting. At this point, we cannot conclude whether these various CTL populations contribute to effective anti-RCC immune responses occurring in vivo. Answering this question will certainly require to identify CTL-defined target structures at the molecular level. This will allow us to analyse the expression of candidate antigens and the frequency of specific CTL in RCC patients after allogeneic HSCT, and to correlate these findings with graft-versus-tumor and graft-versus-host events.

O134
The role of GM-CSF in adult haplo-identical transplant who cannot benefit from NK allo-reactivity

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Haplo-identical transplant is now established as a procedure of choice for patients who lack a compatible donor. It might even be the best choice for AML, provided there is a GvH NK allo-reactivity. However, patients are still referred too late, heavily pre-treated, at very advanced stages. We initiated a three-step phase I study trying to improve transplant related mortality, relapse rate and immunity: (1): G-CSF + DLI; (2): GM-CSF + DLI; (3): patient and disease adapted strategy. Thirty-six consecutive leukemia patients, aged 18-55, were investigated (20 very poor risk, 12 poor risk and 4 better risk). GvH type NK alloreactivity was chosen when possible (21/36) and balanced across the 3 groups. In the first 9 patients, G-CSF was used post-transplant and prophylactic DLI were given at month 1, 2 and 3. The use of G-CSF and 1 to 3 DLI (10^6 CD3/kg) was found safe. It resulted in faster CD4 recovery and a low rate of infections. However, it was insufficient to induce a protective GVL effect. In the next 12 patients, GM-CSF was used plus 1 DLI (10^6 CD3/kg) at day 30 unless aGvHD (3 pts). The comparison between the 2 first groups can be summarized as follows: G-CSF + DLI: TRM at day 100: 0; RR: 6/9; severe aGvHD:0; GM-CSF + 1 DLI group: RR: 1/12; TRM at day 100: 3; aGvHD grade 2 or more: 9/12; price to pay: aGvHD resulting in 5 deaths in total. Median time to relapse in the 21 first patients was 6 months range (4 – 9).

Step 3 (17 patients) consists of a patient adapted strategy: no more aspecific DLI (selected anti-CMV and aspergillus DLI planned in all patients); in myeloid disorders with NK allo-reactivity: no GF. In the other cases, GM-CSF (at a reduced total dose of 500 mcg) is given from day 5 to day 9. The follow-up of patients alive in CCR (12), although promising (3 relapses), is currently short (median 8 months), compared to the median of relapse in the 2 first groups (6 months).

Overall, TRM at day 100 is 2/29, reflecting the good tolerance of the conditioning in a heavily pre-treated population (median age: 43). Overall relapse rate for all patients treated with GM-CSF, without the benefit of NK-alloreactivity, is 6/16 (median FU: 15 months). We conclude that the third strategy might improve the outcome and the relapse rate without exposing patients to unnecessary severe GVHD.

O135
Safety of intrathecal donor lymphocytes injection for the treatment of refractory lymphomatous meningitis

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Background: Central nervous system (CNS) relapse after allogeneic stem cell transplantation (SCT) is associated with a poor prognosis. Methotrexate, Cytarabine and steroids are the first options to treat intracranial (IT) administration but refractory patients rapidly die from their CNS relapse. Donor lymphocytes infusion (DLI) is now a classical salvage approach for systemic relapses after allogeneic transplantation but DL don’t cross the blood brain barrier. On the other hand, IT injection of DLI has never been reported to our knowledge. Therefore, having obtained the informed consent of the patient, we decided to treat the relapsed and refractory lymphomatous meningitis with intraventricular injections of DL. Patient: A 43 years old woman who presented a cutaneous stage IV pleiomorphic large T cell lymphoma, in third complete remission, was treated with an allogeneic familial SCT. She had a past history of CNS involvement treated by CNS RT and IT MTX + AraC with complete clearance of the tumor cells before transplant. Five months after SCT, the patient presented a neurological deterioration and the investigations revealed a meningeal relapse and a leucoencephalopathy. A treatment combining IT Temozolomide, VP16 and BCNU was performed without response and with a new neurological deterioration. After having received an informed consent from the patient and her family, we decided to perform an intraventricular DLI injection. Doses of IT DLI injection were respectively: 2 x 10^6 on day 1 and 2; 6 x 10^6 on day 14; 18 x 10^6 on day 30. We observed a neurological improvement and further analysis of cerebrospinal fluid were negative for lymphomatous cells. During the next months, the patient presented a partial recovery of ataxia and dysarthria but important locomotor dysfunctions persisted. 18 months after BMT, she presented a serious infection at home and refused to be hospitalized. She died without evidence of CNS relapse. Cerebral fluid samples are currently investigated for dosages of cytokines (IL1, TNF, IL2...).

Conclusion: this observation suggests the feasibility and the potential graft-versus-lymphoma effect of intraventricular DLI injection in hematological malignancies. Future studies have to confirm the safety and the efficacy of this procedure as a treatment of lymphomatous meningitis.
Haematopoietic stem cell transplantation (HSCT) in childhood acute lymphoblastic leukaemia (ALL) is reserved only for very high-risk patients in first complete remission (CR1) and majority of relapsed patients. We performed quantitative minimal residual disease (MRD) level assessment prior to HSCT in 31 children (7 girls and 24 boys) with ALL consecutively transplanted at our institution. We used clone-specific immunoglobulin and/or T-cell receptor gene rearrangements (IRG) targeted real-time quantitative PCR (RQ-PCR). The recommendations of the European Study Group on Minimal Residual Disease in ALL (ESG-MRD-ALL) for both methodology and interpretation of the results were strictly observed. We set up IRG-targeted patient-specific RQ-PCR with the sensitivity reaching 10(-4) in 23 patients, in one patient sensitivity reached only 10(-3) and in 7 patients we were not able to set up IRG-RQ-PCR system with adequate sensitivity. However, in 5 of them MRD was monitored using the fusion genes instead (BCR/ABL 1, TEL/AML1 2, ML/LAF4 2). MRD level prior HSCT was determined in 29 children (94%). Two kids had negative, 5 children had the level below 10(-4), two patients had MRD level between 1.0x10(-4) – 9.9x10(-4) and 5 children were highly positive: more than 10(-3). All the patients with undetectable MRD prior to HSCT (n=17) are in continuous CR (follow-up 3-51 months; median 16 months). In the group of patients with detectable MRD (n=12) only 5 patients remained in CR, 5 patients suffered relapses and 2 patients died due to posttransplant complications. The following course of MRD levels was individual and the results of successive examinations (available within 5 days after the sample was taken) showing the dynamics of the clone were used for the indication of the adoptive immunotherapy. When we analysed the prognostic value of the variables, MRD prior to HSCT was the most important prognostic feature in our cohort (p=0.00067), followed by the duration of CR1 (p=0.0083). We confirmed that level of MRD is the most important prognostic factor predicting outcome after allogeneic HSCT in paediatric patients with high-risk ALL. MRD detection before HSCT identifies the patients with the risk of relapse post transplant and allows tailoring of the conditioning regimen, graft-versus-host disease prophylaxis and the adoptive immunotherapy. Grant support: FNM 9735/2004, GAUK 62/2004.

O138
Molecular remission in relapsed/refractory chronic lymphocytic leukaemia and follicular lymphomas treated with RIC-allogeneic stem cell transplantation correlates with a better disease-free survival
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Allogeneic stem cell transplantation (allo-SCT) can be an effective salvage treatment for chronic lymphocytic leukemia (CLL) and relapsed follicular lymphomas (FLC). RIC regimens decreased conventional transplant-related morbidity/mortality and made allo-SCT a relatively safe option for pts with low-grade lymphomas. Aim of this phase II trial was to evaluate whether allo-SCT after RIC could induce durable clinical and molecular remissions (MR) in pts with relapsed/refractory CLL or FCL. Forty-four pts (23CLL and 21FCL) were enrolled in the study, median age was 54 years (32-69 years). All pts had been pre-treated with at least 1 chemotherapy regimen, 25% pts had failed an autologous SCT. Before transplant: 14pts (32%) were chemorefractory; only 10pts (23%) were in complete remission (CR). The RIC regimen included fludarabine and cyclophosphamide. All pts had a HLA matched sibling donor. Bc-2 or immunoglobulin heavy chain rearrangements were used as molecular marker. After allo-SCT, serial BM samples were analyzed for minimal residual disease by nested- PCR. Thirty-eight of 44 pts attained CR after transplant, 25 of them had a molecular marker (bcl-2=11, IgH=14). Twenty-five of 44 pts (57%) developed aGVHD (grade III-IV: 18%). cGVHD was observed in 22 of 42 evaluable patients (52%). There was no significant difference in the incidence of GVHD between PCR-positive and PCR-negative pts (p=0.63). Overall, at a median follow-up of 20 months, 9 pts (20%) experienced a progression. Seven of them never developed GVHD. At a median follow-up of 20 months (6-62 months) 15 of 25 pts (60%) with an available molecular marker were alive in molecular remission (MR), 9 of them never attained MR, 1 showed an intermittent pattern of monitoring was performed by real-time quantitative IgH PCR using allele-specific primers (ASO RQ-PCR). Results: 476 blood samples from 46 patients who had an informative primer and repeated follow-up material available were analyzed by ASO RQ-PCR with a minimum sensitivity of 1E-4. In 145 cases, BM samples obtained on the same occasion were analyzed in parallel. Blood and BM showed highly significant correlating MRD values (p=.0001; slope 0.9), with a tendency to higher MRD levels and higher sensitivity in BM. Myeloablative treatment with SCT resulted in a strong reduction of the CLL load in the blood (median MRD level 6.6E-3 pretransplant vs. 1.0E-4 at 90-180 days post transplant; p<.0001). Whereas MRD levels had no prognostic impact during the first 6 months after SCT, stable or decreasing MRD kinetics below 1E-4 between 6 and 12 months after SCT were strongly predictive for a favourable outcome (4-year progression-free survival (PFS) 100%). In contrast, PFS of those patients who had increasing MRD levels at this time was significantly poorer (4-year PFS 37%; p 0.02; n = 25). Patients with increasing MRD levels early post transplant almost exclusively belonged to the subgroup with unmutated VH.

Conclusions: Myeloablative SCT can lead to substantial additional reduction of the tumor load in patients with CLL in remission. The cause of the dismal post transplant outcome of patients with unmutated VH is faster regrowth of the leukemic clone rather than less effective reduction of MRD levels immediately after SCT.
PCR positivity after chemotherapy. Three of the persistently PCR positive pts relapsed after a median time of 9 months. These were all CLL pts and never developed GVHD before. The estimated-probability of DFS at 2 years for PCR-negative and PCR-positive patients was 100% and 57% respectively (p<0.01). This study suggests that: i) MR can be attained by RIC allo-SCT in the majority of relapsed CLL/FCL pts; ii) GVT can be rarely separated by GVHD; iii) PCR-positive patients without GVHD are at high risk of relapse.

