25. Paediatric diseases

P736
Large scale purification of progenitor cells by AC133+ selection
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In order to eliminate potentially CD34-positive tumor cells from autologous grafts, we established a clinical scale AC133 (CD133) selection procedure. Purity of AC133+CD34+ cells and contamination with residual neuroblastoma cells were evaluated.

Fresh peripheral blood stem cell apheresis products (PBSC) with $>$20x10^6/kg CD34+ from 2 pediatric patients with neuroblastoma and 8 cryopreserved PBSC cells were equally aliquoted and incubated with either magnetic bead conjugated AC133 or CD34 (QBEND 10) antibodies. Positive cells were selected using the Miltenyi SuperMACS or CliniMACS device. The 6 cryopreserved PBSCs of previously deceased patients were experimentally contaminated with a neuroblastoma cell line. Flow cytometric assessment of the selection product was on a EPICS XL-MCL (Beckman Coulter). Residual neuroblastoma cells in the PBSC and in the purified progenitor cells were determined by nested RT-PCR as well as by quantitative real time PCR for tyrosine hydroxylase and by immunocytochemistry.

Purity of freshly isolated PBSC was 98.0% after AC133 selection and 97.3% after CD34 selection in accordance with the mean CD34+ purity of 96.8%±plusmn;4.2% in 71 previous CD34 selections in our laboratory. AC133 selection of cryopreserved PBSC achieved a purity of 91.8%&plusmn;8.0% (n=6) and CD34 selection led to a purity of 87.5%&plusmn;7.6% (n=6). Neuroblastoma cells were detected in PBSC of one patient and in all experimentally contaminated cryopreserved PBSCs prior to but not after AC133 nor CD34 selection.

Large scale positive AC133 selection is feasible and achieves equivalent high purity of progenitor cells as previously reported for CD34 selection with effective tumor depletion. Therefore, AC133 purification may be a superior option to purge autologous grafts in patients with potentially CD34 positive tumor cells.

Supported by "Hilfe für Krebskranke Kinder Frankfurt e.V."

P737
Antibody dependent cellular cytotoxicity after transplantation of allogeneic purified stem cells
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Transplantation of highly purified CD34+ stem cells from mismatched related and matched unrelated donors is a well established method in our institution, which makes it possible to prevent GvHD due to indirect T-cell depletion without enhanced risk of graft failure. However, relapses still represent a major problem after successful transplantation in children with high risk leukemias. Therefore, immunotherapeutical strategies are needed to treat a minimal residual disease (MRD). Natural killer (NK) cells are able to lyse leukemic blasts with reduced HLA class I expression, whereas high expression of HLA class I renders them resistant to NK cells. However, NK cells can be enabled to lyse even those blasts by using the ADCC (antibody dependent cellmediated cytotoxicity) with the help of appropriate antibodies. ADCC was measured after transplantation against 2 B-precursor leukemic cell lines (Raji, MHH4; moAb used: anti CD20) in 16 patients and against fresh leukemic blasts (moAb used: anti CD19) in 5 patients. 14/16 patients could lyse both NK-insensitive cell lines in the ADCC. Cytotoxic activity could be further enhanced by additional stimulation with low dose Interleukin (IL)2. 5/5 patients were able to lyse fresh leukemic blasts.

Development of ADCC was monitored up to 2 years. Clinical activity levels were measured 1 to 4 months after transplantation, corresponding to the NK cell wave at this time. Clinical results: 2 patients, who relapsed after transplantation with CD19 or anti CD20 antibodies. Remissions could be obtained in both cases. However, in patient 1, loss of the CD20 antigen occurred. Patient 2 developed severe GvHD after an add back of T-cells and relapsed during intensive immunosuppressive treatment.

Conclusions: After transplantation of allogeneic, purified CD34+ stem cells, NK cell based ADCC against B-cell precursor leukemias was possible in most of our patients. This ability might be used for well tolerated, posttransplant treatment of MRD.

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Immune recovery after haematopoietic cell transplantation (HCT) in children: immunophenotypic analysis and factors affecting the speed of reconstitution
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Immune reconstitution was studied prospectively in 66 children, who underwent 77 haematopoietic cell transplantations (HCT): 46 autologous HCT in 39 children with hematologic malignancies (HM; n=22) or solid tumors (n=17) and 31 allogeneic HCT in 29 children with HM (n=20) or non malignant diseases (n=9). The aim of the study was the dynamic analysis of immune recovery with regard to potential factors affecting its speed, including age, type of HCT, diagnosis, GvHD and CMV reactivation. Absolute counts of different lymphocyte subsets and immunoglobulin levels were determined in peripheral blood of the patients on day +16 and at 1, 2, 3, 4, 6, 9, 12, 18 and 24 months post transplant.

Common patterns of immune recovery after both allogeneic and autologous HCT were identified: 1. CD4+CD45RO+ peripheral T cell expansion on day +16; 2. inverted CD4+CD8+ ratio from day +30 onwards; 3. rapid NK cell (CD16+CD56+) count normalisation. We observed prolonged T cell lymphopaenia (CD3+, CD3+CD4+, CD4+CD45RA+) until 24 months after autologous HCT, whereas in allogeneic setting CD3+CD4+ cells, including naive CD45RA+ cells returned to normal values at 9 months post transplant. Age > 10 years and coexistence of GvHD and CMV reactivation were associated with a substantial delay in T- (CD4+, incl. CD45RA+) and B cell recovery after allogeneic HCT. Both B- and NK cell recovery, but not T cell recovery, were delayed in

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older children post autologous HCT. Multidrug Gvhd prophylaxis resulted in impaired T- (CD4+), CD4+CD45RA+ and B cell reconstitution only in the early phase after allogeneic HCT (until 4 months).

Our results demonstrate, that T cell recovery is severely impaired in children after autologous HCT. It should be emphasized, that specific approaches to enhance immune reconstitution are necessary to control minimal residual disease and avoid the risk of infectious complications in the autologous setting. Thymic involution after allogeneic HCT seems to be associated with age and coexistence of GvHD and positive CMV status.

