



# Unrelated bone marrow transplantation in children: outcome and a comparison with sibling donor grafting

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## Summary:

The clinical course of 59 children, who underwent BMT during 1988–1998 with a matched unrelated donor (MUD), was compared with 59 case controls receiving a sibling donor marrow. Thirty-eight patients had haematological malignancies while 21 had a nonmalignant disorder. The cumulative incidence of acute GVHD grade II–IV was 28% for MUD recipients vs 11% ( $P = 0.014$ ) for sibling recipients. Extensive chronic GVHD was rare in both groups. The 5-year probability of survival was 52% for MUD vs 77% for sibling recipients ( $P = 0.014$ ). For children with malignancies the 4-year probability of survival was 52% for MUD vs 67% for sibling recipients with a RFS of 49% vs 62%. In the ALL patients the survival of the MUD recipients was 77% and equalled that of the sibling group. For SAA survival was 43% vs 86% ( $P = 0.09$ ) and for metabolic disorders 63% vs 89% ( $P = 0.025$ ). The transplant-related mortality was higher in the MUD group, while death due to relapse was equally distributed. These results of MUD BMT in children compare favourably with most previous reports, and support the use of alternative donors in cases who lack an HLA-identical siblings. *Bone Marrow Transplantation* (2000) 25, 1059–1065.

**Keywords:** bone marrow transplantation; children; unrelated; leukaemia; inborn errors of metabolism; graft-versus-host disease

Allogeneic BMT or stem cell transplantation (SCT) is curative in several life-threatening diseases in children, eg leukaemia, severe aplastic anaemia (SAA), inborn errors of metabolism and immunodeficiencies.<sup>1–8</sup> Since not more than one-third of patients who require SCT have an HLA-identical sibling, there is a need for alternative donors. The chance of finding a well-matched unrelated donor (URD) has increased along with the expanding world-wide pool of donors. Moreover, better tissue typing techniques as well as improved conditioning and immunosuppressive regimens and the growing experience of URD SCT might

further improve the prognosis for patients in need of alternative stem cell sources.

Yet, there are few reports on the clinical course after URD SCT in children as a group, rather than in selected diseases. Furthermore, comparisons of the outcome after unrelated and sibling SCT have mainly been performed in relation to historical controls and between centres rather than in a case-control manner under uniform conditions. In this single centre study we present all children transplanted with a matched unrelated donor (MUD) and compare their outcome with a control group of children receiving sibling grafts.

## Patients and methods

### Patients

The outcome of 59 of children, who received a MUD graft between June 1988 and December 1998, was compared with the outcome of 59 case controls receiving a sibling donor graft. Patients and controls were matched according to diagnosis, stage of remission, time for BMT, age and sex, in that order. Thirty-eight patients had a malignant disease and 21 had a non-malignant disease; SAA, SCID, WAS and different inborn errors of metabolism. The median age was 8 years (range 1–17) in the MUD group vs 9 years (1–17) in the sibling group. Patient characteristics of the MUD and sibling groups are presented in Table 1.

Not included in the case control study were children transplanted with mismatched donors (MMUD) and two MUD recipients with inborn errors of metabolism due to lack of an appropriately matched sibling control. This study has been approved by the human research ethics committee at Huddinge Hospital, Karolinska Institutet.

### Donor histocompatibility

All patients were transplanted with HLA-A, -B, -DRB1 compatible unrelated or sibling donors. For class I antigens, HLA-A and -B typing was performed serologically and HLA class II antigens were determined using genomic methods. Initially RFLP was used with the help of cDNA probes to determine the DRB, DQA and DQB alleles. Since July 1992 PCR, using sequence specific primer pairs, has been used to define the DRB1–5, DQA1, DQB, DPB1 and more recently the DPA1 alleles.<sup>9</sup>