O139 Characterisation of CD30+ cells in PBPC grafts in patients with Hodgkin’s lymphoma
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Previously we have found that CD34+ cell enrichment efficiently removes CD30+ cells from the PBPC graft in patients with Hodgkin’s lymphoma (HL) and that the removal of those cells results in an apparently lower relapse rate. The present study addresses whether the CD30+ cells in PBPC grafts are Hodgkin cells. Therefore, we analysed single CD30+ cells from the diagnostic tissue as well as from the PBPC grafts of three patients. Those cells were isolated with the Laser Capture Microscope after antibody staining for HLA-A2. Single-cells were subsequently analysed by PCR using primers to amplify the immunoglobulin heavy chain (IgH) complementary terminating region 3. The PCR products were then sequenced. In the three cases we found IgH rearrangements in 9/57 cells, 4/76 cells and 0/31 cells, respectively, isolated from the PBPC product. This is substantially lower than that obtained from CD30+ cells isolated B-cells, 15/37 cells, 5/38 cells and 3/31 cells, respectively. In addition, none of the CD30+ cells of the PBPC had the same sequence as the original Hodgkin cell clone. Also, multiparameter flow cytometric analysis of PBPC from additional HL patients revealed that most of the CD30+ cells in PBPC express macrophage markers. We therefore conclude, unexpectedly, that most CD30+ cells in PBPC do not represent Hodgkin cells but likely represent macrophages that my either specifically or non-specifically bind to anti-CD30. It is intriguing that the removal of those macrophages from PBPC results in a lower relapse rate, suggesting a tumour promoting role for those cells.

Infectious diseases 1

O140 Analysis of the adenovirus specific T-cell response, using MHC-I peptides of the adenoaviral hexon-protein for the detection and isolation of specific T-cells
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Human adenovirus (HAdV) infections remain a major problem in children and adults undergoing allogeneic stem cell transplantation (SCT), associated with high morbidity and mortality. Since the drug therapy available is still unsatisfactory, infection has to be controlled by regenerating T-cells. In patients receiving grafts from unrelated donors or haplo-identical donors, a prolonged period of reduced immunocompetence after SCT leads to a vulnerable phase for infection/reactivation of HAdV. In this situation a specific HAdV directed immunotherapy is desirable to control the virus and to prevent progression to potentially fatal disease. However, the specificity of the T-cell response to HAdV is still unclear. The adenoaviral Hexon protein has been shown to be a potent T-cell stimulatory agent. Recently a panel of MHC-restricted Hexon peptides have been published, but the MHC-restriction as well as the immunodominance of the described epitopes remained controversial. HLA-A2 restricted epitopes have not been published so far. We conducted an alignment of the amino acid sequence of the Hexon protein from clinical relevant HAdV serotypes. Using MHC-restriction algorithms and a prediction algorithm for proteasomal cleavages we predicted and synthesized 39 candidate nona- and deca-peptides of the adenoaviral Hexon protein for MHC-I restriction HLA-A1, -A2 and -A24. Peptides were analyzed for T-cell stimulatory capacity using IFN-γ Elispot, flowcytometric intracellular cytokine staining and CSFE assays. We identified four new relevant epitopes, with a reactivity in more than 60% of tested healthy donors. Two of the identified immunodominant peptides were HLA-A2 restricted, the most common HLA-I type. These identified epitopes are located within the highly conserved regions of the Hexon protein and ensure therefore crossreactivity among the different HAdV serotypes. Furthermore we observed an interindividual variety in the response pattern to Hexon epitopes. In children surviving HAdV infection post SCT, peptide specific T-cells could have been detected, suggesting in vivo relevance of the identified epitopes. Additionally HAdV specific T-cells were generated from healthy donors with the identified peptides using clinically grade protocols. In conclusion we detected new relevant HAdV T-cell epitopes. This paves the way for an improved detection of HAdV specific T-cells following SCT and enables the generation of virus specific donor lymphocyte infusions.

O141 Intestinal adenovirus infection with increasing viral load in stool is an early indicator of impending disseminated disease in children undergoing allogeneic stem cell transplantation
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Disseminated adenovirus (AdV) disease is associated with high mortality in allogeneic stem cell transplant (SCT) recipients. We have recently demonstrated that monitoring detection of AdV in peripheral blood (PB) precedes the onset of life-threatening virus disease (Lion et al., Blood 2003). In most instances, detection of AdV in stool preceded the onset of AdV viremia, thus raising the possibility that intestinal infections represent a potential source of virus dissemination. To address this question, we have performed another study in 100 consecutive pediatric patients transplanted at our center. Patients were monitored for the presence and the load of AdV in stool and in PB by a novel real-time PCR approach covering the entire spectrum of currently known human AdV serotypes in two PCR reactions (Ebner et al., submitted). Thirty six patients (36%) tested positive in serial stool samples, revealing adenoviruses of nearly all subgenera, with strong predominance of subgenus C. Some of the patients had diarrhea, but its occurrence did not seem to correlate with the AdV load detected. Nineteen patients revealed only low levels of AdV positivity, not exceeding 1x10^3 copies/g of stool and generally showed very slow virus proliferation kinetics. None of these patients displayed viremia during the subsequent post-transplant course. Seventeen patients had peak AdV levels in stool ranging from 5x10^5-10^6 copies/g. In this subset of patients, 12 (70%) showed rapidly increasing AdV levels in stool, and viremia was later detected in all instances. Seven patients with viremia developed signs of disseminated
Sequential emergence of multiple adenovirus serotypes after paediatric stem cell transplantation

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Objectives: Adenovirus infections have the potential to cause fatal disease after allogeneic stem cell transplantation (SCT), in particular in children. Adenoviruses occur in a variety of at least 50 serotypes and earlier studies relied only on serotyping of initial viral isolates, if analysis of serotypes was included at all. Insight into simultaneous occurrence of serotypes is technically difficult, while sequential occurrence of serotypes could be approached by a sustained analysis of viral isolates through a longer period. This study intended to analyze serotypes at regular intervals in a cohort of adenovirus infected adenovirus-infected patients.

Methods: 28 consecutive pediatric SCT recipients treated in Leiden and positive for adenovirus in culture were included. From these patients, 246 different specimens (feces, throat swabs and urine) were positive for adenovirus. 96 viral isolates from specimens taken at the start, during and at the end of an infection episode were serotyped. Episodes were differentiated by the occurrence of at least 2 culture-negative fecal specimens and were at least one month apart. Serotyping was carried out by classical neutralization with pooled antisera on A549 cells, followed by type-specific neutralization. Genotyping by sequence analysis was added for 19 isolates.

Results: 43 different adenovirus isolates were detected in 28 patients, including 4 isolates without an assigned serotype. In 18 patients (64%) only one serotype was detected, 10 patients (36%) showed multiple serotypes (2 in 7 patients, 3 in 2 patients, 4 in 1 patient). In 6 patients with multiple infections, serotype 31 was the initial serotype, only 1 single-serotype infection was caused by this virus. Feces and throat swabs of the same sample date (n=10) always showed identical serotypes, simultaneously obtained feces and urine (n=8) isolates were different in 2 cases. Genotyping by sequencing demonstrated identity at the adenovirus subgroup level in 100%, at the serotype level in 80%, when conclusive results could be obtained.

Conclusion: More than one single serotype of adenovirus can be detected after SCT in a substantial proportion of cases. A sequential emergence of dominant serotypes is observed during immunological reconstitution. This finding will be relevant for diagnostic purposes, for immunotherapeutic interventions and for insight in the pathogenesis of adenovirus infections after SCT.

Isolation of donor derived CMV specific T-cells for the treatment of CMV disease in patients after in-vitro T-cell-depleted allogeneic stem cell transplantation

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Despite the introduction of new anti-viral drugs, human cytomegalovirus (CMV) infection remains a major cause of morbidity and mortality in patients undergoing allogeneic stem cell transplantation (allo-SCT). To allow the application of allo-SCT in older patients and in haploidentical donor/patient pairs transplantation protocols have been developed using T cell depleted stem cell grafts to reduce the risk of inducing severe graft-versus-host disease. A drawback of this transplantation regimen is the delayed reconstitution of CMV-specific T cell responses resulting in increased incidence of CMV reactivation.

Rapid selection of CMV specific T cells based on their ability to produce cytokines in response to virus-specific peptides or proteins may result in large-scale generation of CMV specific T cells for adoptive immunotherapy. In the current study we selected HLA-A*0201 and HLA-B*0701 positive CMV-seropositive donors, isolated peripheral blood mononuclear cells (PBMC), and specifically stimulated the circulating CMV-specific memory cells with CMVpp65-derived peptides. After overnight culture CMV-specific T cells were isolated using the interferon-gamma (IFNg) secretion assay and MACS separation, and expanded in-vitro in the presence of autologous feeder cells and IL-2. We performed a feasibility study in a cohort of 10 donors with frequencies of circulating CMV-A*0201 or CMV-B*0701 tetramer positive CD8+ T cells ranging from 0,1-0,7%. CD8+ T cell lines could be generated containing 55-95% CMV-tetramer positive T cells. These T cells could be expanded in-vitro 10-50 fold within a period of 10 days. In cytotoxicity assays we demonstrated CMV specific cytotoxicity.

CD4+ helper T cells are probably relevant for in vivo persistence of CMV responses. However, specific HLA-class II binding CMV peptides essential for the isolation of CD4+ T cells are not available. Therefore, to allow the isolation of CMV specific CD4+ helper cells, experiments are being performed in which we stimulate CMV-seropositive donor PBMC with human recombinant pp65CMV protein. After overnight stimulation we were able to isolate 0,1-0,5% CD4+ and CD8+ T cells which specifically produced IFNg in response to the addition of the CMV protein.

In conclusion, we have demonstrated that it is feasible to generate sufficient numbers of CMV-specific T cells for adoptive transfer. These T cells will be used to treat patients with persistent CMV reactivation after allo-SCT.

Quantification of the HCMV-specific CD4+ and CD8+ T-cell immune response in children receiving allogeneic haematopoietic stem cell transplantation

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Objective: To study the development of HCMV-specific CD4+ and CD8+ T cells in pediatric patients in the first year after allogeneic hematopoietic stem cell transplantation (HSCT). Methods: A new technique was developed to simultaneously detect (by IFN-g intracellular staining) HCMV-specific CD4+ and CD8+ T cells using HCMV-infected autologous dendritic cells as antigenic stimulus. We prospectively quantified on a monthly basis HCMV-specific T cells until 6 months after transplantation. Patients enrolled into this study were monitored for HCMV infection in blood by quantitation of either virus disease and died despite treatment with cidofovir administered upon detection of AdV in PB. Our observations therefore indicate a high risk of progression of intestinal to invasive AdV infection in patients with high viral load and rapidly expanding virus copy numbers in stool. Quantitative monitoring of AdV in stool and early onset of antiviral treatment based on virus proliferation kinetics may be warranted in attempts to prevent progression to life-threatening systemic infection.
antigenemia or DNAemia, and, in the presence of viral infection, were given pre-emptive therapy. Fifty patients, receiving HSCT from either an HLA-identical sibling (n=21), or an unrelated donor (n=20), or a T cell-depleted HSCT from a haploidentical relative (n=9), were prospectively monitored.