(supported by grant KBN 4PO5E 08714)

P739

Haploidentical Peripheral Blood Stem Cell transplantation (PBSC) in children with advanced diseases: preliminary data

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Between January 1996 and May 2000, 15 patients affected by acute leukaemia (11), lymphoma (2), MDS (1) or life threatening autoimmune disorder (1) received a haploidentical PSC. Patients were selected because lacking a suitable donor and/or an urgent need for transplantation. The conditioning regimen was fractionated TBI (12 Gy in 13 cases), thiopepa 10 mg/ kg, fludarabine 200 mg/m2 (10 patients) or Cy 120 mg/ kg (3 patients). The remaining 2 patients received TLI 7,5 Gy or melphalan 140/mg/m2 instead of TBI. All patients received ATG at 3 mg/kg/die for 5 days and cyclosporine 1mg/ kg/die for 9 days before day 0. Donors were stimulated with filgrastim subcutaneously at a dosage of 15 mg/kg until the day of the last leukapheresis. PBSC was collected by leukapheresis starting on day 4 of G-CSF administration. T cell depletion was obtained by selection of CD 34+ cells (Clinimacs = 11; Isolox = 3; Cellpro = 1 procedures). No GVHD prophylaxis after transplantation was given. A median of 3 apheresis (range 1-4) was performed. The final product contained from 50 to 92% of the initial population of CD 34+ cells, with a purity of 50-90%. A T cell depletion of 3-6 log 10 was obtained. The final inoculum contained a median CD 34+ cells of 12 x 106 /kg (range 5-52), and a median CD 3+ cells of 0.2 x 105 /kg (range 0.02-1 ). All patients achieved sustained engraftment, with neutrophil count more than 0.5 x 109/L and platelets more than 50 x 109/L after a median time of 10 days (range 8-15) and 16 days (range 12-13), respectively. All patients engrafted with full donor type chimerism. No graft failures were observed. In 13 out of 15 patients no signs of acute GVHD were observed. Two patients developed acute GVHD of grade II and IV, respectively. No chronic GVHD was observed. Four patients died because of progression of disease from 1 to 4 months after transplantation. Three children died for infections within 3 months from infusion. Two patients developed PTLD (one with concomitant acute GVHD grade II) before day 100 and died. Four out of 15 patients (27%) are in CCR at 5, 8,14,15 months respectively. In conclusion, the results obtained in this small series of patients at high risk are in encouraging and allow us to extend the use of T cell depleted haploidentical transplant also in patients at better risk when an HLA identical donor is lacking.

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IV glutamine in children undergoing SCT, its influence on VOD

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Hepatic Veno-Occlusive Disease (VOD) is a complication following high-dose chemotherapy for bone marrow transplant. Liver injury is believed to occur following free radical damage to endothelial cells of the sinusoids and small hepatic veins. Glutathione, the main antioxidant of the cytosol, becomes depleted following chemotherapy. Glutamine infusion can maintain glutathione levels and protect against free radical injury. L-glycyl L-glutamine, are soluble dipeptides of glutamine. The purpose of our study is to review our results after introducing iv glutamine (as L-glycyl L-glutamine), as part of the supportive treatment in the preventive of VOD. Methods: since june 1998, high doses IV glutamine (0.5 GR/KG/Day) supplementing parenteral nutrition have been administered to all patients undergoing SCT. Glutamine was started the firts day of the conditioning regimen and maintained until discharge or oral tolerance. We selected the patients who underwent matched-related allogenic or autologous conditioned with busulfsan-containing regimens. Patients with similar type of SCT and conditioning regimens admitted in our unit between june 1996 -june 1998 (when glutamine supplementation was not used) constituted the control group. Results: 15 children of 31 in the glutamine group and 20 children of 33 in the control group received busulfan. VOD was observed in 2 children of 15 (13%) versus 5 children of 20 (25%) respectively. Comments: the use of IV glutamine seems to decrease the incidence of VOD. a large series is needed to establish significant conclusions.

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The surface expression of the hematopoietic stem cell antigen CD34 on neuroblastoma cells can be reduced by retinoic acid

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Neuroblastoma is the infantile solid tumor with the highest incidence. Most children with neuroblastoma stage III and IV die within a few years. Since autologous peripheral blood stem cell transplantation has become a therapeutic strategy for these patients the assumption has been implicated that especially the peripheral blood is free of tumor cells. Moreover, using the methods of positive selection of mobilized peripheral blood stem cells it has been thought that the tumor cells do not express the hematopoietic stem cell antigen CD34. Recently, we have demonstrated the occurrence of the CD34 antigen in neuroblastoma cells at protein and mRNA level. Here, we present data that the well known differentiation-inducing agent all trans-retinoic acid (ATRA) can reduce the CD34 surface expression on neuroblastoma cells. The expression of the CD34 antigen was investigated in the neuroblastoma cell lines IMR5, IMR32, CHP134, SK-N-AS, SH-SY5Y and SR (SR was established in our
In our children bone marrow transplant (BMT) unit, the strategy of unrelated bone marrow donor searches accepts HLA class I mismatched donors, provided that Thymoglobulin is part of the conditioning regimen. We have previously shown, by using homozygous typing cells, that DRw11 can be subdivided in several cellular subgroups (1). Molecular biology typing allowed us to deduce that HLA-DRB1*1101 does not recognize HLA-DRB1*1104, but not reciprocally. This was further confirmed in several other pairs, by using the mixed lymphocyte reaction assay. Here, we report the clinical results of two BMT in children recipients suffering from hematological malignancy who expressed HLA-DRB1*1101, but lacked HLA-DR matched donors. Due to our biological results, donor searches included donors expressing HLA-DRB1*1104. Two donors were found. In addition to the permissive HLA-DR mismatch (DRB1*1101 towards DRB1*1104), these donors expressed HLA class I mismatches with the recipients, which consisted of two HLA-C Ags in the former pair, and one HLA-A Ag in the second pair. MLR was negative in the GVHD direction in the former donor/recipient pair, or slightly positive in the second pair, probably due to an additional HLA-DP mismatch. BMT were performed in 1995 and 1998, respectively. At present, both patients are alive. Conditioning regimen consisted of fractionated TBI, chemotherapy, Thymoglobulin Sangstadt, and Cyclosporin A. Both patients engrafted with no GVHD. A grade II GVHD occurred in the first recipient but resolved. Whereas no Thymoglobulin adversary effect was noticed in this patient, a cervical B cell lymphoma appeared in the second patient. It was successfully treated with anti-CD20 mAb. Both patients are currently in complete remission. In conclusion, in this study we show that cellular permissivity may exist among HLA Ags, including HLA-DR. Detecting the permissive pairs should allow extension of donor searches and contribute to treat more patients.

