Allogeneic peripheral blood stem cell transplantations in children – a single center experience*

S. ŠUFLIARSKA[1], J. HORÁKOVÁ[1], I. BOĎOVÁ[1], J. MARTINKA[2], M. HRUBIŠKO[2], L. KOVÁCS[1], K. JANČOVIČOVÁ[1], J. LUKÁČ[1]

[1]Bone Marrow Transplantation Unit, e-mail: jozefsuf@cdicon.sk, Department of Pediatrics, Comenius University Medical School, 833 40 Bratislava, and [2]Department of Hematology and Transfusion, University Hospital, 851 07 Bratislava, Slovak Republic

Received October 3, 2003

We analyzed 30 peripheral blood stem cell transplantations (PBSCT) from 25 human leukocyte antigen (HLA) matched sibling donors (MSD) and 4 HLA-matched unrelated donors (MUD) in 29 patients, done between November 1996 and March 2003. Patients aged 3 to 17 years underwent allogeneic PBSCT for malignant (16 patients) and non-malignant (13 patients) diseases. Sibling donors aged 3 to 23 years were given granulocyte colony-stimulating factor (G-CSF) 5–10 μg/kg/day for 4 to 5 days. All but one of the 29 donors underwent one single leukapheresis for stem cell collection. The patients received a median of $4.2x10^6$ CD34+ cells/kg of body weight, they all engrafted after a median of 13.5 days (range 10–25 days). Acute graft-versus-host disease (GVHD) grade II to IV developed in 11 of 26 MSD transplants and in all 4 patients after MUD PBSCT. Eleven of 27 evaluable patients experienced chronic GVHD. After a median follow-up of 662 days, 20 out of 29 patients (69%) are alive, three of them need systemic immunosuppression for chronic GVHD. Six patients experienced relapse of their underlying malignant disease, one of them still alive in complete remission. Two patients died of grade IV acute GVHD and two others due to an opportunistic infection. Based upon our experience, PBSCT is a feasible and safe method for both pediatric donors and patients. It is associated with rapid engraftment, no greater incidence of acute but a higher incidence of chronic GVHD as compared to bone marrow transplantation (BMT) and therefore suitable mainly for children suffering from malignant diseases.

Key words: allogeneic peripheral stem cell transplantation, G-CSF mobilization, graft-versus-host disease, transplant-related mortality

Cytokine mobilized peripheral blood stem cells (PBSC) collected in healthy donors have recently become increasingly popular for use in related as well as unrelated allogeneic hematopoietic cell transplantation. There has been a steady increase in PBSCT; in 1998, 38% of all allografts reported to the European Group for Blood and Marrow Transplantation (EBMT) used donors' peripheral blood as the source of stem cells, whereas in the year 2000, it was already 53% of all allogeneic transplantations [18, 19].

Reports of the first studies of allogeneic PBSCT have clearly demonstrated that this was a feasible and effective approach, resulting in rapid and sustained trilineage engraftment, without an increased incidence of acute GVHD [7, 23, 28 41]. Majority of the further comparative studies of allogeneic PBSCT with BMT confirmed faster engraftment, with no greater incidence of acute GVHD [5, 8, 10, 39, 44]. One randomized study and a meta-analysis showed that PBSCT was associated with an increased risk of acute GVHD [12, 40]. Some reports found a significant increase in the risk of chronic GVHD, when compared with BMT and the probability of having chronic GVHD one year after PBSCT was about 65% [8, 10, 12, 40, 42], though other have failed to show significantly increased incidence of chronic GVHD [5, 6, 8, 11, 32]. Transplant-related mortality (TRM) does not show striking differences, overall survival seems to be higher and relapse rate lower in patients receiving PBSC, mainly in those transplanted with advanced myeloid malig-

*This work was supported in part by the grant 1/9287/02 from VEGA (Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences).

nancy [6, 10, 33, 39]. However, there is still limited number of studies evaluating PBSCT in children mostly reporting results on small cohorts of patients [4, 13, 24, 25, 27, 29, 42]. Majority of these studies found allogeneic PBCST safe for both the pediatric donors and recipients and indicated that PBCST might be considered as an alternative to bone marrow allografts. Those studies also showed a trend toward higher incidence of chronic GVHD [4, 43], however to clarify the antileukemic potential and the overall benefits of PBSCT further studies with long-term follow up are required. Therefore, we decided to carry out an analysis of the results of PBSCT undertaken in children at the pediatric BMT Unit of Comenius University Medical School, specifically looking at the numbers of CD34+ cells infused, kinetics of engraftment, incidence of acute and chronic GVHD, TRM and overall survival.

Patients and methods

Patients and transplantation. Between November 1996 and March 2003, twenty-nine patients (16 males and 13 females) from 3 to 17 years of age (median 11 years) received an allogeneic PBSCT for the treatment of malignant diseases as well as for non-malignant hematological disorders. Twenty-fours patients received stem cells from fully HLA-matched sibling donor, one from a syngeneic donor and four from HLA-matched unrelated volunteer. Written informed consent was obtained from the parents of the patients and their sibling donors, indicating their voluntary participation in this treatment procedure. The main characteristics of the patients, conditioning regimens and GVHD prophylaxis are summarized in Table 1. Unmanipulated PBSC with a median of $4.2x10^6$ CD34+ cells/kg of recipients body weight (range $1.6–12.4x10^6$ CD34+ cells/kg) and a median of $3.4x10^8$ CD3+ cells/kg of recipients body weight (range $1.5–11.5x10^8$ CD3+ cells/kg) were infused after completion of conditioning regimen. Post-transplant G-CSF (5 μg/kg/day) was given in 27 (90%) cases, starting day + 5 with a median duration of 8 days (range 3–17 days). Three patients received no cytokine support post transplant.