Results: In 29/34 (85.3%) HCMV-seropositive HSCT recipients HCMV-specific T cells were detected in blood within day +60. At that time, HCMV-specific CD4+ T cell count was comparable to that of controls, while HCMV-specific CD8+ T cell count was higher, with no significant changes thereafter. HCMV infection was detected in blood of 22/29 patients. It was either self-limiting (n=12) or required ganciclovir course (n=10) which was shorter (median 7 days, range 5-16) than that of patients with delayed immune reconstitution (n=5; median 49 days, range 31-82, p<0.001). None of these patients developed HCMV disease or late HCMV infections. Neither the type of donor employed nor manipulation of the graft influenced recovery of virus-specific immunity. HCMV-specific response was detected in only 3 (18.8%) out of the 16 HCMV seronegative recipients (one of whom developed detectable HCMV infection in blood).

Conclusion: Effective HCMV-specific T cell immunity can promptly develop in children given HSCT (regardless of donor type, T-cell depletion of the graft or donor HCMV serological status), particularly in seropositive recipients in whom latent virus may be a major antigenic stimulus for rapid reconstitution of T cell immunity. On the other hand, our data suggest that maintenance of memory T cell immunity transferred from seropositive donors to seronegative recipients seems to be a rare event. In view of these results, it is reasonable to hypothesize that antiviral intervention be decided also taking into account reconstitution of HCMV-specific T cell immunity.

O145
Evaluation of cytomegalovirus-specific cytotoxic T-lymphocytes in patients with HLA-A*02 or HLA-A*24 phenotypes undergoing haematopoietic stem cell transplantation
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Cytomegavirus-specific cytotoxic T-lymphocytes (CMV-CTL) are essential for the control of CMV reactivation and CMV disease. To accurately monitor the quantity and function of CMV-specific T-cell immunity, haematopoietic stem cell transplantation (HSCT), two dominant CMV pp65 epitope peptides that bind to HLA-A*0201 NLVPVMVATV (A*02NLV) and HLA-A*2402 QYDPVAAFL (A*24QYD) were evaluated for immunological potential. The samples from 63 patients with HLA-A*02 or HLA-A*24 serotype were analyzed by 1) tetramer assay for evaluating the number of CMV-CTL, and 2) intracellular cytokine staining and ELISPOT assay for the function. We disclosed that the number of A*02NLV-specific CMV-CTL was significantly higher than that of A*24QYD (3.51% vs 0.06% /CD8). The frequency of IFN-g producing cell was also higher, with no significant changes thereafter. At that time, HCMV-specific CD4+ T cell count was comparable to that of controls, while HCMV-specific CD8+ T cell count was higher, with no significant changes thereafter.

O146
Risk factors for development of CMV disease after allogeneic stem cell transplantation in the era of viral load monitoring
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Background: Viral load measurements have been introduced in the routine management of allogeneic SCT recipients and a higher viral load and the rate of increase in viral load have been suggested to influence the risk for CMV disease.

Objective: The aim of this study was to analyze risk factors for CMV disease in the era of preemptive therapy based on quantitative PCR.

Patients and methods: 164 consecutive patients were monitored by quantitative PCR. Peripheral blood leukocyte samples were analyzed weekly by a real-time PCR technique. Preemptive therapy was introduced on varying viral loads with the aim to establish a safe cut-off level.

Results: 106 of 164 (65.8%) patients had CMV DNA detected at least once by PCR. 94/164 (57.3%) patients representing 88.6% of patients with CMV DNA detected received antiviral therapy. Ten of 164 (6.1%) patients developed CMV disease: three patients had interstitial pneumonia, four patients gastrointestinal disease, and three patients developed CMV retinitis. The median time to CMV disease was 96 days (39-164 d). Four patients developed CMV diagnosed after day 100. Patients who developed CMV disease had higher peak viral load (10log 3.5; SE +/- 0.26; p=0.02) than patients without CMV disease (10log 2.7; SE +/- 0.09), while initial viral load, viral load at initiation of therapy, or delta viral load (defined as 10log at initiation of therapy - 10log initial viral load) had no impact. Donor/patient serologic status before SCT (D+/R- and D-/R+ > D+/R+) and grade of acute GVHD significantly influenced the viral load while donor type, conditioning, and stem cell source did not. In multivariate analysis acute GVHD and donor type were independent risk factors for CMV disease. Viral load was included in different ways into the multivariate models (initial viral load, peak viral load, viral load at initiation of therapy, and delta viral load) and none of the measurements had a significant impact on the risk for CMV disease when corrected for GVHD and donor/recipient status.

Conclusion: We conclude that viral load has no impact on the risk for CMV disease when corrections are made for other risk factors.

O147
Molecular serotype analysis of adenoviruses: clinical implications
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Adenovirus (AdV) infections are a major cause of morbidity and mortality in allogeneic stem cell transplant (SCT) recipients. Human adenoviruses represent a large family, currently including 51 serotypes, which are divided into 6 species (A-F). We have recently demonstrated by a species-specific RQ-PCR approach covering the entire spectrum of human adenoviruses that molecular detection of AdV in
peripheral blood precedes the onset of life-threatening virus disease, and provides a basis for early preemptive treatment (Lion et al., Blood 102(3):1114-20, 2003). An association of AdV species with clinical manifestation and response to antiviral therapy has been reported, but little is known about the clinical role of individual AdV serotypes. In immunocompromised patients, the use of serological testing for identification of adenovirus serotypes is limited due to the impaired immune response. We have therefore determined the nucleotide sequence of the complete AdV hexon gene in all 51 human serotypes, and identified regions permitting rapid serotyping at the molecular level. Serotypes belonging to the species A, B, C, E, and F, can be determined by fragment length analysis of a single PCR product, respectively. Serotype identification within the largest AdV species D requires sequencing of a single 300bp PCR amplicon. In view of the great predominance of species C in our region, we have also established real-time PCR tests permitting identification of its four serotypes. Analysis of all AdV C positive cases within more than 6,000 clinical specimens investigated at our center over the past years revealed the highest prevalence for serotype 2 (57%), followed by the serotypes 1 (39%) and 5 (4%). In some instances, two different AdV C serotypes were present simultaneously. We have demonstrated that the identification of specific virus strains within individual AdV serotypes, which may be required for investigation of possible transmission of infections within the hospital, can be achieved by sequencing of PCR products derived from an appropriate AdV target region within the hexon gene. The possibility of rapid molecular serotype and strain analysis provides a basis for studies on adenovirus epidemiology, and may in future have implications for the selection of the most appropriate antiviral treatment.

Severe aplastic anaemia / Autoimmune diseases

O148 Clonal T-cell populations attacking the haematopoietic tissue are responsible for immune-mediated aplastic anaemia and pathogenically related marrow failure syndromes

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Inhibitory cytokine network activity in the marrow of aplastic anaemia patients with active and quiescent disease

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IFNγ and TNFα mediate the damage on haemopoietic cells in Aplastic Anemia (AA). IL-12 stimulates T-cells to secrete IFNγ. In 19 AA pts [13 M, 6 F, mean age 16.5 yrs; 9 with Active Disease (AD) - i.e. at onset or relapse - 10 with Quiescent Disease (QD) - i.e. still responding to IS] and in 10 normal controls (ctrl) we investigated: 1) Marrow intra-lymphocyte (CD3+) and intra-monocyte (CD14+) expression of TNFα, IFNγ. IL-12 (only in CD14+) and IL-4 (only in CD3+) by flow cytometry and MoAb. 2) Percentage difference in BFU-e growth before and after block of TNFα (by anti-TNFα molecule Etanercept) and IFNγ (by anti-IFNγ MoAb) effects. Cytokine expression: Data are as median percentages of positive cells. IFNγ. CD3+ / IFNγ+ cells were significantly higher in both AA groups (17% for AD and 19.5% for QD; p < 0.001 for both) than in ctrl (5%), while CD14+/IFNγ+ cells were significantly higher in QD pts (10% vs. 4.5% - p=0.0005-) and borderline higher in AD (10%, p=0.05). CD3+/TNFα+ and CD14+/TNFα+ cells were significantly increased both in AD (10% vs. 2% of ctrl - p=0.0008 and 0.003 respectively). CD14+/TNFα+ cells were significantly increased only in AD vs. ctrl (10 vs. 4.5%, p=0.03).

IL-12 was significantly increased both in AD (78%, p=0.0006) and in QD (77%, p=0.006) compared to ctrl (24.5%, IL-4). No differences was observed between ctrl and either AD or QD pts. COLONY ASSAY Neutralisation of both TNFα and IFNγ, caused a percentage increment of BFU-e growth significantly greater in both AD (13.4%- p=0.01) and QD (53.3%- p=0.007) pts vs. ctrl (0%). Neutralisation of IFNγ incremented BFU-e more in AD (35.4%) and QD (10.4%) pts, than in ctrl (1.75%); statistical significance was reached only for QD pts (p=0.046). TNFα
block induced a not significant BFU-e increment in AA pts over ctrl.

Conclusion: This study:
1) Confirms that IFNγ and TNFα are overproduced in the marrow lympho and monocytes of AA pts both with Active and Quiescent disease.
2) Shows the novel data that also IL12 is overexpressed in marrow monocytes of AA pts both with Active and Quiescent disease. This infers that IL12, as an IFNγ stimulator may participate to the harmful cytokine network on hemopoetic cells.
3) Show that blocking IFNγ and TNFα effects improves erythropoiesis in vitro thus confirming the key role for these cytokines in marrow damage.
4) Show that also pts responding to IS still retain a cytokine pressure which may possibly explain the disease recurrence.