(1) Freidel C, Gebuhrer L, Betuel H, Farre A and al. Human. Immunol.30:183, 1991

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Analysis of severe acute and late toxicity following Total Body Irradiation (TBI) in 262 children

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Patients: From 1980 to 1998, 262 children (pts) underwent intensive chemotherapy with TBI (12 Gy in 6 fractions in most pts), Age range 1 to 15 years. 134 pts were included in a program of autologous bone marrow and 128 patients underwent allogenic BMT. 23 pts had non Hodgkin’s Lymphoma, 86 pts bad Neuroblastoma, 104 pts had acute Leukaemia, 9 pts had chronic Leukemia, 10 pts had Ewing sarcoma, and 30 pts had others diseases. Results: The 5 years overall survival were 83% for Ewing sarcoma, 58% for Non Hodgkin Lymphoma, 46% for Acute myeloid Leukemia, 45% for Chronic Myeloid Leukaemia, 44% and 28% for Acute Lymphoid Leukaemia, and Neuroblastoma. At the time of analysis 145/262 (55%) pts have died: 92 pts from disease and 53 pts from complications. We have analysed severe acute toxicity: 18/262 pts (6%) had veno occlusive disease
whether procedure consisted of ATG
+ (med. 3,4 x 10^8 BM-MNC/kg) have been transplanted. For GvHD prevention CsA (day -1 to 20 mg/10 kg and CY 4 x 50 mg/kg. Methylprednisolone 1,0 mg/kg was given before each ATG-dose. 2,4-5,0 x 10^8 BM-MNC/kg seronegative donors. Interval from diagnosis to BMT was 2-20 mths (med. 3,5 mths). Preparative regimen consisted of ATG 5 x

complications is acceptable. The incidence of second cancer continues to be a severe and fatal complication for long

This retrospective study shows that the incidence of severe acute and late complications is acceptable. The incidence of second cancer continues to be a severe and fatal complication for long survivals specially for children’s.

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GVHD prophylaxis and GvL effect in children undergoing BMT from matched related donor (MRD - BMT) for ALL in II CR
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Total antileukemic effect of allogeneic BMT depends not only on action of preparative regimen for BMT, but also on GvL reaction. However, GvL effectiveness in children with ALL is still controversial. Aim: To evaluate whether changes of GvHD prophylaxis intensity demonstrates an impact on GvHD occurrence and relapse rate in children with ALL in II CR undergoing MRD-BMT. Patients and methods: Between 1994-2000 seventeen children with ALL in II CR have been transplanted with bone marrow from matched related donor (MRD). Median age was 10 (range 6-18) years. Depending on the intensity of GvHD prophylaxis two groups of children were separated in two groups. In Group I (n = 6) GvHD prophylaxis consisted of at least two immunosuppressants, i.e. CsA (3 mg/kg)/+/-“short” MTX/+/-Pred. In Group II (n = 11) CsA (3 mg/kg) was given alone for GvHD prevention. Besides in Gr. I CsA was administered 150-180 days post BMT, whereas in Gr. II only till day +130. All 17 patients have been conditioned with FTBI (12 Gy in 8 fractions) supplemented with CY 60 mg/kg/day for 2 days (in 4/6 pts from Gr. I and in 5/11 pts from Gr. II) or with VP 60 mg/kg/day (in 2/6 pts from Gr. I and in 6/11 pts from Gr. II). Results: There was no deaths related to transplant complications. In Gr. I (n = 6) - with more intensive GvHD prophylaxis - GvHD II-IV occurred in only 1 pts (16,7%), while relapse in 4 (66,7%) (6,5 years pLFS = 33%). On the contrary in Gr. II (n = 11) - CsA alone prophylaxis - aGvHD II-IV was diagnosed in 5 pts (45,5%), while relapse only in 2 children (18,2%) (3,5 years pLFS = 78%). Among 5 relapses that occurred in both groups, only one was observed despite less intensive prophylaxis and GvHD II-IV. There was significant difference in pLFS between two groups (33% vs 78%, respectively p = 0,03). This difference was even more evident in children from both group demonstrating no GvHD (pLFS 20% vs 80%, p = 0,02). Conclusions: 1. In children undergoing MRD-BMT for ALL in II CR the less intensive GvHD prophylaxis, i.e. CsA alone, results in more frequent GvHD occurrence, but diminishes risk of relapse. 2. The impact of GvHD prophylaxis intensity on ALL relapse rate appeared most evident in children without any symptoms of GvHD. 3. Observation concerns small, but homogeneous group of patients and could advocate participation of GvL reaction in total antileukemic effect of MDR-BMT in children with ALL in II CR. Supported by Grant KBN 4 PO5E 108 18

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MRD-BMT after ATG-CY conditioning in heavily pretransfused children with SAA transplanted from matched related donor late from diagnosis
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According to Passweg et al. (1997) between 1986-1992 survival after MRD-BMT for SAA was negatively associated with time from diagnosis to transplantation (>2 months) and with number of previous blood product transfusions (>20). Aim: To evaluate whether procedure consisted of ATG-CY as preparative regimen, CsA-MTX for GvHD prophylaxis and r-HuG-CSF is effective in heavily pretransfused children with SAA undergoing MRD-BMT late from diagnosis. Patients and method: Between 1993-1999 ten children (5 boys, 5 girls) aged 6-15 (med. 11) years with SAA underwent MRD-BMT. In 4 patients (pts) SAA was preceded by liver disease of unknown origin and in other 2 by EBV infection. Prior to BMT all pts were transfused with RBC (range 2-17 U, med. 9 U) and platelets (range 15-178 U, med. 26 U). Donor-recipient sex-mismatch was observed in 5 pairs, while major ABO-incompatibility in 3 BMTs. Prior-BMT CMV-antibody was detected in 8 pts, 2 CMV-seronegative pts received BM from seronegative donors. Interval from diagnosis to BMT was 2-20 mths (med. 3,5 mths). Preparative regimen consisted of ATG 5 x 20 mg/10 kg and CY 4 x 50 mg/kg. Methylprednisolone 1,0 mg/kg was given before each ATG-dose. 2,4-5,0 x 10^8 BM-MNC/kg (med. 3,4 x 108 BM-MNC/kg) have been transplanted. For GvHD prevention CsA (day -1 to +180) and “short” MTX were given. G-CSF was administered from day +1 until ANC reached 1,0 G/l. Last 7 pts were weekly tested for CMV-DNA. Results: The Engraftment was achieved in all pts with ANC > 0,5 G/l obtained within 15-23 days (med. 21 days), platelet count > 50 G/l within 17-116 days (med. 35 days) and reticulocytes > 0,05% within 13-104 days (med. 23 days). Acute GvHD>II was observed in 3 pts. with subsequent extensive cGvHD in 2 of them. Reactivation of CMV infection was observed in 3 of 7 pts monitored for CMV-DNA. However, there was no early or late deaths related to BMT complications. All children are alive with median follow-up of 40 mths (range 19-75 mths) and pEFS is 100%. Conclusion: ATG+CY as conditioning, CsA-MTX for GvHD prophylaxis and r-HuG-CSF is an effective therapeutic procedure even in heavily pretransfused children undergoing MRD-BMT for SAA late from diagnosis, i.e. despite major factors associated in the past with treatment failure. Supported by grant 501-1-05-20.
Clinicin impact of donor T-cell engraftment following T-cell depleted PBSCT versus non T-cell depleted BMT in children