Donors, HLA-typing, PBSC collection. Twenty-five PBSC donors were HLA completely matched healthy siblings with a median age of 10.5 years (range 3–23 years). Four unrelated donors were HLA-A, -B, -C, -DRB1 and DQB1 compatible with the patients. Serological or DNA-based low-resolution typing was used for assessment of class I antigens, whereas DRB1 and DQB1 antigens were assessed by high-resolution molecular genetic typing. Sixteen donors were male and 13 female, with a donor-recipient sex mismatch in 11 cases. Donors' characteristics are summarized in Table 2. For PBSC mobilization all familial donors were given G-CSF 5–10 μg/kg/day subcutaneously once daily in the afternoon. After 4–5 doses, all but one donor sub-

Table 1. Patient and transplant characteristics

Characteristic	*Value*
No. Patients/Transplants	29/30
Patient age (years)	3–17 (median 11)
Patient sex (male/female)	16/13
Diagnosis and disease status at PBSCT	
Malignant diseases:	16
ALL:	6
CR1	1
CR3	4
CR4 (second transplant)	1
AML:	6
CR1	2
CR2	4
CML – CP1	2
NHL – CR2	2
Ewing's tumor	1
Non-malignant diseases:	13
VSAA	9
SAA	1
PRC aplasia	1
Wiskott-Aldrich syndrom	1
MDS	1
Conditioning regimen (total doses given)	
Busulfan 16 mg/kg Cyclophosphamide 120 mg/kg $\pm$ VP 16 40–60 mg/kg	15
Cyclophosphamide 200 mg/kg Antithymocytic globulin	11
Other	4
GVHD prophylaxis	
CS-A, MTX, $\pm$ATG	21
CS-A	8
None	1

ALL – acute lymphoblastic leukemia, CR – complete remission, AML – acute myeloblastic leukemia, CML – chronic myeloid leukemia, CP – chronic phase, NHL – non-Hodgkin lymphoma, VSAA – very severe aplastic anemia, SAA – severe aplastic anemia, PRC – pure red cell aplasia, MSD – myelodysplastic syndrome.

Table 2. Donor characteristics

Sibling donors:	A = 25
Age: median (range)	10.5 years (3–23)
Sex (male/female)	15/10
Sex mismatch	A = 11
G-CSF:	
Dose	5–10 μg/kg/day
Administration	4–5 days
Leukapheresis:	
Single apheresis	A = 28 (96%)
Two aphereses	A = 1 (4%)
Venous access:	
Central venous catheter	A = 17 (68%)
Cubital veins	A = 8 (32%)
Unrelated donors (male/female)	A = 4 (2/2)
Sex mismatch	None

sequently underwent one single leukapheresis with a sufficient number of CD34+ cells obtained, using a continuous flow Cobe Spectra blood separator (Cobe Laboratories, Lakewood, CO, USA) according to the manufacturer's manual. In one donor, the stem cell harvest had to be repeated twice. Donorś vascular access was obtained through a central venous catheter in 17 (68%) and through venipuncture of cubital veins in both arms in eight of the 25 sibling donors, as well as all four unrelated donors. In one case, stem cells were collected in advance, cryopreserved, and later thawed prior to infusion. G-CSF administration, central venous catheter insertion and leukapheresis were well-tolerated and no severe adverse effects, except for mild bone pain in one donor, were reported.

Definition of endpoints and statistical analysis. The study was focused on hematopoietic recovery, acute and chronic GVHD incidence, TRM and overall survival analysis. Neutrophil engraftment was defined as the first day of an absolute neutrophil count (ANC) $>0.5x10^9$/l, maintained for 3 consecutive days. Platelet engraftment was defined as the first day of platelet count $>20.0x10^9$/l, maintained for 3 consecutive days without transfusion support. Further, donor chimerism, as evidence of bone marrow graft acceptance, was established in sex-mismatched donors by dual-color fluorescence *in situ* hybridization (FISH) for sex chromosomes heterochromatic regions. In patients with sex-matched donors the samples for chimerism study underwent polymerase chain reaction (PCR) to analyze the variable number of tandem repeats (VNTR) [3]. Acute GVHD was evaluated in patients with evidence of engraftment and was diagnosed clinically and graded according to the GLUCKSBERG criteria [17, 35]. Chronic GVHD was assessed in all patients surviving to day 100 and defined as limited chronic GVHD with localized skin and/or mouth and/or liver involvement or extensive chronic GVHD, that did not meet the definition of limited chronic GVHD [2, 38]. TRM was defined as any cause of death other then relapse or progressive disease irrespective of the time after transplantation. Statistical analysis was done using Statistical Package for Social Scientists computer program (SPSS, Chicago, IL, USA). Engraftment kinetics was analyzed using linear regression analysis and Student's t-test. The Kaplan-Meier method was used to estimate overall survival and probability of chronic GVHD [22].

Results

Engraftment and hematopoietic recovery. All patients engrafted neutrophils as defined above. The range in days to an absolute neutrophil count of $0.5x10^9$/l was 10–25 days with a median of 13 days for the whole group of patients. The median time to neutrophil engraftment was 11 and 17 days in the related and unrelated setting, respectively. No statistically significant differences were found in the median time to neutrophil recovery in patients transplanted for malignant (11 days) or non-malignant diseases (16 days), as well as in patients receiving MTX as GVHD prophylaxis (14 days) or not receiving MTX (11 days). We found no correlation in the number of CD34+ cells infused and speed of engraftment. Both methods for chimerism analyses revealed complete donor chimerism in all but one of the patients after engraftment, who was non-informative. Twenty-eight patients were evaluable for platelet engraftment and the range in days to a non-transfused platelet count of $20x10^9$/l was 9–44 days with a median of 14 days. Two patients died after having developed grade IV acute GVHD without ever being transfusion independent.