O150
In vivo anti-CD52 monoclonal antibodies in bone marrow transplantation for acquired aplastic anaemia

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Chronic graft versus host disease (GVHD) remains a major problem in bone marrow transplantation (BMT) for aplastic anaemia (AA). We report results using anti-CD52 monoclonal antibodies (MoAb), Campath-1G (1989-1998) and Campath-1H (1999-2004) for BMT performed for acquired AA at St George's Hospital, from HLA identical sibling donors (n=33) and unrelated donors (UD) BMT (n=10); Unmanipulated bone marrow was used in all but one patient. For sibling BMT, conditioning was with cyclophosphamide (CY) 50mg/kgx4 and anti-CD52 MoAb 0.75-1mg/kg. GVHD prophylaxis was cyclosporin (CSA) alone in 19 patients; 14 also received anti-CD52 MoAb. For UDBMT, conditioning was CY 50mg/kgx4 and Campath-1G (n=6); CY 50mg/kgx4, fludarabine (FLUD) 30mg/m²x5 and Campath-1H (n=1); CY300mg/m²x4, FLUD 30mg/m²x5 and Campath-1H (n=3). All received CSA, except one with renal impairment who received mycophenolate instead. 20/43 patients (14 sibling and 8 UD) had failed prior ATG therapies. For sibling BMT, graft failure occurred in 8 (4 early and 4 late), of whom 4 survive, 3 with autologous recovery. Cumulative incidence of acute and chronic GVHD was 14 and 4%, respectively. There was only one case of chronic GVHD (Gd I) 5 year survival was 81% (CI 68-96%) with median follow up of 59 months. Survival improved for BMT after 1995 (93%) compared with 74% survival before 1995. Of 19 recipients positive for CMV, CMV reactivation occurred in 5 (26%) within 100 days. There was no late CMV reactivation. Fatal autoimmune cytopenia occurred in one case and EBV lymphoproliferative disease in one, which responded completely to CSA withdrawal and DLI. Performance status for all patients is 100% except for one who developed avascular necrosis of the hip. For UDBMT, 8/10 are alive. All patients were fully HLA matched on high resolution DNA typing, except in 2, who both died. Graft failure occurred in 5 (4 early and 1 late) but 4 are alive with autologous recovery. There were no cases of chronic GVHD and only one case of acute (Gd I) GVHD. There was one case of pneumocystis. One case developed immune thrombocytopenia purpura at 11 months post BMT. We conclude that anti-CDMoAb is associated with a low incidence of acute GVHD and almost absent chronic GVHD. Graft failure was relatively high but many patients were rescued by autologous recovery. Graft failure appears to be reduced when using CSA alone as GVHD prophylaxis.

O151
Late complications and quality of life after allogeneic stem cell transplantation for severe aplastic anaemia

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Objectives: The aim of this study was to assess long-term outcome in patients with SAA undergoing SCT and to identify the factors associated with late LC and QOL.

Patients and Methods: A questionnaire was send by mail for participation to patients aged under 14 at the time of HLA-matched SCT performed from 1991 to 2001. Detailed data on 138 patients were submitted from 41 institutions in Japan. This study focused on 109 out of 138 patients who survived for at least 6 months and who did not experience graft rejection or receive second transplant. 82% of patients received marrow or peripheral blood from related donors, while 18% received marrow from unrelated donors.

Results: With a median follow-up of 88 months (range 48 to 144 months) the 10-years survival was 92%. The assessment of QOL with SF-36 questionnaire revealed that the scores of three domains (role-physical, role-emotional, and social-functioning) remained lower than those of general population during the first 2-4 years after SCT, then returned to normal thereafter. No significant differences were observed in the scores of other domains between the patients and general population. Chronic graft-versus-host disease (CGVHD), which had developed in 33.6% at 6 years after SCT, was the most frequent late event. The most frequent LC was infection requiring hospitalization (31%) followed by eye (11%), liver (11%), endocrine (7.3%), pulmonary (6.5%), bone/joint (6.4%), and others (5.5%). Secondary malignancies were observed in 4 patients (3.7%). Both Karnofsky and SF-36 scores correlated with the presence of LC (P<0.0001). If LC of each organ were analyzed separately, all except for the liver also significantly associated with lower scores (infection p=0.011, eye p=0.017, lung p=0.019, bone p=0.0042, endocrine p=0.011). Multivariate analysis identified CGVHD, frequent transfusion, male sex, and acute GVHD as the risk factors of developing secondary malignancies were identified. Conditioning (Cytophosphamide; CTX + ATG/ALG vs. CTX + radiation) had no significant effect on the incidence of late complications (54% vs. 52%) and secondary malignancies (4% vs. 2%) Conclusion: LC after SCT for SAA remain as a major cause of impaired QOL in the minority patients, and this retrospective study suggests that efficient control of CGVHD and timely transplantation, but not conditioning, could reduce LC and improve QOL.

O152
Autologous haematopoietic stem cell transplantation in patients with severe and refractory Crohn's disease

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Crohn’s disease (CD) is an immunologically mediated inflammatory disease of the gastrointestinal tract. A high mortality and morbidity is expected in severe refractory cases and a new treatment approach is needed. In theory, maximum immune ablation followed by autologous hematopoietic stem cell transplantation (HSCT) can induce remission. We conducted a phase I HSCT study in 12 patients with severe CD. Candidates were less than 60 years old with a Crohn’s Disease Activity Index (CDAI) of 250-400 despite conventional therapy including infliximab. Peripheral blood stem cells were mobilized with cyclophosphamide and granulocyte colony-stimulating factor and CD34+ enriched.
The immune ablative (conditioning) regimen consisted of 200mg/kg cyclophosphamide and 90mg/kg equine antithymocyte globulin. The procedure was well tolerated with anticipated cytopenias, neutropenic fever, and disease-related fever, diarrhea, anorexia, nausea, and vomiting. The median days for neutrophil and platelet engraftment were 9.5 (range 8-11) and 9 (range 9-18), respectively. The initial median CD4A was 291 (range 250-358). Symptoms and CD4A improved prior to hospital discharge, while radiographic and colonoscopy findings improved gradually over months to years following HSCT. Eleven out of twelve patients entered a sustained remission defined by a CD4A < 150. After median follow-up of 18.5 months (range 7-37), only one patient has developed a recurrence of active CD which occurred 15 months post-HSCT.

Autologous HSCT may be performed safely and has a marked salutary effect on CD activity. A randomized study will be needed to confirm the efficacy of this therapy.

O153 Immune reconstitution after autologous bone marrow transplantation in systemic sclerosis

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Objective: To characterize the determinants of clinical response after hematopoietic stem cell transplantation (HSCT) in systemic sclerosis (SSc), we analysed hematopoietic and immune reconstitution in 7 patients the year after HSCT.

Methods: Two groups of patients were retrospectively constituted according to the observed clinical responses (group A, n=4) or none or relapse (group B, n=3) after HSCT. T cell reconstitution was analyzed every 3 months prior to potential reintroduction of immunosuppression using lymphocyte immunophenotyping, Immunoscope profile for TCR beta chain CDR3 analysis and TREC measurements by real time quantitative PCR.

Results: (mean ± SD, Mann-Whitney U rank test and linear regression slope (LRS) analysis). Patient characteristics were similar at entry except for a lower Rodnan’s modified skin score (p = 0.03) and SHAQ (p = 0.05) in group A versus B. Absolute values of CD3+, CD4+, CD8+, CD4+CD45RO+, CD4+CD45RA+ were normal for all patients before HSCT. CD3+ T cells remained low thereafter and CD8+ values were back to normal at 3 months in both groups. B cell numbers were below normal up to 9 months in group A and back to normal at 3 months in group B. Two immune reconstitution profiles appeared with sustained CD3+ defect in group A (LRS = -2.29) with opposite trend in group B (LRS = 2.87) plus more rapid CD4+ reconstitution in group B (LRS = 3.15) than A (LRS = 0.90). B cell evolution varied slightly in group A (LRS = 1.23) and rapidly increased from 3 to 6 months after HSCT in group B (LRS = 17.71). CD4+CD45RO+ profiles were similar, but CD4+CD45RA+ reappearance pattern was faster in group B (LRS = 4.45) than group A (LRS = -0.87). The alphabeta T-cell repertoire was disturbed with overexpression of skewed and negative families before and up to at least one year after HSCT. TREC levels before HSCT were lower in all patients compared to controls (r = 0.009). TREC values correlated with C-reactive protein (CRP) levels (r = -0.41, p = 0.001) and CD19+ (r = 0.35, p = 0.001) and CD20+ (r = 0.34, p = 0.002) lymphocyte counts.

Conclusion: B and T lymphocyte populations remained disturbed for at least 1 year after HSCT in SSc patients suggesting the persistence of an underlying disease mechanism.

O154 Autologous haematopoietic stem cells transplantation in severe scleroderma: ISAMAIR four years after, long-term results from the French multicentre phase II-I study

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Objectives: To report the long term results from a national, non-randomised, open phase I-II trial of Autologous Hematopoietic Stem Cells (HSC) transplantation with CD34+ selection in severe scleroderma (SC).

Methods: Eligible patients with severe diffuse SC as defined in the ISAMAIR study (1) underwent HSC mobilization with cyclophosphamide (CYCLO) (4g/m²) + G-CSF (5microg/kg/day) or G-CSF alone (10 microg/kg/day) if left ventricular ejection fraction (LVEF) < 40% until last apheresis to obtain at least 2.5x 106 CD 34+ /kg. Conditioning used CYCLO (200 mg/kg) or melphalan (140 mg/m²) if LVEF <40% prior to CD34+ HSC reinfusion. Evolution was recorded daily up to the end of the aplasia and then every 3 months. We analysed: 1) the feasibility of HSC mobilization, CD34+ selection and of the intensification procedures; 2) the short and long term clinical response segregating major (MR), partial (PR) and no response (NR), disease progression (PD/RO) or relapse (REL) (2) by modified major (MR2) or partial (PR2) responses according to repeated evaluation of functional (Performance status and SHAQ) and major organ (skin, heart, lung and kidney) involvement (1).

Results: (median, range): 14 SC were included in 40 months. HSC mobilization was successful in 12 SC. Yield was not enough to allow CD34+ selection in 1 SC. Autologous peripheral (12) or bone marrow (1) transplantations were performed with 1 procedure related death. Nine patients responded within 6 (3-12) months: 6MR, 3PR. No response was observed in 3 patients. After 45 (36-52) months of follow-up, 8 patients are alive of whom: two in MR1, one in MR2 9 months after successful treatment of relapse by Cellexcept and four patients are in PR2 after relapse occurring within 12 (6-18) months after PBSCT responded to reintroduction of immunosuppression. Four patients died from PRO at 12 (6-18) months.

Conclusion: Autologous HSC transplantation is feasible with low toxicity and significant clinical benefits in SC, underlying the need to compare HSC to monthly CYCLO in European phase III ASTIS trial (www.astistrial.com).

1) Farge D et al BJH 2002; 114-119

O155 Long-term follow-up in patients with multiple sclerosis after high-dose chemotherapy and autologous stem cell transplantation

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Objectives: During the last several years high dose chemotherapy+autologous stem cell transplantation (HDCT+ASCT) is more often used as a therapeutic option for multiple sclerosis (MS) patients. Data on treatment outcomes at long-term follow-up are worthwhile to examine. The aim of this study was to provide clinical and quality of life (QoL) monitoring in MS patients before and at different time-points after HDCT+ASCT.