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In order to minimize GvHD T-cell depleted PBSCT has been used increasingly during the last years. Additional ATG pre-transplant is frequently being used as graft rejection prophylaxis. To evaluate the role of PBSCT with different degrees of T-cell depletion and the influence of ATG on donor T-cell engraftment and clinical outcome, a retrospective evaluation of data obtained by flow cytometric phenotype analysis has been performed. We compared CD3+/CD4+, CD3+/CD8+ and CD56+ count in PB at day +1, +14, +28, +56, +100, +180 and +360 in 50 paediatric patients after SCT. Patients with mixed T-cell chimerism were excluded. Source of stem cells was PBSC in 21 and BM in 29 cases. The median number of transplanted T-cells/kg aw 1 x10/4 (PBSC, T-cell depleted), 1 x10/7 (PBSC, T-cell reduced) and 8 x 10/7 (BM, unmanipulated). All patients, except those with MSD underwent ATG containing conditioning regimens. Patients after PBSCT had a significantly lower CD3+ count between day + 14 and +180 (260/yl vs 850/yl, p=0,0175). There was a significantly lower CD3+ count after PBSC with T-cell depletion compared to PBSC with T-cell reduction up to day + 56. Patients transplanted with unmanipulated BM who received ATG in the course of their conditioning regimen showed a significantly delayed T-cell engraftment compared to those conditioned without ATG on day +14 (0/yl vs 250/yl, p=0,0002) and on day +28 (median 100/yl vs 280/yl, p=0,0008). Cd56+ count was higher after PBSC on day +14 and +28. There was no difference in CD3+/CD4+ ratio between the different patient groups. Concerning clinical outcome, we found a strong correlation between delayed T-cell engraftment and viraemia. Patients with PCR proven viraemia had a significantly lower CD3+ count on day +28 and +56 and patients with less than 100/yl CD3+ cells on day +28 showed a higher incidence of viraemia (52,5% vs 10%, p=0,015) and TRM (54% vs 15%). There was a trend towards a higher CD3+ count between day + 28 and + 56 in patients with a-GvHD. Concerning relapse no difference in CD3+ count could be observed. Patients with T-cell depleted PBSCT and/or ATG containing conditioning regimens show a delayed T-cell engraftment. ATG seems to be an independent factor for delayed T-cell engraftment. The number of circulating T-cells after allogeneic SCT seems to have an impact on the incidence of viral infections and TRM but not on relapse rate.

High risk Ewing/Pnet sarcoma treated with meagtheraphy followed with ashsct rescue. Comparison with conventional therapy

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Between 1992 and 1999 thirty four high risk Ewing’s sarcoma (HR ES) pts (22 males, 12 females) were enrolled in the transplantation program. The median age was 13,4 yrs. Primary tumor site was pelvic in 15, femur 8, spinal column - 5, humerus 2, metarsus 1, clavicule 1, ulna 1, tibia 1 pts. Eight of them had metastases (lymphnodes and lungs). Induction treatment: POG/CCG (20pts), (EI)CESS (10 pts), T-11 (3 pts), St.Jude (1 pt). All but one child achieved complete remission, the last child was in PR at the end of induction. High dose chemotherapy: VP-16 (160 mg/m2 x 4, carboplatin 250 mg/m2x4a melphanal 70 mg/m2x 2), TBI in 3 pts. Twenty-two pts received periferal blood stem cell (PBSC) rescue, 6 pts autologous bone marrow (BM) and 6 pts PBSC + BM. Median engaftment was 11 days (range 11 and 32). The median 3 yrs overall survival is 64%, EFS 55%.

In the historical group 34 HR ES pts (12 males, 22 females) were treated by conventional therapy. Primary tumor site was pelvic in 18 pts, femur 4 pts, spinal column 9 pts, ribs 2 pts and mandible 1 pt. Nine of them had metastases. Therapeutic regimens:T-11, T-2- 9 pts, (EI)CESS-92 - 7, Cisplatin/ADR in 2 pts. Radiotherapy in 25/34 pts. Overall survival is 20,6 %, EFS 11,75%.

In conclusion: In spite of the fact that those patients represent probably a group who has received a more aggressive primary treatment and the relapse could be more severe; the results indicate that the group that is presented now yielded better EFS than the group previously reported (1983-1991).

Allogeneic versus autologous BMT for ALL in second remission in 111 children. GETMON (1992-98)

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We are comparing the results of allogeneic BMT (alloBMT) with those obtained with autologous BMT (autoBMT) in 111 children for ALL in second remission. Thirty-six patients received alloBMT and seventy-five autoABMT between 1992 and 1998, in seven spanish centres. Both groups were homogeneous with respect to the following: age, sex, immunophenotype, duration of first remission, leukocytes number and risk at diagnosis, percentage of early and late relapses and marrow or extramedullary involvement prior to transplant, time interval from second remission to transplant, chemotherapy treatment for relapse and conditioning regimens for transplant. The source of the stem cells was predominantly bone marrow in both. Median time follow-up of alive patients is 50 months in alloBMT group and 55 months in autoBMT group.

The most significant results are: 1.- The 8-year actuarial event-free survival (EFS) was 0.48 +/- 0.08% in alloBMT vs 0.35 +/- 0.05% in auto BMT (p=NS). 2.- High risk at diagnosis carries a poorer prognosis in EFS in alloBMT (0.27 +/- 0.10 in high risk vs 0.60 +/- 0.11 in standard risk, p=0.003) and also in autoBMT (0.25 +/- 0.07 vs 0.45 +/- 0.08, p=0.03). 3.- Relapse probability was higher in autoBMT 0.60 +/- 0.06 vs 0.36 +/- 0.09 in alloBMT (p=0.03). 4.- Isolated or combined bone marrow relapse denotes a poorer prognosis in autoBMT group (0.27 +/- 0.05 in BM relapse vs 0.61 +/- 0.14 in extrBM, p=0.05).