Graft-versus-host disease. All patients were evaluable for acute GVHD. Fifteen out of 30 transplanted children developed acute GVHD grade II to IV. It was 42% (11/26) and 100% (4/4) in the related and unrelated setting, respectively. Acute GVHD was the cause of death in two patients (6%), the only two who developed grade IV GVHD with gut involvement. Eleven (40%) out of 27 patients followed for at least 100 days have developed chronic GVHD. Onset of chronic GVHD occurred between days 100 and 359. Two patients died before day 100 and one patient has been followed only for 70 days yet. Nine of the 11 patients with chronic GVHD had extensive form with lung (4), skin and eyes (1), joints $\pm$ skin (2), liver + gut (2) involvement. Three patients developed late onset de novo chronic GVHD. Treatment of these 11 patients varied but generally included CS-A, steroids, ursodeoxycholic acid and mycophenolate-mofetil, one patient received psoralen and ultraviolet A-radiation (PUVA) for chronic skin GVHD. After the therapy chronic GVHD resolved in 5 patients (45%), whereas it was still present at the time of last follow up or at time of death in 4 and 2 children, respectively. One patient needed repeated courses of immunosuppressive treatment. The overall cumulative probability of developing chronic GVHD by two years was 58% and is shown in Figure 1.

TRM, relapse and survival. There were four (13%) treatment related deaths in our cohort. Two patients, both transplanted for aplastic anemia died of grade IV acute GVHD and related complications on days 55 and 68. One patient died of a gram-negative sepsis and multiorgan failure and one died of disseminated aspergillosis after graft failure due to CMV infection on days 159 and 136, respectively. After a median follow-up of 662 days (from 70 to 2092 days) 20 out of 29 patients (69%) are alive. The Kaplan-Meier overall survival estimate of the whole cohort is 43% at 6 years and is shown in Figure 2. Six (37%) out of 16 children transplanted for malignant disease relapsed and 5 subsequently died, one of them being transplanted twice for ALL. One boy who experienced extramedullar relapse of T-cell non-Hodgkin

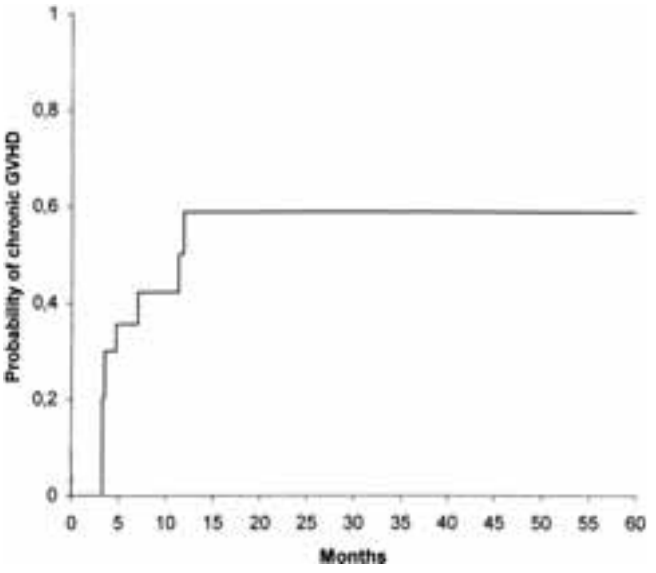

Figure 1. Cumulative probability of chronic GVHD.

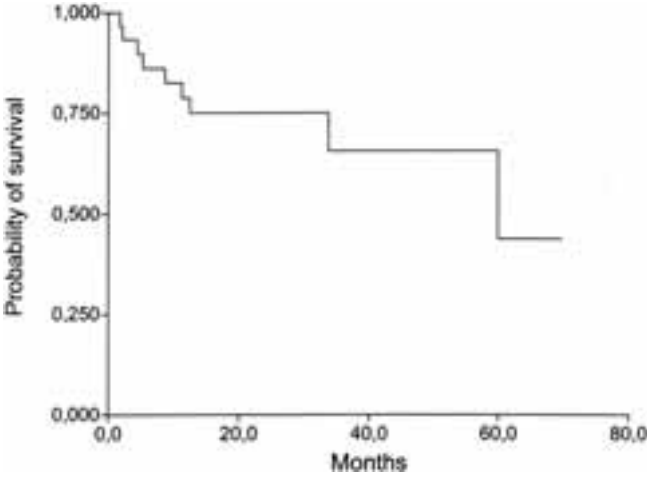

Figure 2. Probability of overall survival of the whole cohort.

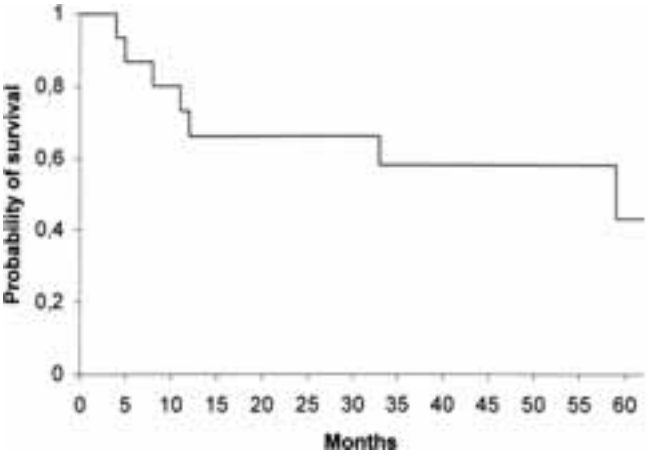

Figure 3. Probability of overall survival of malignant diseases.