Methods: Thirteen patients with MS were included in the study. All patients previously underwent conventional transplantation with CD34+ selection in 1 SC. Autologous peripheral (12) or bone marrow (1) transplantations were performed with 1 procedure related death. Nine patients responded within 6 (3-12) months: 6MR, 3PR. No response was observed in 3 patients. After 45 (36-52) months of follow-up, 8 patients are alive of whom: two in MR1, one in MR2 9 months after successful treatment of relapse by Cellexcept and four patients are in PR2 after relapse occurring within 12 (6-18) months after PBSCT responded to reintroduction of immunosuppression. Four patients died from PRO at 12 (6-18) months.

Conclusion: Autologous HSC transplantation is feasible with low toxicity and significant clinical benefits in SC, underlying the need to compare HSC to monthly CYCLO in European phase III ASTIS trial (www.astistrial.com).

1) Farge D et al BJH 2002; 114-119
There was also a significant age effect, the actuarial survival treatment. Clinical and QoL evaluation was provided at baseline, at discharge, at 3, 6, 9, 12 months, and then every 6 months after HDCT+ ASCT. MRI was conducted at baseline, at 6, 12 months, and at the end of follow-up. QoL was assessed by FACT-BMT and FAMS. Integral QoL index was assessed by the method of integral profiles. Median EDSS at base-line was 6.0 (range 1.5 - 7.5). The median follow-up duration was 36.3 months (range 8-66 months).

Results: Clinical examination and MRI revealed disease stabilization (DS) in eleven patients and disease progression (DP) in two patients. EDSS decreased in eight patients, increased from 6.5 to 7.0 and from 6.0 to 6.5 in patients with DP, and remained stable in three patients. Significant QoL improvement was observed in all patients with DS at the end of follow-up. 66 month disease-free period was observed in patient F. (female; 38 years old; cerebral-spinal secondary progredient subtype; EDSS – 5.0; ASCT 31.05.99) with maximum follow-up. In addition, integral QoL index doubled which pointed to good QoL response.

Conclusion: HDCT+ASCT in MS patients resulted in DS in eleven out of thirteen patients under observation. Along with clinical stabilization dramatic improvement of QoL took place. Long term follow-up of patients with disease-free period and good QoL response confirms that HDCT+ASCT can be considered an effective treatment for MS patients.

### Working Party: Aplastic anemia

#### 173 Treatment of severe aplastic anemia with antilymphocyte globulin, cyclosporine and granulocyte colony stimulating factor 5 µg vs 10 µg/kg: final analysis of a GITMO prospective randomised study

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Background. In a previous study we showed encouraging outcome in severe aplastic anaemia (SAA) patients treated with anti-lymphocyte globulin (ALG), cyclosporin (CyA) and G-CSF 5 µg/Kg/day. However failure to respond, delayed responses, partial responses, relapses and early deaths remain significant problems. The aim of the present study was to test whether an increased dose of G-CSF (10 µg/Kg/day) would reduce these complications.

Aim of the study. This is a multicenter prospective trial in 77 SAA patients treated with horse ALG (15 mg/kg/day day 1-5) and CyA (5 mg/kg/day day 1-180). Patients were randomized to receive G-CSF 5 µgKg/day (n=38, group A) or 10 µg/Kg/day (n=39, group B) from day +1 to day +30. All patients then received G-CSF 5 µg/Kg/day from day +31 to day +90. Primary end point was response at day +120. Secondary end points were early deaths, blood counts at day +120, and survival.

Results. At day +120 responses were classified as absent, partial, complete in 12, 22, 4 patients in group A and in 23, 7, 9 patients in group B (p=0.001). At last follow up these figures were respectively 9,12,17 vs 19,2,18 (p=0.004). Thirteen patients (5 in group A and 8 in group B) died before day 120 (p=0.3). Median peripheral blood counts an day 120 were respectively 9,12,17 vs 19,2,18 (p=0.3). Neutrophils 2.4 vs 1.9x10^9/l (p=0.4) and platelets 42 vs 36 x10^9/l (p=0.3). The actuarial survival at 4 years being 81%, 79%, 34% for patients aged 0-20, 21-40 and over 40.

Conclusions. Increasing the dose of G-CSF does not appear to reduce early deaths, does not improve peripheral blood counts nor survival, and may reduce the response rate in patients with SAA receiving ALG and CyA.

This trial highlights the uncertainties in the use of growth factors for patients with marrow failure: at present we would urge Centers to enter patients in the ongoing EBMT randomized trial comparing ALG,CyA with or without G-CSF 5 µg/Kg. The expected 360 patients who will be enrolled in that study may give us a definitive answer as to the place of G-CSF in the management of this rare and complex hematologic disorder.

#### 176 H-Y, a relevant transplantation antigen in HvG and GvH direction. An analysis in HSCT patients with aplastic anaemia

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Background: Increased GvHD risks are well documented for HSCT from female donors into male recipients. Single cases of graft rejection mediated by H-Y in female recipients have also been reported, but no study has yet documented an effect of H-Y recognition in HvG direction.

Patients and methods: We retrospectively investigated the outcome of 1443 T-cell replete HSCT from HLA-identical donors performed for SAA between 1971 and 2002. Only patients receiving conditioning with chemotherapy with or without radiation and post-grafting GvHD prophylaxis were included.

Patients were analyzed in two categories: M into F (M->F, n=199) were compared to F into F (F->F, n=181); F into M (F->M, n=310) patients were compared to M into M (M->M, n=340). Because of interactions, patients receiving conditioning with ATG (n=413) were investigated separately. The Kaplan-Meier estimator was used for univariate, Cox proportional hazards models for multivariate analysis.

Results: In female patients without ATG conditioning, overall survival was worse in the M->F group compared to the F->F group. Kaplan-Meier estimated overall survival was 69% for F->F patients, compared to 61% for M->F patients (p=0.12). In a multivariable model accounting for other pre-transplant risk factors (interval diagnosis/transplant, donor type, conditioning, GvHD-prophylaxis, stem cell source, patient age), the relative risk of death of M->F patients compared to F->F patients was 1.62 (95% CI 1.31-2.31, p=0.009). Increased mortality of M->F patients was associated with a higher risk of late graft failure. In male patients without ATG conditioning, survival was worse in the F->M group compared to the M->M group; Kaplan-Meier estimated overall survival was 65% for the M->M group vs 49% in the F->M group (p<0.001). In the Cox model, the relative risk of death for F->M vs M->M was 1.76 (95% CI 1.36-2.29, p< 0.001). Increased mortality was attributable mainly to higher incidence and severity of GvHD.

Conclusions: The increased risk of rejection (HvG) in female patients with male donors and the increased risk of death from GvHD in male patients with female donors confirms the clinical relevance of H-Y encoded gene products in transplantation. It appears that ATG as part of the conditioning regimen mitigates these effects.
Working Party: Infectious diseases

204 Recommendations for vaccination of stem cell transplant recipients
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Over the last 25 years the numbers of hematopoietic stem cell transplant (SCT) patients have increased rapidly. Infections have been major obstacles for successful transplantations. Thus, infection prevention is very important in transplant recipients. As the results of transplantation have improved, the number of long-term survivors has increased. Vaccination is a potentially important strategy for reducing the risk for vaccine-preventable infections after stem cell transplantations. Important considerations for a vaccination program are efficacy of vaccination in the immunocompromised situation and the risks for side effects. The EBMT produced recommendations for vaccination of SCT recipients published in Bone Marrow Transplantation in 1995. The Infectious Diseases Working Party of the EBMT has updated the recommendations based on current knowledge. The new recommendations will be presented.

205 Comparison between early and late immunisation with three doses of an heptavalent pneumococcal conjugate vaccine in allogeneic stem cell transplant recipients (EBMT IDPW01 trial)
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S. pneumoniae invasive infection remains a significant cause of death among allogeneic stem cell transplanted patients. The polysaccharide 23-valent vaccine (PS23) induces a limited antibody response in the SCT population. Conjugate vaccine are more immunogenic than PS vaccines in the normal population. The goal of this study is to compare the immune response to early (d100) versus late (9 months) post-transplant immunization with three doses of the heptavalent pneumococcal conjugate vaccine (Prevenar®, Wyeth-Lederlé) given at one month interval, in allogeneic stem-cell recipients.

This is an EBMT prospective, randomised, open study conducted in 15 EBMT centers of 7 countries, and supported by Wyeth-Lederlé. Randomisation is done between day 85-day 109, to receive 3 injections of Prevenar® at one month interval, beginning either on d100 ± 10 (Early Group), or at 9 months ± 10 days (Late Group), followed by one dose of PS23 administered at 12 and 18 months post-transplant, respectively. The primary endpoint is the percentage of responders, defined as an antibody titer >= 15 µg/ml, to all the 7 serotypes present in the conjugated vaccine, one month after the third dose of Prevenar®. Patients transplanted with a non-myeloablative regimen are not included, as those with S. pneumoniae infection between transplant and d100. S. pneumoniae invasive infections are collected until 24 months post-transplant. Up to November 15, 2004, 150 patients were included.

This study has been supported by Pfizer.

206 An internet register for invasive pulmonary mycosis
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Background: Estimates of how many cases of invasive pulmonary mycosis develop amongst recipients of an HSCT vary because there is no adequate mean of registration. To meet this need we designed and tested an electronic register called DiFend employing the definitions of the EORTC/MSG consensus group (Ascioglu et al Clin Infect Dis 2002;34:7-14) to record cases of IPM that occurred in each of our 3 hospitals.

Methods: The database was constructed by PharmaNetX BV and divided into 3 parts: 1) recruitment of patients with unexplained fever or who were receiving antifungal therapy 2) demographic data and 3) diagnosis, treatment and outcome which included Host factors, clinical features (chest X-ray, CT scan, physical symptoms), mycology (microscopy, culture, galactomannan antigen), EORTC/MSG class (Proven, Probable, Possible, Not classified).

The programme was accessed using Internet Explorer (Microsoft) with each user having a unique user name and password and all data were entered without infringing the patient's identity. The project was financially supported by Pfizer BV.

Results: Thus far 63 adult cases have been entered registered of which 20 (32%) were considered probable or proven IPM

Conclusion: The DiFend programme is sufficiently user friendly and secure and will be extended to other hospitals interested in registering IPM.
211 Correction of severe primary immunodeficiencies by bone marrow transplantation: report from the the Italian Association of Haematology and Oncology (AIEOP) registry
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Italian children affected by severe primary immunodeficiencies who underwent bone marrow transplantation (BMT) are registered within the Italian Association of Haematology and Oncology (AIEOP) registry since 1986. Severe combined immunodeficiency (SCID) affected 71 children, while 45 children (30 Wiskott-Aldrich syndrome, 7 chronic granulomatosus diseases, 2 HLA class II deficiency, 2 Chediak-Higashi syndrome, 2 XLA, 2 CID) were affected by non-SCID primary immunodeficiencies. As concerns SCID patients, 11 children required a second transplant. In this group of patients, mean time between diagnosis and BMT was 4 months, while in non-SCID patients was 20 months.