In conclusion: In spite of the fact that those patients represent probably a group who has received a more aggressive primary treatment and the relapse could be more severe; the results indicate that the group that is presented now yielded better EFS than the group previously reported (1983-1991).
Prolonged remission and autologous recovery in a pediatric patient with Ph+ CML after graft failure of unrelated cord blood transplantation

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Allogeneic stem cell transplantation is the best curative treatment for patients with Ph+ CML. Following BMT, engraftment, remission or relapse must be monitored. A 10-year-old boy was diagnosed in July 1997 of Ph+ CML in chronic phase. The molecular genetic study showed bcr/abl positivity with b3a2 rearrangement. As an HLA matched sibling was not available, an unrelated BMT donor was searched for 1 year without success, while the patient was under treatment with hydroxyurea. Finally, an unrelated cord blood transplant with a 5/6 "broad" compatibility was performed. Conditioning consisted in busulfan (16 mg/Kg), cyclophosphamide (120 mg/Kg) and ATG. Cyclosporine and methotrexate were given for GVHD prophylaxis. The number of nucleated cells infused was 6.3x10e7/Kg and of CFU-GM and CD34+, 3.7x10e4/Kg and 0.2x10e6/Kg respectively. No engraftment was achieved on day +40, cyclosporine was discontinued and treatment with G-CSF at a dose of 10 mcg/Kg/day was initiated. Bone marrow repopulation was confirmed on day +53 with neutrophil count of 0.5x10e9/l. Platelet count reached >20x10e9/l on day +70. Neither GVHD nor CMV infection were detected. Two months after transplant, autologous reconstitution was confirmed by karyotype, erythrocyte antigens and HLA antigens study. Bone marrow aspiration showed complete hematologic remission and bcr/abl rearrangement was undetectable.

Two years after transplant the patient remains in complete clinical and hematologic remission. Bcr/abl rearrangement has consistently been negative. This finding may have resulted from a successful elimination of leukemic clones by the conditioning regimen and bone marrow reconstitution from a pool of residual normal hemopoietic cells. The prolonged remission of this patient may be due to the proliferative advantage of Ph- progenitors and the antileukemic effect of lymphocytes in the graft.

Extended family studies for the identification of Bone Marrow (BM) donors in Jewish and Arab patients

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Background: The most suitable donor for a patient in need of an allogeneic BMT is an HLA genotypically identical donor which is available for only a small portion of the patients. When a HLA identical core family member is not found, it is possible to perform an extended family search.

Methods and Results: During the last 10 years (1990-1999), 356 patients and 3,015 of their family members were tissue typed in order to find a matched donor; 239 (67%) were Jewish and 117 (33%) were Arabic. An HLA identical donor was identified for 168 (47%) patients of which 49 (29%) had more than one potential donor. Of the Jewish patients, 95 (38.7%) had a matched donor while for the Arabic patients, genotypically identical donor was identified for 73 (62.3%). In 38 families where a matched sibling was not identified, an extended family search (grandmother/father, cousins, uncles, etc.) was performed. Among 5 Jewish families, only one HLA genotypically identical donor was found while 21 out of 33 (63.6%) Arab patients had an HLA genotypically identical donor in the extended family. We conclude that extended family search for potential identical HLA donors is worthwhile especially when performed in distinct ethnic populations such as Israeli-Arab where consanguinity often prevails, thus offering substantial chance of finding donors other than core family members.

Technique for PBSC harvesting in children of weight under 10 kg

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BACKGROUND: Peripheral blood stem cells (PBSC) are routinely used as hematopoietic support after high-dose chemotherapy for various malignancies in children. Nevertheless, limited data are available on PBSC collection in the smallest pediatric patients weighed under 10 kg. Collection using automated or semi-automated devices (e.g. COBE Spectra) is almost impossible because of two main technical problems: volume of the disposable PBSC separation set is more than 25% of total blood volume (TBV), flow capacity of vascular access is not sufficient for leukapheresis. Therefore, we developed a simple manual technique for PBSC harvesting.

PATIENTS AND METHODS: Two children (T.R. and M.N., aged 7 and 7.5 months, weight 6.3 and 8.6 kg) with newly diagnosed brain tumors were planned to be treated with six sequential courses of high-dose chemotherapy, each followed by PBSC support. PBSC were mobilized using cyclophosphamide 2.5 g/m2 followed by G-CSF 10 mcg/kg/day. Harvests were initiated when CD34+ cell count in peripheral blood exceeded 50x106/l. Prior to collection, children were treated with i.v. heparin. About 50 ml of blood (8 ml/kg, 10% of TBV) was drawn from venous catheter, injected into 150 ml transfer bag containing 5 ml ACD-A, and centrifuged (120 g, 15 minutes). After centrifugation,uffy coat was carefully obtained and injected into collection bag containing solution of 4% human serum albumin in Hank’s salt solution. Remaining plasma and erythrocytes were mixed and reinfused. Next procedure started downnight after reinfusion. Buffy coats were pooled in collection bag until cryopreservation. Samples were taken from the collection bag to assess a number of so far collected progenitors. The procedure was repeated until satisfactory number of CD34+ cells was reached for six PBSC supports and back up.

RESULTS: PBSC collections started on day 8 (patient T.R.) and 9 (patient M.N.) from cyclophosphamide infusion, lasted 3 days for each patient, and 9 and 13 procedures were performed. Seven bags containing 2.67-3.96 and 1.79-2.54 x 106 CD34+ cells/kg were frozen for each patient. All samples collected to detect bacterial contamination (before freezing and after thawing) were negative.

CONCLUSION: The above-mentioned simple technique seems to be safe and effective way for PBSC harvesting in very small children when used after potent mobilization regimen.
PBSC collection using COBE spectra in small children - single center experience

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BACKGROUND: Autologous peripheral blood stem cells (PBSC) are now routinely used as hematopoietic support after high-dose chemotherapy even in small children with various malignancies.

PATIENTS AND METHODS: Twenty-two small children with solid tumors, aged from 22 months to 10 years, with median weight of 15.7 kg (10-26) were mobilized using either combination of chemotherapy and G-CSF or G-CSF alone and underwent PBSC collection between May 1998 and August 2000. Cyclophosphamide, VP 16, and doxorubicin were the most frequently used drugs as mobilizing chemotherapy. PBSC harvest has been started when CD34+ cell count after nadir has reached at least 5x10^6/l in heavily pretreated children and 15x10^6/l in others. Collections were performed using a COBE Spectra cell separator. The extracorporal line was primed with irradiated red blood cells when volume of the apheretic disposable set exceeded 20% of the patient's total blood volume.