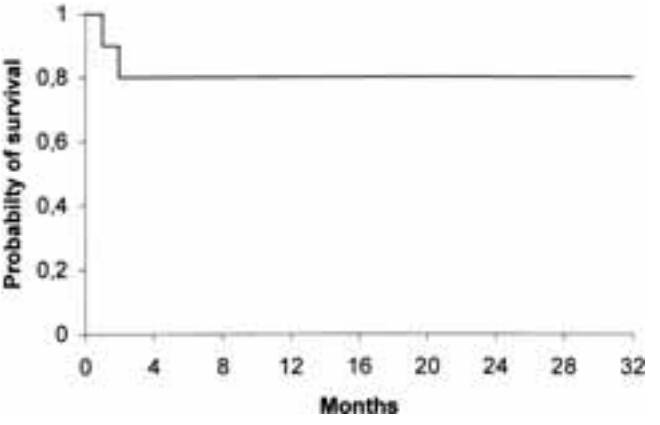

Figure 4. Probability of overall survival of non-malignant diseases.

lymphoma is still alive after a short course of conventional chemotherapy, with complete donor chimerism and a quiescent chronic GVHD. The Kaplan-Meier overall survival estimate of patients with malignant diseases is shown in Figure 3. Eleven out of 13 patients transplanted for non-malignant disorders are alive, one remaining on immunosuppressive therapy for extensive chronic GVHD. The Kaplan-Meier overall survival estimate of these patients is shown in Figure 4.

Discussion

Several retrospective and prospective randomized studies comparing allogeneic peripheral blood and bone marrow transplantation have indicated that PBSCT results in faster engraftment and similar incidence of acute GVHD [7, 8, 28, 41, 44], but an increased risk of chronic GVHD [10, 12, 40, 42]. Though some studies have failed to confirm the above statements of acute and chronic GVHD [6, 9, 11, 32]. Recently published study of RINGDÉN et al [37] gives further evidence of rapid engraftment, no difference in acute GVHD and higher incidence of chronic GVHD.

In our study, all but one of 29 pediatric patients who underwent allogeneic PBSCT patient engrafted both neutrophils and platelets. The observed hematopoietic recovery was rapid with median times to neutrophil and platelet engraftment of 13 days and 14 days, respectively. These results are in accordance with the published data of pediatric patients [13, 25, 27, 29, 45]. There was no statistically significant difference between the median time to engraftment in patients transplanted for malignant and non-malignant diseases, in patients transplanted from related or unrelated donors, as well as in patients receiving or not

receiving MTX for GVHD prophylaxis, though there was a trend toward a more rapid engraftment in patients with malignant diseases who did not receive MTX [37]. We found no relationship between the number of CD34+ cells infused and speed of engraftment. Although MIFLIN et al found a more rapid engraftment when more than $4x10^6$/kg of CD34+ cells were infused, the engraftment in our cohort was fast enough even with lower number of CD34+ cells infused [20, 28].

Grade II to IV acute GVHD was observed in 50% of patients, whereas grade III or higher only in 3 children (10%). Two of them with grade IV acute GVHD, who received CS-A without MTX as GHVD prophylaxis died due to this complication. Otherwise, the lack of MTX in GVHD prophylaxis did not significantly increase the risk of acute GVHD in our patients. All patients in our cohort, grafted from a full matched unrelated donor developed at least grade II acute GVHD. The incidence of grade III and IV acute GVHD we observed in the MSD transplants was comparable with published data suggesting no difference of acute GVHD in PBSCT compared to BMT [4, 24, 25, 43]. We found a higher incidence of acute GVHD in the unrelated setting, compared with the published data, as all our patients transplanted from a MUD experienced acute GVHD [24, 36].

The overall cumulative probability of developing chronic GVHD by two years was 58% in our series of pediatric patients. It is clearly higher as compared with the results of a large retrospective analysis providing data on chronic GVHD in childhood [46], that shows 27% probability of chronic GVHD 2 years after transplantation. Some studies focused on pediatric patients have demonstrated an impressive reduction in relapse probability in children who developed chronic GVHD, particularly those with ALL [20, 46]. Recently published data in adult recipients of PBSCT with hematological malignancies found higher incidence of chronic GVHD, but could not confirm a better antileukemic effect of PBCS with lower relapse rate, even though they observed improved survival [11, 14]. Some studies report even shorter survival, higher relapse incidence, as well as chronic GVHD that may be more difficult to control after PBSCT [15, 16, 34].

TRM of 13%, reported in our study seems to be relatively low, when compared with other reports of allogeneic PBSCT in children, despite some heavily pre-treated patients in more then second complete remission of ALL [4, 20, 29].

Our experience with pediatric donors was favorable. Twenty out of 25 MSD were less than 18 years old with the youngest donor being 3 years old. Majority of the donors needed a central venous catheter insertion for stem cell collection with no complication related to this procedure and all but one donor underwent just one single leukapheresis with sufficient number of stem cells collected. G-CSF administration was well-tolerated and no adverse effects except for mild bone pain in one donor, were reported. The possible long-term side effects of G-CSF administration need to be evaluated after longer follow-up of the PBSC donors, though recent data suggest PBSC donation appears to be reasonably safe with no greater risk of leukemia of the donors [1, 26].