Among the SCID children 15 had a family matched donor (MFD), 23 a matched unrelated donor (MUD), 33 a partially matched family donor (PMFD), and 23 an unrelated donor (MUD), 33 a partially matched family donor (PMFD).

Among non-SCID children, 16 had a MFD, 22 a MUD, 7 PMFD. As concerns stem cell source 102 received marrow cells, 5 cord blood, 2 PBSC and 7 BM+PBSC. In SCID patients only 14 children weren't conditioned, 26 received BU+EDX, the rest a combination of different drugs with a prevalence of fludara, thiopeta containing protocols. In non-SCID patients Bu containing protocols were administered in all but 2 children. As concerns acute GVHD in SCID children only 5 children on 71 (7%) presented severe GVHD grade III/IV (1 MFD, 3 MUD, 1 PMFD), while 2 presented moderate GVHD; in non-SCID patients 7 children out of 45 (15%) presented severe GVHD grade III/IV and 6 SGVD (3 moderate, 3 severe).

Overall survival in all patients projected at 10 years exceeds 70% (75% SCID, 72,7% non-SCID). On the whole the registry report demonstrates that the number of registered children is far too low as compared with the expected numbers for the country suggesting misdiagnosis or non registration of the procedures; moreover the time interval between diagnosis and BMT remains a major concern.

212 X-linked adrenoleukodystrophy - effects of haematopoietic stem cell transplantation on genotype and protein expression in different brain regions and extraneural tissues
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X-linked adrenoleukodystrophy (X-ALD) is the most common inherited peroxisomal disease characterized by impairment of ALD protein (ALDP) which is involved in the beta-oxidation of very-long-chain fatty acids. The clinical course of X-ALD is highly heterogenous and characterized by cerebral dysmyelination, myelopathy, peripheral neuropathy and adrenal insufficiency of variable degrees. There is no clear genotype-phenotype correlation, even disregardless of family history. Some patients have successfully been treated with hematopoietic stem cell transplantation (HSCT) in order to prevent the progression of cerebral X-ALD. However the molecular and cellular mechanism of the therapeutic effect is poorly understood.

Here we report on mutation and protein expression analyses before and after HSCT in a family affected with X-ALD. Three members (2 female heterozygotes, 1 male) were asymptomatic, 1 boy showed adrenal insufficiency, and 2 boys suffered from cerebral X-ALD and underwent HSCT. Of these, 1 pt. is alive with no neurological deterioration, whereas his brother died due to complications at day +76. After autopsy, genomic DNA from 12 different brain and 11 extraneural tissues were analysed for X-ALD mutation. Furthermore, paraffin-embedded tissues were stained with 2 different ALDP-antibodies using enzyme catalysed amplification techniques. Regardless of clinical manifestation, all 6 family members revealed a so far undescribed large deletion in the ALDP gene (948-9463del) that included parts of exon 1 and complete exon 2. The negative immunofluorescence assay of cultured skin fibroblasts indicated that the mutation results in lack of protein expression. Neutrophils engulfed (ANC>500/μl) at day +16 (pt. 1) and +10 (pt. 2) respectively, and consecutively both pts showed a complete chimerism in blood. Both deleted and undeleted X-ALD-sequences were detected in each of the 23 tissues of pt. 2, indicating mixed chimerism.

Immunohistochemistry showed punctuate staining of blood cells, bone marrow stem cells, glia cells but also neurons in brain. The immunohistochemical staining pattern for restored tissues of pt. 2, indicating mixed chimerism. Furthermore, genomic DNA from 12 different brain and 11 extraneural tissues were analysed for X-ALD mutation. Furthermore, paraffin-embedded tissues were stained with 2 different ALDP-antibodies using enzyme catalysed amplification techniques. Regardless of clinical manifestation, all 6 family members revealed a so far undescribed large deletion in the ALDP gene (948-9463del) that included parts of exon 1 and complete exon 2. The negative immunofluorescence assay of cultured skin fibroblasts indicated that the mutation results in lack of protein expression. Neutrophils engulfed (ANC>500/μl) at day +16 (pt. 1) and +10 (pt. 2) respectively, and consecutively both pts showed a complete chimerism in blood. Both deleted and undeleted X-ALD-sequences were detected in each of the 23 tissues of pt. 2, indicating mixed chimerism.

Immunohistochemistry showed punctuate staining of blood cells, bone marrow stem cells, glia cells but also neurons in brain. The immunohistochemical staining pattern for restored ALDP showed no apparently different pattern compared to healthy control tissues.

In conclusion, our analyses provide molecular evidence that the therapeutic effects of HSCT in X-ALD is mediated at the cellular level by differentiation of transplanted stem cells in affected tissues and thus, restoration of normal ALDP expression.

213 Thymic function among paediatric stem cell transplant recipients
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Introduction: Recovery of thymic function is an essential part of immunoreconstitution. Delayed and/or insufficient thymic recovery materialises as an increased risk of post-transplant complications (e.g. viral infections). TREC (T-cell receptor excision circles) levels in peripheral blood mononuclear cells (MNC) serve as an indicator of thymic recovery and reconstitution of recipient T-cell function.

Material and Methods: Between 6/01-3/04 a total of 66 (autologous 15 and allogeneic 51) patients with a median age of 7.6 yrs with either a malignant (53) or non-malignant (13) disease were transplanted using matched unrelated (31) or sibling donors (20) or given autologous stem cell rescue (15). TBI was used in 7 autologous and 46 allogeneic cases. T-cell depletion was not employed. Thymic function was evaluated by quantifying TREC levels in peripheral blood MNCs prior to transplant and every three months during the first 18 months post-transplant. Quantification of thymic signal-joint TCR delta excision circle (TREC) was done by real-time quantitative-PCR. TREC values were corrected according to the percentage of CD3+ cells in the sample and then expressed as the numbers.

Results:
Pretransplant: No significant difference in TREC-values between allogeneic and autologous recipients was observed. Posttransplant: TREC-values decreased in all three groups during the first three months post-transplant. The data indicates a difference in the kinetics of thymic recovery post-transplant between the recipients of sibling and URD grafts and those of autologous stem cell rescue. At 6 months the difference between recipients of sibling and URD grafts was of borderline significance (P=0.06). The follow-up continues and the statistical analysis of the data at 9 and 12 months is pending.

Conclusions: Thymic function seems to recover in all the three groups at least by 18 months post-transplant. In many cases the recovery is evident only after 6 months post-transplant. Beyond 6 months the recovery appears more rapid among recipients of autologous stem cell rescue and those of sibling grafts.

214 Onset of thymic recovery and plateau of thymic output are differentially regulated after stem cell transplantation in children

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Thymus-dependent T-cell regeneration is a major pathway for immune reconstitution after stem cell transplantation in children. Therefore, we prospectively assessed T-cell dynamics and thymic function in 164 pediatric patients between 1 and 124 months post-transplant by measuring T-cell-receptor excision circles (TRECs) and spontaneous expression of Ki67 in peripheral T-cell subsets. We analyzed the impact of recipient age, conditioning regimen, type of donor and graft, stem cell dose, and graft-versus-host disease (GVHD) on the onset as well as on the plateau of thymic output. A high rate of spontaneous proliferation in early reconstituting naive and memory T-cells inversely correlated with total T-cell numbers. Accordingly, TREC-content was diminished in early appearing naive T-cells. A multivariate analysis revealed that the onset of thymic recovery was inversely correlated only with recipient age (P<0.002), whereas the plateau of thymic output was higher in patients receiving increased stem cell numbers (P=0.0022). Donor type, stem cell source and conditioning regimen influenced none of the analyzed parameters. In conclusion, lymphopenia-driven proliferation is important for T-cell homeostasis in children early after stem cell transplantation, but might result in underestimation of thymic function. Onset and plateau of thymic activity are independently regulated by different transplant-related factors.

215 Reconstitution of lymphocyte subpopulations in children with lysosomal diseases after haematopoietic cell transplantation

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Introduction: Several studies have investigated immunological reconstitution after hematopoietic cell transplantation (HCT) and reported that immunological recovery is delayed after T-cell depletion particularly in recipients of unrelated grafts. However, data on immune recovery in children after graft manipulation by positive selection are limited. We prospectively evaluated reconstitution of lymphocyte subpopulations in 9 children with lysosomal diseases (6 mucopolysaccharidosis type I-H or Hurler syndrome and 3 late-onset globoid-cell leukodystrophy or Krabbe disease) who underwent 11 allogeneic HCT (10 unrelated, 1 familiar donor) following CD34+ immunomagnetic enrichment. Limited T-cell addback and additional in vivo B-cell depletion with rituximab.

Results: Absolute lymphocyte count recovery was slow to cross the 5th percentile (p5) occurring at a median of 10 and 13 months after HCT in patients with full and partial chimerism, respectively. As expected, natural killer (NK) cells were the first lymphoid cells to emerge and represented only 90% of the total lymphoid population during the first three months. CD4+ lymphocyte recovery occurred 9 to 18 months after HCT in all patients in each group. In most patients, CD8+ lymphocyte recovery was slow and comparable to that of CD4+ lymphocytes. The CD4+/CD8+ ratio normalized by 2 to 6 months after HCT in 50% of the patients. CD8+ lymphocyte recovery was enhanced in patients with viral reactivation. Reconstitution of lymphocytes was particularly delayed in patients treated with rituximab. All patients who achieved complete or partial chimerism became independent of immunoglobulin replacement therapy at a median time of 10 months (range, 6 to 19 months) after successful HCT.

Conclusions. These patients were at risk for significant infections for up to two years after HCT. More and detailed immune reconstitution data are needed on a larger number of patients with lysosomal disorders to guide possible post-HCT interventions to improve results in this field.

Working Party: Late effects

219 Cardiac and pulmonary function late effects after bone marrow transplantation in childhood: prospective study on behalf of EBMT LEWP Group

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Introduction: Patients(pts) undergoing bone marrow transplantation(BMT) for haematological childhood disorders might present long-term cardiac and pulmonary function(CPF)sequelae,which could impair the quality of life. The aim of the current study was to assess the incidence and the risk factors of CPF by a prospective multicentre study.

Patients and Methods: The study set up by 9 EBMT centres from March 1994 to December 1997, enrolled 220 consecutive children(130 males, median age at BMT 9.5 yrs) who underwent BMT(162 allogeneic and 58 autologous) for malignant (193) and non malignant (27) diseases. TBI based conditioning regimen was used in 120/220 pts. A total of
133/220 pts were alive at the end of the study with a reliable CPF assessment which consisted of pulmonary function tests (PFTs) and M-Mode echocardiography performed at pre BMT phase, at 1st year post BMT and yearly. No sufficient information were available for remaining 32 long term survivors. Statistical analysis to evaluate the difference between pre BMT and post BMT CPF was performed by paired t-test, while the assessment of risk factors was based on univariate analysis.