RESULTS: A total of 68 aphereses was performed with a median of 3 aphereses (1-5) per patient. The extracorporeal line was primed with irradiated red blood cells in 14 patients with body weight less than 18 kg (40 aphereses). In 15 patients with lower body weight heparin as anticoagulant therapy has been used. As a venous access, we have used combination of Hickman catether (access) in subclavian vein and peripheral catether (return) in 17 patients, double lumen catether in femoral vein in 3 patients, double lumen Hickman catether in one patient, and in one patient combination of single lumen catether in subclavian vein and peripheral catether. The inlet flow ranged between 19 and 40 ml/min (median 24 ml/min). A median of 7.5x10^6/kg (1.3-108.9) CD34+ cells, and 103.3x10^4/kg (18.4-525.9) CFU-GM were collected. No hypotensive, hypothermic or bleeding complication as well as no signs of complication due to citrate toxicity has been observed so far. Twenty out of 22 children were accompanied by their relatives during the procedures and overall tolerance was very good.

CONCLUSION: We conclude that leukaphereses using COBE Spectra could be safely performed even in very small children. Citrate toxicity assessment could be very difficult in small children. The use of heparin instead of citrate seems to prevent this potential complication.

Optimum timing of apheresis following G-CSF in autologous setting: monitoring of CD34+ cell concentration in the peripheral blood of children within 24 hours before apheresis

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To collect a maximum amount of CD34+ cells for autologous transplantation in childhood, it is desirable to know the circadianic kinetic of CD34+ cells in the peripheral blood to time apheresis after G-CSF (Neupogen (R), Amgen) application on collection day.

In our attempt to improve the efficiency of stem cell apheresis after stimulation with G-CSF, we have monitored the increase of CD34+ blood progenitor cells in the peripheral blood of 12 pediatric patients (6 neuroblastoma, 2 osteosarcoma, 1 PNET, 1 ALL, 1 rhabdomyosarcoma and 1 Ewing-s sarcoma. Average age of the patients was 8 years (3/4-17) with a mean body weight of 34,1kg (9,1-71). In the 12 hour interval between two daily applications of G-CSF (2×times;5 µg/kg) during the night prior to the first apheresis, we determined the concentration of CD34+ blood progenitor cells in peripheral blood by flow cytometric analysis using an EPICS XL-MCL (Beckman Coulter) every one or two hours in triplicate. An automated leukocyte count was also performed.

In our pediatric patients the leukocytes showed minimum concentration about two hours after G-CSF, while the CD34+ progenitor cells reached their nadir about four hours after G-CSF (mean: 79.7 ±plasm; 60 CD34-cells/µl). Surprisingly, the CD34+ cells reached their maximum level of concentration between 8 and 10 hours after G-CSF dose (mean: 119.7 ±plasm; 81 CD34+ cells/µl).

We conclude that the optimal time for apheresis of CD34+ blood progenitor cells in the peripheral blood of children differs significantly from the four-hour-rule used in adult apheresis schedules. We suggest a timing of apheresis for pediatric patients adapted to the eight-hour-peak of CD34+ stem cell concentration found in our twelve patients, and recommend an individual monitoring of every patients prior to apheresis (e.g. by two analyses of CD34 cell concentration about four and eight hours after G-CSF) to determine the best time for apheresis. This strategy may help to optimize peripheral blood stem cell collection for a successful transplantation and a better clinical outcome of pediatric patients. Supported by “Hilfe für Krebskranke Kinder Frankfurt e.V.” and “Amgen GmbH, München”.

Levels of in vivo generated complement activation products and C1 inhibitor (C1-inh) in a C1 inh concentrate-treated patient with Capillary Leak Syndrome (CLS) following bone marrow transplantation

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C1-inh concentrate has been shown to have beneficial effects in CLS, We report on our first experience in a bone marrow transplanted (BMT), C1-inh treated child with CLS. We measured the levels of CH50, C4, C3 as well as in vivo generated complement activation products (C5b-9, C3bBbP, C1rC1sC1inh,C4d) and the concentration and activity of C1-inh in two BMT patient, one is treated with C1-inh for CLS and other without this complication. C5b-9 levels were higher in the patients compared to the healthy controls (11.3±1.9 and 3.9±1.1 p=0.002). Strong correlation was found between the levels of C3bBbP and C1rC1sC1inh complexes (r=0.97, p<0.0001), indicating that complement activated through both pathway, The levels of C4d, C5b-9 were higher in the patient with CLS, than in the patient without CLS (C4d:17.9±1.3 ng/ml and 14,1±0.7 ng/ml, p=0.03, C5b-9: 16.2±1.9 U/ml and 5.5±2.1 U/ml, p=0.003). By contrast the activity of C1-inh was lower in the former patient (104.6±3.3 and 116.8±1.1, p=0.01) After infusion of C1inh the concentration of levels of complement activation products decreased, it
was the most striking with C1rC1sC1inh (from 1320 U6Ml to 198 U/ml). In CLS patient treatment with C1-inh concentrate resulted a rapid clinical stabilization. Our data also supported that complement has a pathological role in the development of CLS. The results point to the amplifier function of the alternative pathway, indicating that blocking both major pathways with combined complement inhibition might be an effective therapeutic possibility in the treatment of CLS.

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The use of donor-type red blood cells to manage abo-major-incompatible BMT in children

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Infusion of ABO-major-incompatible bone marrow may lead to severe hemolysis due to recipient isohemagglutinins directed against donor-type RBCs. Red cell depletion of bone marrow or reduction of isoagglutinin titers by plasmapheresis or transfusion of donor-type RBCs are ways to avoid this problem.

Within 8 years from 1992, 30 children underwent ABO-major-incompatible BMT or PBSCT at our hospital. Their median age was 7 yr (range 4mo-18yr), male/female ratio was 2:1, diagnoses were ALL(10), CML(4), AML(3), NHL(3), MDS(3) and others(7). Isoagglutinin titers at admission, examined by indirect antiglobulin test at 37°C, were between 1:2 and 1:32 in 7 patients who then received 1 incompatible transfusion. At higher titers, the marrow was red-cell depleted, PBSCs were given unmanipulated. One boy with a titer of 1:128 was transfused twice.

Donor-type red cells were given 9 times to 8 patients several days before BMT. Three moderate reactions (urticaria, nausea, fever, macrohematuria) were seen. No severe reaction requiring catecholamines occurred. Isoagglutinin titer controls revealed a drop to 0 - 1:8 in 7/8 patients. During the subsequent BMT, no transfusion reaction was observed in any of the children.

Conclusion: In ABO-major-incompatible BMT in children the transfusion of donor-type RBCs before BMT is a safe method to reduce isoagglutinin titers. Thus, red cell depletion of the marrow with possible loss of stem cells can often be avoided.