In conclusion, allogeneic PBSCT is safe for pediatric donors and recipients, it results in rapid neutrophil and platelet recovery as compared to bone marrow, with a similar incidence of grade III and IV acute GVHD and a slightly higher incidence of grade II acute GVHD, mainly in the unrelated setting. The chronic GVHD rate appears to be high but as the anticancer effect of chronic GVHD is expected, it seems reasonable to use PBCST in malignant diseases. However, for nonmalignant disorders it should be used cautiously, as these patients do not benefit from chronic GVHD, which might be the major cause of late morbidity and mortality after allogeneic stem cell transplantation.

We are grateful to the following physicians for referring their patients to our center: Dr. KAISEROVA (Dept. of Oncology, Bratislava), Dr. BUBANSKA, Dr. STANCOKOVA (Center of Pediatric Oncology, Banska Bystrica), Dr. ORAVKINOVA (Dept. of Oncology, Košice).

References

[1] ANDERLINI P, RIZZO JD, NUGET ML, SCHMITZ N, CHAMPLIN RE, HOROWITZ MM. Peripheral blood stem cell donation: an analysis from the International Bone Marrow Transplant Registry (IBMTR) and European Group for Blood and Marrow Transplant (EBMT) databases. Bone Marrow Transplant 2001; 27: 689–692.

[2] ATKINSON K, HOROWITZ MM, GALE RP, LEE MB, RIMM AA, BORTIN MM. Consensus among bone marrow transplanters for diagnosis, grading and treatment of chronic graft-versus-host disease. Committee of the International Bone Marrow Transplant registry. Bone Marrow Transplant 1989; 4: 247–254.

[3] BADER P, HOLLE W, KLINGEBIEL T, HANDGRETINGER R, BENDA N et al. Mixed hematopoietic chimerism after allogeneic bone marrow transplantation: the impact of quantitative PCR analysis for prediction of relapse and graft rejection in children. Bone Marrow Transplant 1997; 19: 697–702.

[4] BENITO AL, GONZALEZ-VICENT M, GARCIA A, BALAS A, QUINTERO V et al. Allogeneic peripheral blood stem cell transplantation (PBSCT) from HLA-identical sibling donors in children with hematological diseases: a single center pilot study. Bone Marrow Transplant 2001; 28: 537–543.

[5] BENSINGER W, CLIFT R, MARTIN P, APPELBAUM FR, DEMIRER T et al. Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: Aretrospective comparison with marrow

transplantation. Blood 1996; 88: 2794–2800.

[6] BENSINGER W, MARTIN P, STORER B, CLIFT R, FORMAN S et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. New Engl J Med 2001; 344: 175–181.

[7] BENSINGER W, WEAVER C H, APPELBAUM F, ROWLEY S, DEMIRER T et al. Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony - stimulating factor. Blood 1995; 85: 1655–1658.

[8] BLAISE D, KUENTZ M, FORTANIER C, BOURHIS JH, MILPIED N et al. Randomised trial of bone marrow versus lenograstim – primed blood cell allogeneic transplantation in patients with early stage leukaemia: A report from the Société Francaise de Greffe de Moelle. *J* Clin Oncol 2000; 18: 537–546.

[9] BROWN RA, ADKINS D, KHOURY H, VIJ R, GOODNOUGH LT et al. Long-term follow-up of high-risk allogeneic peripheral blood stem cell transplant recipients: Graft-versus-host disease and transplant-related mortality. J Clin Oncol 1999; 17: 806–812.

[10] CHAMPLIN RE, SCHMITZ N, HOROWITZ M, CHAPUIS B, CHOPRA R et al. Blood stem cells versus bone marrow as a source of haematopoietic cells for allogeneic transplantation. Blood 2000; 95: 3702–3709.

[11] COUBAN S, SIMPSON D, BARNETT MJ, BREDESON C, HUBESCH L et al. Arandomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. Blood 2002; 100: 1525–1531.

[12] CUTLER C, GIRI S, JEYAPALAN S, PANIAGUA D, VISWANATHAN A et al. Acute and chronic graft-versus host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. J Clin Oncol 2001; 19: 3685–3691.

[13] DIAZ MA, ALEGRE A, VILLA M, BENITO A, BERNARDO MR et al. Allogeneic peripheral blood progenitor cell (PBPC) transplantation in children with haematological malignancies. Br J Haematol 1997; 96: 161–164.

[14] ELMAAGACLI AH, BASOGLU S, PECENY R, TRENSCHEL R, OTTINGER H et al. Improved disease-free-survival after transplantation of peripheral blood stem cells as compared with bone marrow from HLA-identical unrelated donors in patients with first chronic phase chronic myeloid leukemia. Blood 2002; 99: 1130–1135.

[15] FLOWERS M, PARKER P, JOHNSTON L, MATOS A, STORER B et al. Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term follow-up of a randomized trial. Blood 2002; 100: 415–419.

[16] GARDERET L, LABOPIN M, GORIN NC, POLGE E, FOUILLARD L et al. Patients with acute lymphoblastic leukaemia allografted with a matched unrelated donor may have a lower survival with a peripheral blood stem cell graft compared to bone marrow. Bone Marrow Transplant 2003; 31: 23–39.

[17] GLUCKSBERG H, STORB R, FEFER A, BUCKNER CD, NEIMAN PE et al. Clinical manifestation of graft-versus-host disease in human recipients of marrow from HLA matched sibling donors. Transplantation 1974; 18: 295–304.

[18] GRATWOHL A, PASSWEG J, BALDOMERO H, HERMANS J, URBANO-ISPIZUA A. Hemotopoietic stem cell transplantation in Europe 1998. The Hematology Journal 2000; 1: 333–350.