Results: Median follow up of evaluable pts was 5 yrs (range 4-8 yrs). Fiftyfive/220 patients deceased, none for late CPF abnormalities. Fifteen of 108 evaluable pts for cardiac function (=13.9%) presented late pathological shortening fraction (not significant t-test) and 14/15 were affected by malignancies. According to univariate analysis TBI was the only factor significantly associated (p=0.008) with impaired cardiac function. Eighteen of 96 of evaluable pts for pulmonary function (18.8%) presented pathological PFTs (9 restrictive, 8 obstructive and 1 mixed pattern). As to univariate analysis C-GVHD was the only significant factor (p=0.02). All patients with CPF alterations were asymptomatic (median Lansky index >90 in all).

Conclusions: The current prospective study is the first focusing a relatively low incidence of only asymptomatic CPF late effects in children transplanted for haematological disorders. Overall both cardiac and pulmonary functions show some recovery over time. TBI for cardiac function and C-GVHD for pulmonary function are the most important risk factors. Despite most of the pts displays a normal CPF from the beginning of BMT up to 5th year of median follow up, it seems reasonable to perform a continue surveillance at least in adolescent age to prevent more serious future damages.

220 Late mortality and complications following allogeneic HSCT are associated with cGVHD severity: long-term follow-up of the IMUST Study

The International Unrelated Search and Transplant (IMUST) Study prospectively accrued 345 unrelated donor (URD) and 699 matched sibling donor (MSD) SCT between 1989 and 1992. We now compare late events occurring in 108 URD and 355 MSD recipients surviving >2 yrs (2.0-13.9) after SCT. Late deaths occurred in 105/463 patients surviving >2 yrs, relapse related transplant related 29, second malignancy 4, other 2 and unknown 6. Extensive C-GVHD was present in 84 recipients surviving >2 yrs, limited C-GVHD in 179 and no C-GVHD in 200. The cumulative incidence (CI) for survival at 12 yrs in patients surviving >2 yrs with C-GVHD was 49% (44-55) in the MSD cohort and 59% (51-70) in the URD cohort. The CI for extensive C-GVHD was slightly higher in the URD than MSD recipients, 49% (44-55) versus 30% (22-42), p=0.1. Twelve yr survival was 77+/-5% for the MSD and 67+/-11% for the URD cohort, p=0.1. For patients with extensive C-GVHD survival was 49+/-14%, 80+/-7% for limited C-GVHD, and 82+/-6% no C-GVHD, p=0.0001. In extensive C-GVHD patients 12 yr survival was similar after MSD and URD transplants, p=0.89. Cox regression showed C-GVHD was the only variable significantly associated with survival >2 yrs, p=<0.0001. At 12 yrs for bone necrosis and second malignancy was reported as less than 8% in both cohorts. However extensive C-GVHD predicted bone necrosis, CI at 12 yrs 11% (6-22), p=0.0002, and second malignancy, CI at 12 yrs 15% (7-34), p=0.0002. Cataracts and endocrine failure were frequent in survivors >2 yrs. CI for cataracts at 12 yrs was 43% (33-57) in the URD cohort and 28% (24-34) in the MSD cohort, endocrine failure 28% (19-40) in the URD and 29% (23-35) MSD cohort. Cox regression showed cataract formation was independently associated with TBI conditioning, RR 5.5 (2.7-11.3), p=<0.0001 and URD, RR 1.6 (1.0-2.3), p=0.03. In Cox regression endocrine failure was independently associated with female gender, RR 2.7, (1.8-4.1), p=<0.0001 and young age RR 2.1 (1.2-3.6), p=0.008.

We conclude that late mortality and late complications following allogeneic SCT are closely associated with C-GVHD but not donor type.

222 Increased risk for secondary thyroid carcinoma after haematopoietic stem cell transplantation: an EBMT Late Effect Working Party study

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Background: The effect of hematopoietic stem cell transplantation (HSCT) on thyroid carcinogenesis needs to be determined in a large population. This study evaluates the incidence of secondary thyroid carcinoma (STC) and the related risk factors in patients transplanted in the Centers affiliated to the EBMT group.

Patients and Methods: Retrospective investigational study on secondary thyroid cancer incidence, comparing the data obtained by a two-step questionnaire approach from 166 (39.3%) centers who replied, and the data reported to the EBMT-Registry on their transplant activity. During the follow-up period (1985 – 2003), 31 incident cases of STC were found within the EBMT cohort of 68,936 transplanted subjects. Observed STC were compared to age and gender specific incidence rates of the European population (1993-1997).

Results: The risk of STC in the transplanted population was 3.16 in comparison to the European population. Multivariate analysis revealed that young age at transplant was the strongest risk factor for STC (RR=567.43 for age group 0-10 years; RR=38.81 for age group 11-20). Other risk factors were irradiation (RR=3.11), being male (RR=2.79) and chronic graft-versus-host disease (RR=2.55). Eight patients had no clinical signs of thyroid illness at the time of diagnosis. Total thyroidectomy and iodine ablation was the standard treatment in the majority of patients, and only one patient died due to STC progression.

Conclusions: A careful follow-up of the thyroid status including annual ultrasound examination and fine-needle aspiration in case of suspected thyroid nodule are recommended for an early detection and treatment of the tumor especially in patients transplanted during childhood and adolescence.

Acute leukaemia 2 / Myelodysplastic syndromes

O227 Retrospective comparison of reduced-intensity conditioning and conventional myeloablative conditioning for allogeneic haematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes

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In this multicenter retrospective study, the outcomes of 993 patients with MDS who underwent transplantation between 1997 and 2001 from HLA-identical siblings were analyzed
according to the type of conditioning (RIC (n=215) vs. CONV (n=621), and 157 unclassifiable). As expected, baseline characteristics differed in some important variables, such as age >50 years (73% in RIC vs. 26% in CONV, p<0.0001). Patient age, stem cell source, and disease status at transplant were also found to be significantly different in the Cox model. Multivariate analysis was focused on 1-year outcomes: non-relapse mortality (NRM), disease relapse (REL), OS and progression-free survival (PFS). 2-year treatment failure incidence, and survival. Use of RIC reduced the median duration of neutropenia and thrombocytopenia by 2 and 7 days, respectively. The incidence of grades 2-4 acute GVHD was lower in the RIC group (24% vs. 39%, respectively, P<0.01), but the 1-year incidence of extensive chronic GVHD was identical. One-year NRM was significantly reduced with RIC (HR, 0.61; 95% CI, 0.46-0.95; P= 0.03), but the 1-year REL incidence was significantly increased with RIC (HR, 1.67; 95% CI, 1.22-2.29; P = .001). One-year OS and PFS, however, did not differ between conditioning groups. Variables that improved OS were: age >50 years, diagnosis other than secondary acute leukemia, high CD34+ cell dose infused, and high-risk MDS in first CR at transplant. In conclusion, based on the higher risk of relapse, patients with no strong contraindications for CONV conditioning should not receive a RIC outside of prospective randomized trials. However, the reduced 1-year NRM with RIC (in patients usually with a contraindication for a CONV transplant) suggests that the goal of reducing early NRM with RICs has been accomplished. In this retrospective analysis we could not adjust for co-morbidity factors. Therefore, prospective trials are needed to establish the position of RIC based transplantation in the treatment of patients with MDS.

FAB subtype M2/M4 and cytogenetic inv(16)/(18;21) were prognostic in AML-10; the number of cytopenias and the duration of antecedent hematologic disorder (AHD) >6 months were prognostic in the CRIANT study. Based on these results a prognostic score was designed for both studies. The AML-10 study distinguished 3 groups with an estimated 3-year survival rate of 69%, 39%, and 18% resp. The CRIANT study distinguished 3 groups with a 4-year survival rate of 72%, 41%, and 8%. In conclusion: these prognostic scores identify a large group of 42% MDS pts and a smaller group of 23% AML pts, with a 4-year survival less than 20%. Our finding that different variables are of prognostic importance in MDS and AML pts supports the hypothesis that MDS and AML are intrinsically different disorders.

O229 Impact of cytogenetics on the outcome of patients undergoing allogeneic HSCT from HLA-identical siblings for MDS or secondary AML: a retrospective analysis of the EBMT-CLWP
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Cytogenetics (CG) represent one of the most important factors in determining survival for pts with MDS, being therefore required by the IPSS to predict life-expectancy. However, the impact of CG on the outcome of pts undergoing allo-HSCT for MDS is unknown. The aim of this EBMT-CLWP study was to carry out a retrospective analysis of the impact of CG on OS, RFS, REL, and TRM in pts with MDS/sAML undergoing allo-HSCT from HLA-identical siblings. Data from 2,077 pts with MDS/sAML who underwent allograft from HLA-identical siblings from 1984 to 2004 reported to EBMT were considered. The following covariates were included: CG (good- vs standard- vs high-risk according to the IPSS); stage at HSCT (untreated vs treated in CR vs treated not in CR); FAB classification (RA/RARS vs RAEB/CMML vs RAEB-t/AML); age (as a continuous covariate in the COX models); time from dx to HSCT (<5 vs 5 to 8 vs > 8 months); calendar year in which HSCT was performed; CG (standard vs RIC); source of stem cells (BM vs PB). A complete information dataset was available in 402 pts, who are the subject of this analysis. 16% of pts had RA or RARS, 26% had RAEB or CMML, and 59% had RAEB-t or sAML. Age was >50 years in 40%. CG classified 50% of pts as good- 25% as intermediate-, and 25% as high-risk. At the time of HSCT, 36% were untreated; among treated pts, 163 were in CR. A RIC regimen was administered to 108 pts (27%). Source of stem cell was PB in 63%. By univariate analysis, subdivision of pts in the IPSS risk-categories for CG associated with significantly different OS (at 36 mos, alive pts in good-, interm-, and high-risk groups were 55%, 40% and 30%, respectively), RFS (alive pts at 36 mos 50%, 38% and 20%, respectively), relapse probability (35%, 41% and 67% at 36 mos, respectively) and TRM (24%, 37% and 40% at 36 mos, respectively). By multivariate COX, CG associated with OS, RFS, REL together with stage at HSCT and calendar year (for RFS and OS); CG was the only factor associated with TRM. Concerning REL, the interval between dx and HSCT as well as the source of stem cells were also significant, with the hazard associated with PB being twice as high as for BM in comparison to BM.

In summary, this study provides evidence that CG have strong prognostic impact on outcome of pts undergoing allo-HSCT
from HLA-identical siblings for MDS/sAML and should be taken into consideration when selecting candidates for this treatment strategy. Detailed analyses will be presented.