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Transplantation for Congenital Amegakaryocytic Thrombocytopenia (CAMT): report of two cases with reduced toxicity conditioning regimens

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OBJECTIVE: We report on our experience with the transplantation of two boys with CAMT. In both patients a homozygous mutation in the TPO-receptor gene c-mpl had been identified (G305C leading to R102P; Ballmaier et al.; Blood, in press). Due to the non-malignant character of this disease, a conditioning approach with reduced toxicity was used in both patients.

CASE 1: This patient developed increasing transfusion dependency and bleeding episodes. His mother was found to be a suitable donor with an HLA-DRB1 mismatch. Conditioning was busulfan (2x4 mg/kg), fludarabine (6x30 mg/m2) and cyclophosphamide (2x80 mg/kg). Rejection prophylaxis was OKT3 and prednisone from days -4 to +10. 29.6x10(6)/kg CD34-selected PBSC were transfused. Engraftment was at day +10 following administration of G-CSF. There were no episodes of severe infection, mucositis or GvHD. During follow-up, 3 DLI with a maximum number of 5x10(5) CD3-T-cells were necessary due to autologous reconstitution up to 47% in the peripheral blood. More than a year after transplant, the boy is well with a stable donor chimerism of 100% and no GvHD.

CASE 2: This boy also developed signs of bone marrow failure. An HLA-identical unrelated donor was identified. Conditioning and rejection prophylaxis were identical to case one. 9.5x10(6)/kg CD34-selected PBSC were transfused. In this case, no G-CSF was administered. After initial engraftment on day +11, graft rejection occurred on day +15. A re-transplantation was performed with a second HLA-identical unrelated donor and an intensified conditioning regimen. Despite rapid regeneration, the boy developed ARDS and died on day +11 after re-transplantation.

CONCLUSION: Our conditioning regimen with the use of a T-cell-depleted transplant allowed rapid engraftment with little toxicity in both patients with CAMT. However, graft rejection occurred in one patient, indicating that a higher stem cell dose and/or intensified immunosuppression may be required for stable engraftment.

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Treosulfan followed by CY + ATG as conditioning for allogeneic BMT in a child with Wiskott-Aldrich syndrome - a case report

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Treosulfan (TRS) demonstrates activity against committed as well as early hematopoietic stem cells (Ploemacher et al., 1999). TRS was used, with success, in combination with fludarabine by Casper et al., (2000) in preparative regimens for allo-HSCT in 5 adult patients, who had increased risk of toxic side effects because of age, infections and second transplantation. Therefore we decided to substitute busulfan with TRS in a 3-year-old boy (UPN 97) with WAS. He was referred to our Unit after completion of his treatment of Hodgkin disease (type II NS). At the time of admission, he suffered from malabsorptive syndrome, hepatitis C and recurrent lower respiratory tract infections. The conditioning regimen consisted of TRS (10 g/m2 on days -8, -7 and -6), CY (50 mg/kg from day -5 till -2) and ATG (from day -5 till -2, total dose 375 mg). ATG was given not to a fixed dose, but up to the desirable effect, that is CD3 count below 0,1% of WBC. On day “0” the child was infused with 7,6 x 106 CD34+/kg. As GvHD prophylaxis he received CsA (3 mg/kg) + MTX (10 mg/m2 on day-1). G-CSF (5 mcg/kg) was given intravenously from day +1 until WBC exceeded 1,0 G/l (+11). Neutrophil level >0,5 G/l was achieved on day +9, whereas platelet level >20 G/l on day +12. Acute GvHD II (skin) treated with methylprednisolone (max. 4 mg/kg) was observed from day +7 until +17. The only undesirable effect observed during hospitalization was the elevation of alanine aminotransferase activity (up 3 x normal), but since the boy was infected with hepatitis C virus, the origin of this laboratory finding is unclear. During the whole peritransplant period, the boy had pneumonia (of unknown origin) on clinical and X-ray examinations, with prominent bronchoconstriction since engraftment. All the complications resolved completely until discharge from hospital on day +27. Complete donor chimerism was reached by day +73. On day +112 (last observation) the boy is doing well with no infections and stable haematopoiesis of
Severe congenital dyserythropoietic anemia treated with intrauterine transfusions and bone marrow transplantation

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We report the case of a one-year-old boy who was diagnosed intrauterine of severe anemia presenting as hydrops fetalis. Parents were cousins and the mother had 4 pregnancies before with one dead fetus. All etiologic studies of anemia were negative as well as the tests of matriofetos iso-immunization. The patient needed five intrauterine transfusions from 21 weeks of gestation (Hb: 1.6 g/dl), until birth at 34 weeks by caesarean section (Hb: 6.9 g/dl). Birth weight was 1,915 g. Pallor, mild jaundice and generalised edema were present. The patient required phototherapy, mechanical ventilation in newborn period and was transfused every 3-4 weeks. Bone marrow examination showed increased cellularity with erythroid hyperplasia and dyserythropoietic features like binnucleated erythroblasts. An ultrastructural study suggested the diagnosis of atypical congenital dyserythropoietic anaemia (CDA). Ham test was negative. The patient was allografted from an HLA-identical sibling at 14 months of age. Regimen consisted of Busulfan (14 mg x kg) and Cyclophosphamide (200 mg x kg). Cyclosporine A was used as GVHD prophylaxis. A total number of nucleated cells 7.72 x106/kg, CD3+ cells 16.73 x106/kg and CD3- cells 3.2 x106/kg were infused. Neutrophil engraftment on day +11 was observed. DNA studies showed total chimerism at the same day. The patient is now alive and free of red cell transfusions.

Conclusion: This case report highlights the possibility to reach delivery in a patient with hydrops fetalis by means of periodic intrauterine transfusions. A postnatal diagnosis of CDA allowed us to treat the patient with a potentially curative option such as allogeneic BMT.

Immune reconstitution following sibling marrow or cord blood progenitor cell transplantation (a preliminary report)

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The aim of this study was to evaluate the pattern of immune reconstitution in children after fully matched sibling marrow or cord blood cell transplantation. Between November 1997-November 2000 immunological reconstitution was analysed in twelve children allografted either by matched sibling marrow (n:10) or cord blood(n:2) progenitors. Among bone marrow allografted ten patients (4 girls,6 boys) 5 were AML in 1 st remission while remaining 5 have different diagnosis (1 SCID,1 FFA,1 SAA,1 KMM1,1 SCA). The age range was 6 month-17 year with a median of 10 years. Cord blood progenitor recipients (n:2) were both patients with Beta-Thalassemia Major (a girl-2.5 years; a boy-4.5 years old). Peripheral blood (PB) lymphocyte regeneration was investigated prior and at hematological reconstitution whenever achieved and at 2,3, 6,12, 18 and 24 month following transplant. The distribution of lymphocyte subsets expressing CD3, CD4, CD8, CD56, CD16 and lymphocyte activation markers CD69, CD25 and CD54 were evaluated by using direct immunofluorescence method and dual color staining in flow cytometry (EPICS XL-MCL). NK cells and CD8+ T cells were the first two populations which came up early after allogeneic marrow transplantation. NK cells and CD8+ cells remained as the prominent populations till 3 and 12 post transplant months respectively. However CD4+ T cells were reached to reasonable levels by 12 month and B lymphocyte numbers seemed to be normalised at 6 months after transplantation in marrow recipients. Early recovery of CD8+ T cells, NK cells and faster CD4+ T and B lymphocyte reconstitution have been detected in patients who had received cord blood cell transplantation.