[19] GRATWOHL A. The role of the EBMT activity survey in the management of hematopoietic stem cell transplantation. European Group for Blood Marrow Transplantation. Int J Hematol 2002; 76 Suppl 1: 386–392.

[20] GUSTAFSSON JERNBERG A, REMBERGER M, RINGDÉN O, WINIARSKI J. Graft-versus-leukaemia effect in children: chronic GVHD has a significant impact on relapse and survival. Bone Marrow Transplant 2003; 31: 175–181.

[21] HELDAL D, TJONNFJORD G, BRINCH L, ALBRECHTSEN D, EGELAND T et al. A randomized study of allogeneic transplantation with stem cells from blood or bone marrow. Bone Marrow Transplant 2000; 25: 1129–1136.

[22] KAPLAN EL, MEIER P. Nonparametric estimation from incomplete observation. J Am Stat Assoc 1958; 53: 457–481.

[23] KÖRBLING M, PRZEPIORKA D, HUH YO, ENGEL H, VAN BESIEN K et al. Allogeneic blood stem cell transplantation for refractory leukaemia and lymphoma; potential advantage of blood over marrow allografts. Blood 1995; 85: 1659–1665.

[24] LAWS HJ, NURNBERGER W, SCHMITZ M, ENCZMAN J, GOBEL U, DILLOO D. Increased incidence of chronic GVHD post related and unrelated allogeneic peripheral blood stem cell transplantation in childhood – a single center experience. Bone Marrow Transplant 2001; 27 Suppl 1: S176 (abstr. P493).

[25] LEVINE JE, WILEY J, KLETZEL M, YANIK G, HUTCHINSON RJ et al. Cytokine-mobilized allogeneic peripheral blood stem cell transplants in children result in rapid engraftment and a high incidence of chronic GVHD. Bone Marrow Transplant 2000; 25: 13–18.

[26] LOCASCIULLI A, ARCESE W, LOCATELLI F, DI BONA E, BACIGALUPO A et al. Treatment of aplastic anaemia with granulocyte-colony stimulating factor and risk of malignancy. Italian Aplastic Anaemia Study Group. Lancet 2001; 357: 43–44.

[27] MATSUBARA H, MAKIMOTO A, TAKAYAMA J, HIGA T, SAITO T et al. Possible clinical benefits of the use of peripheral stam cells over bone marrow in allogeneic transplantation setting for the treatment of childhood leukemia. Jpn J Clin Oncol 2001; 31: 30–34.

[28] MIFLIN G, RUSSELL NH, HUTCHINSON RM, MORGAN G, POTTER M et al. Allogeneic peripheral blood stem cell transplantation for haematological malignancies – an analysis of kinetics of engraftment and GVHD risk. Bone Marrow Transplant 1997; 19: 9–13.

[29] MINIERO R, BUSCA A, PESSION A, RONDELLI R, UDERZO C et al. Allogeneic peripheral blood stem cell transplantation in children with hematologic malignancies. Haematologica 1999; 84: 657–660.

[30] PETERS C, MINKOV M, GADNER H, KLINGEBIEL T, NIETHAMMER D. Proposal for standard recommendation for prophylaxis of graft-versus-host disease in children. Bone Marrow Transplant 1998; 21 Suppl 2: S57–S60.

[31] PETERS C, MINKOV M, GADNER H KLINGEBIEL T, VOSSEN J et al. Statement of current majority practices in graft-versus-host disease prophylaxis and treatment in children. Bone Marrow Transplant 2000; 26: 405–411.

[32] POWLES R, METHA J, KULKARNI S, TRELEAVAN J, MILLAR B

et al. Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: a randomised trial. Lancet 2000; 355: 1231–1237.

[33] POWLES R, SINGHAL S, SIROHI B, TRELEAVAN J, KULKARNI C et al. Enhancement of the anti-tumor efficacy of allogeneic transplantation with the use of blood-derived stem cells: 5-year follow-up of a prospective study comparing marrow and blood allografts. Bone Marrow Transplant 2002; 29 Suppl 2: S227 (abstr. P806).

[34] PRZEPIORKA D, ANDERLINI P, SALIBA R, CLEARY K, MEHRA R et al. Chronic graft-versus host disease after allogeneic blood stem cell transplantation. Blood 2001; 98: 1695–700.

[35] PRZEPIORKA D, WEISDORF D, MARTIN P, KLINGEMANN H-G, BEATTY P et al. Consensus conference on acute GVHD grading. Bone Marrow Transplant 1995; 15: 825–828.

[36] REMBERGER M, RINGDÉN O, BLAU IW, OTTINGER H, KREMENS B et al. No difference in graft-versus-host disease, relapse, and survival comparing peripheral stem cells to bone marrow using unrelated donors. Blood 2001; 98: 1739–1745.

[37] RINGDÉN O, LABAPIN M, BACIGALUPO A, ARCESE W, SCHAEFER UW et al. Transplantation of peripheral blood stem cells as compared with bone marrow from HLA-identical siblings in adult patients with acute myeloid leukemia and acute lymphoblastic leukemia. J Clin Oncol 2002; 20: 4655–4664.

[38] SHULMAN HM, SULLIVAN KM, WEIDEN PL, MCDONALD GB, STRIKER GE et al. Chronic graft-versus-host syndrome in man. A long-term clinico-pathological study of 20 Seattle patients. Am J Med 1980; 69: 204–217.