O230
Achievement of LW or IM-1 risk score prior SCT may be connected with prolonged DFS in patients with advanced MDS

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In the years 1998-2004, fifty-three patients with MDS underwent allogeneic stem cell transplantation in our institute, either from HLA matched related (33 cases) or unrelated donor (20 cases), 6 patients received autologous stem cells harvested in the first CR. Twenty-seven patients with less advanced disease (RA and RAEB <= 10% blasts according to FAB criteria) were transplanted either after standard conditioning (Cy+TBI) or (Bu-Cy) intensified by idarubicin in patients with > 8% blasts) or after reduced intensity conditioning (Flu+Bu) in 5 patients. Median survival, three years disease free survival (DFS), transplant related mortality (TRM) and relapse rate were: 20 months, 70%,29% and 0% for RA patients and 3.5 months, 23%,50% and 10% for RAEB <= 10% blasts patients. In this subgroup, adverse results of SCT may be explained by a high rate of acute GVHD grade III-IV (50%). Twenty-two patients with advanced disease (RAEB>10% blasts and RAEB-T) were pretreated with combination chemotherapy, CR rate was 77% (17 patients), 4 patients (18%) achieved PR, 1 patient had resistant disease. Sixteen pretreated patients were allografted, 6 patients underwent autologous SCT. Ten patients with RAEB>10% blasts or RAEB-T were transplanted immediately after diagnosis with intensified myeloablative conditioning (idarubicin + BuCy or CyTBI or FluTBI). Median survival of pretreated allografted patients was 33,5 months compared to 11,8 months in non-pretreated allografted and to 3 months in autografted patients. Similarly, 3 years DFS was 55%, 23% and 17%, TRM was 19%, 40% and 0% and relapse rate was 25%,33% and 83% for the respective groups. In allografted patients with advanced disease stratified according to IPSS score at the time of starting conditioning, median survival and 3 years DFS were 36,5 months and 55% for patients with low or intermediate-1 risk score and 12,5 months and 21% for those with intermediate-2 or high score. Six out of seven patients who relapsed after allogeneic SCT were transplanted either with disease or with resistant disease with the initial median of bone marrow blasts ranging from 21 to 24% prior to immediate SCT. On the other hand, all but one patient surviving more than 4 years without signs of the disease had low or intermediate-1 score according to IPSS at the time of SCT. We conclude, that the reduction of bone marrow blasts prior to SCT may benefit patients with advanced MDS and contribute to prolonged DFS after transplantation.

O231
Thrombotic thrombocytopenic purpura is still an issue after allogeneic haemopoietic stem cell transplantation

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Introduction: Thrombotic thrombocytopenic purpura (TTP) might constitute a severe complication after allogeneic haemopoietic stem cell transplantation (AH SCT) with an incidence ranging from 0.5 to 30%. Due to some difficulties in diagnosing and treating TTP, a high mortality rate has been generally reported. The aim of this study is to focus the incidence and the outcome of TTP in the era of more complex AH SCTs.

Patients and Results: The study set up by 4 GITMO centres from January 2000 to November 2004, enrolled 687 consecutive patients(pts), who underwent AH SCT (354 from HLA compatible sibling donor, 65 from MM family donor, 273 from HLA compatible unrelated donor). The diagnosis of TTP was performed on the basis of homogeneous clinical and laboratory criteria but here for brevity's sake we chose TTP index (pathological if >20) according to literature. The main characteristics of those pts who developed TTP are listed in Table 1. For one centre (N=4) some data are not available at present time (columns 7-9). One hundred and one/687 pts presented TTP (=14,7%) with a mean TTP index of 34. In 3/4 centres (46/101 pts) TTP onset occurred at a median time of day +45 post AHSCT, the most frequent treatment was Defibrotide alone (38 pts) and the cause of death only related to TTP, 3 to 180 days after its onset, was 13% (6 pts).

Conclusions: The current study underlines that both incidence of TTP (14,7 %) and transplant-related mortality induced by TTP (13%) are still relevant, especially after MUD and MM family donor AHSCT. The early onset of TTP indicates that this complication is probably GVHD mediated. The uncertainty of TTP diagnosis can constitute an obstacle in successfully treating this severe complication, even if the early therapy with Defibrotide seems to be promising.

O232
Early sequential myeloablative allogeneic stem cell transplantation in patients with refractory or relapsed myeloid leukemias during high-dose cytarabine induced cytopenia

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Allogeneic stem cell transplantation (SCT) may offer the only chance of cure for patients with myeloid leukemias in refractory or relapsed disease. Mainly, patients with unfavourable cytogenetics or blast transformation of a myeloproliferative disorder (mpd) have a poor prognosis as a result of a high rate of treatment related mortality and relapse with conventional conditioning regimens. Recently Kolb et al have introduced a sequential protocol utilizing early tbi-based non-myeloablative transplantation following conventional induction therapy in high-risk patients.

We conducted a similar model, were we have treated 22 patients (median age 53 years, range 17-64, 3 female, 19 male) with advanced leukemias according to an intensified sequential high-dose protocol. Diagnoses were AML (primary refractory t1, relapsed 4), MDS (refractory 7, untreated 2) and blast transformation of a MPD (refractory 8, untreated 0). The median blast count at induction was 15% (range 1-63) and 11 patients (50 %) had unfavourable cytogenetics. Induction therapy consisted of fludarabine, high-dose cytarabine with or without idarubicine or ansamycin. ATG, high-dose melphalan with or without high-dose thorapoa was used for conditioning while patients were still cytopenic from the preceding induction therapy. Following transplantation of a median of 6,1x10^6 CD34+ cells/kg (range 0.85-12.0) from related (8) or unrelated (14) donors, the median time to a wbc>1000/µl and plt>20000/µl was 15 days (range 10-24) and 17 days (range 8-
30), respectively. No toxic death was observed during the first 30 days and 95% of patients achieved CR by day 28, 100% by day 50. Seventeen patients (59%) developed aGVHD (13°I-II, 0°III-IV) and 7 patients (32%) at risk have developed chronic GVHD (4 limited, 3 extensive) so far. After a median time of 210 days (range 5 – 708 days) sixteen patients (73%) are in first complete remission. Six patients died, one of infections (5%), two of a chronic GVHD (9%) and three of disease progression (14%). Only one patient (5%) died in the first one hundred days (day 99) after allogeneic SCT by aGVHD.

We conclude that early sequential transplantation during induction induced cytopenia using high-dose melphalan and thiopeta based conditioning is feasible and highly effective in patients with advanced myeloid leukemias and poor prognosis. Although these early results are promising, a longer follow up and comparison with standard regimens are required.

O233 Conditioning regimens for allotransplantation of haematopoietic stem cells: the use of total-body-irradiation in Germany

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The conditioning regimen for allogeneic hematopoietic stem cell transplantation (HSCT) in patients with acute lymphatic(ALL), acute myelocytic (AML) and chronic myelocytic (CML)leukemia may consist in chemotherapy combined with total body irradiation (TBI) or in chemotherapy alone. Several large studies comparing the conditioning regimens TBI plus cyclophosphamide (TBI/Cy) and busulfan plus cyclophosphamide (Bu/Cy) have demonstrated a superiority of TBI/Cy conditioning(lower treatment related mortality and lower relapse rate) in patients with ALL and AML, whereas TBI/Cy and Bu/Cy seem to be equivalent in patients with CML. To get an overview on the frequency of the use of TBI-containing conditioning regimens for allo-transplantation in ALL, AML and CML patients in Germany the data base of the German Registry for Stem Cell Transplantation (DRST) was used. The records (First Report - Minimal Essential Data - A) of 3,573 first transplants (n = 930 for ALL, n = 1487 for AML and n = 1,156 for CML) were evaluable. All transplants had been performed in the years 1998-2002.TBI-containing conditioning regimens were used in 85 % of the ALL patients (794/930), but only in 45 % of the AML (662/1,487) and in 49 % of the CML (561/1,156). Interestingly, the use of TBI was neither correlated to the transplant center size nor to the type of donor used (related vs. un-related, HLA-matched vs. HLA-mismatched) or the stem cell source (bone marrow vs. peripheral blood). Since TBI-containing conditioning regimens seem to be superior in ALL and AML patients according to the literature it seems worthwhile to find out the reason why 15 % of all ALL- and even 55 % of all AML-patients were conditioned with chemotherapy alone. A DRST-based multicenter study addressing this issue is in preparation. This study was supported by the German José Carreras-Leukämie Stiftung

O234 TBI 8 Gy / fludarabine as conditioning for allogeneic HSCT from related or unrelated donors in AML: improved benefit risk ratio for patients in first or second complete remission (CR1/CR2)

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We evaluated a dose-reduced TBI-based conditioning regimen followed by allogeneic hematopoietic stem cell transplantation (HSCT) in patients with acute myeloid leukemia (AML) aiming at lowering non-relapse-mortality (NRM) while preserving anti-leukemic efficacy. 50 patients with AML in CR1 / CR2 (9 / 9), with untreated primary disease / untreated relapse (1 / 7) or with primary / secondary refractory disease (16 / 8) underwent allogeneic HSCT from related (n=26) or unrelated (n=24) donors. Patients were not considered eligible for conventional conditioning. Median age was 53 years (range: 20 - 66 years). The preparative regimen consisted of 8 Gy TBI, fludarabine (30 mg/m² IV x 4), and optional ATG (rabbit, 20 mg/kg/d x 2) followed by immunosuppression with CSA / MTX. Median follow-up was 35.6 months (range: 20 - 59 months). Complete donor engraftment was documented within 8 weeks and sustained in all evaluable patients. Cumulative probabilities of grades II-IV and III-IV acute GVHD were 32% and 8%, respectively. Notably, for patients transplanted in CR and with untreated disease, cumulative NRM at 3 years and beyond was 4%, while it reached 50% for patients transplanted with refractory disease. 3-year estimates for relapse-free survival were 67% for patients transplanted in CR (95% C.I., 43 - 92%), 38% for patients transplanted with untreated primary disease / untreated relapse (95% C.I., 3 - 72%), and 4% for patients transplanted with refractory disease (95% C.I., 0 - 10%). Estimated overall survival rates were 89% (95% C.I., 66 - 100%), 50% (95% C.I., 15 - 85%), and 4% (95% C.I., 0 - 17%), respectively.

We conclude that in AML patients in CR1 / CR2 myeloablative conditioning with 8 Gy TBI / fludarabine +/- ATG improves the benefit risk ratio by reducing NRM while maintaining anti-leukemic activity. Based on this phase II trial, two randomized German Intergroup studies comparing conventional conditioning (12 Gy TBI/cyclophosphamide) to the 8 Gy TBI/fludarabine regimen prior to allogeneic HSCT in patients with AML in CR1 and CR2 were launched and have started recruitment.

Infectious diseases 2

O235 Monitoring of herpesviruses in children and adult allogeneic haematopoietic stem cell recipients

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Objectives: Herpesviruses can cause life-threatening complications in allogeneic hematopoietic stem cell transplant (HSCT) recipients. Therefore rapid diagnosis and treatment are necessary for survival of the patients. The aim of the study was to define the frequency and clinical correlates for seven different viruses.