Incidence of secondary myelodysplastic syndromes following autologous stem cell transplantation in children with malignant diseases

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Secondary myelodysplastic syndromes (sMDS) and secondary myeloid leukemias (sAML) have been reported after autologous hematopoietic stem cell transplantation for various malignancies. We retrospectively reviewed all pediatric hematopoietic stem cell transplantation cases performed in our center from 1985-1999 in order to determine the incidence of sMDS or sAML. Data from 97 and 122 patients who received allogeneic and autologous transplantation respectively were available. None of the allografted patients have developed sMDS or sAML so far. Among 122 autografted patients, two (1.6%) developed sMDS. The incidence did not differ according to the progenitor cells source (1.4% for bone marrow and 1.8% for peripheral blood).

These two patients had acute myeloblastic leukemia (M1) in first complete remission and their ages at transplant were 13 and 6 years respectively. They had not received alkylating agents for the treatment of their leukemia. None of them showed myelodysplastic features before transplantation and cytogenetic analyses were normal in both patients. The transplant preparative regimen was busulfan (16 mg/Kg total dose) and cyclophosphamide (120 mg/Kg total dose) in the two cases. Time between transplantation and diagnosis of sMDS was 13 months for the bone marrow recipient and 6 months for the patient who underwent PBSCT. In both, bone marrow examination showed dysmyeloletopietic features affecting the three lineages and cytogenetic analyses evidenced clonal abnormalities. The ABMT patient received IL-2 for one month and died from disease progression. PBSCT patient was submitted to allogeneic BMT from an HLA matched sibling and died of GVHD and multiorgan failure.

The incidence of sMDS in our series of pediatric patients is similar to the incidence reported in adults. We found not association between alkylating agents therapy and development of sMDS. Time elapsed between transplantation and sMDS was shorter in the patient who underwent PBSC, as previously described in the literature.
Relapse after allogeneic stem cell transplantation for juvenile myelomonocytic leukemia: are there still options?

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From 1997 until now we transplanted 5 children with juvenile myelomonocytic leukemia. Two children were transplanted with HLA-identical sibling donors (one bone marrow, the other cord blood), one with a 1 mismatch family donor, one with an unrelated donor, one haplo-identical peripheral blood stem cell graft. All primary conditioning regimens were busulphan/cyclofosfamide/melphalan except for the haplo-procedure where thiopeta was used instead of melphalan. The unrelated donor did not engraft and after a second procedure the patient rapidly developed a relapse and died. The identical sibling cord blood procedure resulted in complete remission with a current follow-up of 10 months. The non-T-cell depleted mismatch family donor graft procedure resulted in severe acute GvHD (grade III) and extensive chronic GvHD. This patient is in complete remission with a follow-up of more than 2 years now. The cGvHD has resolved.

Three patients developed a relapse post-transplant (60%). One relapse developed very rapidly, no treatment was undertaken. The other two relapsed 7 and 8 months after the transplant. In these cases the relapse was first noticed in chimerism studies, showing reappearance of increasing recipient hematopoiesis. DLI’s were given, leading to temporary improvement in chimerism studies. Hence a second transplant using the same donors was considered feasible.

Conditioning was fludarabine/TBI based.

Toxicity of the second procedure was very limited and they are currently in complete remission with follow-ups of 3 and 4 months respectively.

These results show that juvenile myelomonocytic leukemia has a high probability of relapse after allogeneic stem cell transplantation but that in cases of slowly progressive relapse, mostly apparent in chimerism studies, a second graft can be a therapeutic option.

Safety and efficacy of high-dose G-CSF (24 mcg/kg) for PBSC mobilisation in children

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BACKGROUND: Good mobilisation is essential for PBSC collection with a minimum number of apheresis. Heavily treated patients are more prone to mobilisation failures or to repeated apheresis sessions, which is particularly undesirable in children. There is some evidence suggesting that increased doses of G-CSF have greater mobilisation potential than standard ones. We evaluate the safety and efficacy of high-dose G-CSF for mobilisation in children.

METHODS: Twelve consecutive patients submitted to our bone marrow transplant unit for autologous PBSC transplantation were mobilised with r-met-HuG-CSF (Filgrastim) at 24 mcg/Kg/day divided in two 12-hourly doses, for 4 days. On the 5th day, patients were given the morning dose of G-CSF and CD34 count was determined by flow citometry according to ISHAGE protocol.

Patients with CD34 counts greater than 15/mm3 were submitted to a large volume leukapheresis (LVL) aimed to obtain at least 2 x 10e6 CD34/Kg.

RESULTS: The patients were 5 boys and 7 girls with a median age of 10 y (range: 2.5 to 15.3). Diagnoses were CNS tumors in 6 patients, T-ALL in 2, Hodgkin disease in 2, Neuroblastoma in 1, and Ewing’s sarcoma in 1. All patients had been intensively treated prior to PBSC transplantation.

On the 5th day of mobilisation median WBC count was 36.3 x 10e9/L (range 7.3 to 120), and median CD34 count was 24/mm3 (range: 2 to 104). Ten patients (83%) attained CD34 counts equal or greater than 15/mm3 and were submitted to LVL. All these 10 patients achieved the target dose of CD34 cells with one single apheresis (median CD34/Kg: 3.36, range: 2.22 to 12.7).

No clinical side effects of this high-dose G-CSF schedule were reported by the patients. Two patients (17%) developed leukocytosis greater than 100 x 10e9/L. However, no clinical symptoms attributable to this degree of leukocytosis were present.

Two additional patients presented mild thrombocytopenia (platelet count 114 and 113 x 10e9/L) that could not be attributed to other causes.

CONCLUSIONS: High-dose G-CSF is well tolerated in children, and in combination with LVL it allows PBSC collection with a single apheresis in heavily treated patients.