[39] SCHMITZ N, BACIGALUPO A, HASENCLEVER D, NAGLER A, GLUCKMAN E et al. Allogeneic bone marrow transplantation vs filgrastim-mobilised peripheral blood progenitor cell transplantation in patients with early leukaemia: First results of a randomized multicentre trial of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 1998; 21: 995–1003.

[40] SCHMITZ N, BEKSAC M, HASENCLEVER D, BACIGALUPO A, RUUTU T et al. Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. Blood 2002; 100: 761–767.

[41] SCHMITZ N, DREGER P, SUTTORP M, ROHWEDDER E, HAFERLACH T et al. Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim. Blood 1995; 85: 1666–1669.

[42] STOREK J, GOOLEY T, SIADAK M, BENSINGER W, MALONEY DG et al. Allogeneic peripheral blood stem cell transplantation may be associated with high-risk of chronic graft-versus-host disease. Blood 1997; 90: 4705–4709.

[43] VICENT MG, MADERO L, ORTEGA JJ, MARTINEZ A, GOMEZ P et al. Matched-pair analysis comparing allogeneic PBPCT and BMT from HLA-identical relatives in childhood acute lymphoblastic leukemia. Bone Marrow Transplant 2002; 30: 9–13.

[44] VIGORITO A, AZEVEDO W, MARGUES J, AZEVEDO AM, EID KA et al. A randomized prospective comparison of allogeneic bone marrow and peripheral blood progenitor cell transplantation in the treatment of haematological malignancies. Bone marrow transplant 1998; 22: 1145–1151.

[45] WATANABE T, KAJIUME T, ABE T, KAWANO Y, IWAI A, IWAI T et al. Allogeneic peripheral blood stem cell transplantation in children with hematological malignancies from HLA-matched siblings. Med Pediatr Oncol 2000; 34: 171–176.

[46] ZECCA M, PRETE A, RONDELI R, LANINO E, BALDUZZI A et al. Chronic graft-versus-host disease in children: incidence, risk factors, and impact on outcome. Blood 2002; 100: 1192–1200.

Table 2. Summary of results

Patient no.	specimen T / N*	histology typing	differentiated	staging pT	pN	M	location	chemother. response	telomerase activity	hTERT expression	splicing pattern
1	T	Tubopapillary adenocarc.	Well	T2	N0	M0	Rectum	FU non response	30.3	Yes	n.a.
	N	–	–	–	–	–		–	1.6	Yes	n.a.
2	T	adenomatous polyp	n.a.	n.a.	n.a.	n.a.	Colon descendens	n.a.	11.9	Yes	n.a.
	N	–	–	–	–	–		–	0.0	No	n.a.
3	T	Tubopapillary adenocarc., exulcerated	Moderately	T3	N0	Mx	Colon sigmoideum	n.a.	91.4	Yes	α+β+, β-
	N	–	–	–	–	–		–	1.9	Yes	α+β+, α-, β-
4	T	Tubopapillary adenocarc., metastasis	Moderately	T4	NO	M1	Omentum	n.a.	11.5	Yes	α+β+, α-, β-, α-β-
	N	–	–	–	–	–		–	0.0	Yes	α+β+, α-, β-
5	T	Tubopapillary adenocarc., metastasis	Moderately	T4	N1	M1	Liver	FU response	11.8	Yes	α+β+, β-
	N	–	–	–	–	–		–	0.0	No	0
6	T	Tubopapillary adenocarc., exulcerated	Moderately	T3	N0	M1	Rectum	n.a.	1.3	Yes	n.a.
	N	–	–	–	–	–		–	1.5	Yes	n.a.
7	T	Tubopapillary adenocarc., exulcerated	Moderately	T3	N1	Mx	Rectum	n.a.	18.0	Yes	α+β+, α-, β-, α-β-
	N	–	–	–	–	–		–	0.0	Yes	β-
8	T	Tubopapillary adenocarc.	Moderately	T4	Nx	Mx	Colon descendens	n.a.	20.5	Yes	n.a.
	N	–	–	–	–	–		–	0.0	No	0
9	T	Tubopapillary adenocarc.	Moderately	T4	Nx	Mx	Colon transversum	FU response	46	Yes	n.a.
	N	–	–	–	–	–		–	0.0	No	0
10	T	Tubopapillary adenocarc.	Moderately	T4	N0	Mx	Colon ascendens	FU response	123	Yes	n.a.
	N	–	–	–	–	–		–	0.0	No	0
11	T	Tubopapillary adenocarc., exulcerated	Moderately	T2	N1	Mx	Rectum	n.a.	1.3	Yes	n.a.
	N	–	–	–	–	–		–	0.0	No	0
12	T	Tubopapillary adenocarc., exulcerated	Moderately	T2	N0	M0	Rectum	FU response	6.0	Yes	n.a.
	N	–	–	–	–	–		–	1.6	Yes	n.a.

13	T	Tubopapillary adenocarc., exulcerated	Moderately	T3	N1	M1	Rectum	FU response	0.0	No	0
	N	–	–	–	–	–		–	0.0	No	0
14	T	Adenocarcinoma, exulcerated	Poorly	T4	N1	Mx	Colon descendens	n.a.	29.3	Yes	n.a.
	N	–	–	–	–	–		–	0.0	No	0
15	T	Tubopapillary adenocarc., exulcerated	Moderately	T3	N0	Mx	Caecum	n.a.	8.1	Yes	n.a.
	N	–	–	–	–	–		–	0.0	No	0
16	T	Adenocarc.	Moderately	T3	N0	M0	Colon descendens	FU response	0.7	Yes	n.a.
	N	–	–	–	–	–		–	1.0	Yes	n.a.
17	T	Mucinous adenocarc.	Moderate	T3	N0	Mx	Colon descendens	n.a.	9.4	Yes	n.a.
	N	–	–	–	–	–		–	1.8	Yes	n.a.
18	T	Tubopapillary adenocarc., exulcerated	Poorly	T3	N0	M0	Rectum	FU response	0.0	Yes	α+β+, α-, β-
	N	–	–	–	–	–		–	0.0	No	0
19	T	Adenocarc., exulcerated	Moderately	T4	N2	Mx	Colon sigmoideum	n.a.	18.2	Yes	α+ß+
	N	–	–	–	–	–		–	0.0	No	0
20	T	Tubopapillary adenocarc., exulcerated	Well	T4	N2	M0	Rectum	n.a.	5.0	Yes	n.a.
	N	–	–	–	–	–		–	1.9	Yes	n.a.
21	T	Adenocarc., exulcerated	Moderately	T3	N1	Mx	Rectum	n.a.	0.0	Yes	α+β+, α-, β-
	N	–	–	–	–	–		–	1.8	Yes	α+β+, α-, β-
22	T	Tubopapillary adenocarc.	Moderately	T4	N1	Mx	Colon sigmoideum	n.a.	47.0	Yes	α+β+, β-
	N	–	–	–	–	–		–	0.0	No	0
23	T	Tubopapillary adenocarc., exulcerated	Moderately	T3	N0	Mx	Rectum	n.a.	1.3	Yes	α+β+, α-, β-
	N	–	–	–	–	–		Rectum	1.4	Yes	α+β+, α-, β-
24	T	Carcinomatous polyp	Moderately	T3	N0	Mx	Colon rectosigmoideum	n.a.	195.0	Yes	α+β+, β-
	N	–	–	–	–	–		–	0.0	No	0
25	T	Mucinous adenocarc.	Moderately	T4	N0	Mx	Colon transversum	n.a.	8.3	Yes	n.a.
	N	–	–	–	–	–		–	0.0	No	0
26	T	Tubopapillary adenocarc.	Moderately	T3	N0	Mx	Colon rectosigmoideum	n.a.	12.3	Yes	n.a.
	N	–	–	–	–	–		–	1.8	Yes	n.a.
27	T	Carcinomatous polyp	Moderately	T4	N0	M1	Colon ascendens	FU non response	0.0	Yes	β-, α-β-
	N	–	–	–	–	–		–	0.0	Yes	β-, α-β-

28	T	Tubopapillary adenocarc.	Moderately	T3	N0	Mx	Caecum	n.a.	1.9	Yes	α+β+, α-, β-
	N	–	–	–	–	–		–	1.9	Yes	α+β+, α-, β-
29	T	Adenocarc., exulcerated	Moderately	T3	N0	Mx	Colon descendens	n.a.	2.6	Yes	n.a.
	N	–	–	–	–	–		–	0.0	No	0
30	T	Tubopapillary adenocarc., exulcerated	Moderately	T4	N1	Mx	Colon descendens	n.a.	4.4	Yes	n.a.
	N	–	–	–	–	–		n.a.	0.0	No	0
31	T	Tubopapillary adenocarc., exulcerated	Moderately	T4	N0	Mx	Colon rectosigmoideum	n.a.	1.1	Yes	β-, α-β-
	N	–	–	–	–	–		–	0.0	Yes	α+β+, α-, β-
32	T	Tubopapillary adenocarc., exulcerated	Moderately	T4	N0	Mx	Rectum	n.a.	2.9	Yes	n.a.
	N	–	–	–	–	–		–	1.6	Yes	n.a.
33	T	Tubopapillary adenocarc., exulcerated	Moderately	T3	N1	Mx	Rectum	FU response	0.0	No	0
	N	–	–	–	–	–		–	0.0	No	0
34	T	Mucinous adenocarc.	Moderately	T4	N0	M1	Colon sigmoideum	n.a.	38.5	Yes	α+β+, β-
	N	–	–	–	–	–		–	0.0	No	0
35	T	Mucinous adenocarc., exulcerated	Moderately	T4	N1	M0	Rectum	n.a.	0.0	No	0
	N	–	–	–	–	–	Rectum	–	0.0	No	0
36	T	Adenocarc., exulcerated	Moderately	T3	N0	Mx	Colon sigmoideum	n.a.	3.8	Yes	α+β+, β-
	N	–	–	–	–	–		–	0.9	Yes	α+β+, β-
37	T	Adenocarc., exulcerated	Moderately	T3	N0	Mx	Colon rectosigmoideum	n.a.	4.4	Yes	α+β+, β-
	N	–	–	–	–	–		–	0.0	Yes	β-
38	T	Adenocarc.	Well	T3	N0	Mx	Rectum	n.a.	0.0	No	0
	N	–	–	–	–	–		–	0.0	No	0
39	T	Tubopapillary adenocarc., exulcerated	Moderately	T4	N1	M1	Colon sigmoideum	FU non response	219.0	Yes	α+β+, α-, β-, α-β-
	N	–	–	–	–	–		–	1.0	Yes	α+β+, β-, α-β-
40	T	Tubopapillary adenocarc., exulcerated	Moderately	T3	N0	Mx	Colon descendens	n.a.	147.0	Yes	α+β+, β-
	N	–	–	–	–	–		–	1.1	Yes	β-
41	T	Adenocarc.	Poor	T3	N1	M0	Rectum	FU non response	195	Yes	α+β+, β-
	N	–	–	–	–	–		–	1.5	Yes	α+β+, β-

* T = tumour or suspected tumour specimen, N = normal specimen, n.a. = not analyzed