**Table 1** Patient and donor characteristics and outcome

<i>Patient and donor characteristics</i>	<i>Unrelated donor graft</i>	<i>Alive</i>	<i>Relapse</i>	<i>Related donor graft</i>	<i>Alive</i>	<i>Relapse</i>
Total number	59	34	12	59	46	10
Diagnosis						
ALL	20	16	5**	20	16	4*
AML	9	3	3	9	5	4*
CML	4	1	2*	4	3	1
T cell lymphoma	1	0	1	1	0	1
MDS	3	2	1	4	3	0
Granulosarcoma	1	0	0	0		
Total malignant disease	38	22		38	27	
Aplastic anaemia	7	3		7	6	
Immunodeficiency	1	0		1	1	
WAS	1	1		0		
CGD	0			2	2	
AGU	2	2		0		
HLH	3	1		2	2	
Mb Gaucher	0			4	4	
Sanfilippo	2	1		0		
Sandhof	0			1	0	
MLD	2	2		0		
ALD	3	2		0		
Thalassaemia	0			4	4	
Total metabolic disease	14	9		14	13	
Status of malignancies pre-BMT						
CR 1	12	7	3*	14	7	4*
CR 2	11	9	3*	13	9	4*
CR 3	3	2	2	2	2	0
CR 4–5	1	1	0	0		
Relapse or partial remission	10	4	4*	6	3	2
Recipient						
Age median (range)	8 (1–17)			9 (1–17)		
Gender F/M	28/31			24/35		
CMV serology +/-	33/26			38/21		
Donor						
Age	36 (20–49)			8 (1–32)		
Gender F/M	31/28			37/22		
CMV serology +/-	25/34			30/29		
Immunised F to M	9			0		
Preparative regimen						
Cy/TBI	34			32		
Cy/FTBI	8			2		
BU	14			19		
CY	0			5		
TAI/TLI	3			1		
GVHD prophylaxis						
Mtx + CsA	54			44		
Mtx	0			8		
CsA	0			7		
CsA + prednisolone	1			1		
Years follow up, median (range)	3 (0.6–10.9)			7.7 (0.7–16.6)		

\*Each asterisk denotes one patient alive after post-BMT relapse.

AGU = aspartyl-glucose-aminuria; ALL = acute lymphatic leukaemia; ALD = adrenoleukodystrophy; AML = acute myeloid leukaemia; ATG = antithymocyte globulin; BU = busulfan; CGD = chronic granulomatous disease; CML = chronic myelocytic leukaemia; CsA = cyclosporin A; CY = cyclophosphamide; FTBI = fractionated TBI; GVHD = graft-versus-host disease; HLA = human leukocyte antigen; HLH = haemophagocytic lymphohistiocytosis; MDS = myelodysplastic syndrome; MLD = metachromatic leukodystrophy; SAA = severe aplastic anaemia; SCID = severe combined immunodeficiency; TBI = total body irradiation; TLI = total lymph node irradiation; WAS = Wiskott–Aldrich syndrome.

### Stem cell source

Unmanipulated bone marrow was used except in one MUD recipient who received a T cell-depleted (TCD) graft (see third case under rejection) as did two recipients of sibling grafts who participated in a TCD study. Moreover in five of the MUD and in one of the sibling cases, PBSC were used.<sup>10</sup>

### Conditioning

Patients with leukaemia were prepared with cyclophosphamide (CY) 60 mg/kg/day for 2 days (total dose 120 mg/kg) and busulfan (BU) 1 mg/kg × 4/day for 4 days (total dose 16 mg/kg) or 10 Gy of single fraction TBI. In eight MUD and two sibling recipients 12–14.4 Gy of fractionated TBI was given.<sup>5</sup> Antithymocyte globulin (ATG) or

OKT3 was given for 5 days before BMT to patients with URD.<sup>11</sup> SAA patients were prepared with CY 50 mg/kg/day for 4 days (total dose 200 mg/kg) and ATG or OKT3. Patients with inborn errors of metabolism received BU 4 mg/kg/day for 4 days, followed by 4 days of CY 50 mg/kg/day. In patients with haemophagocytic lymphohistiocytosis (HLH), leukaemia in incomplete remission or Ph+ ALL, etoposide (900 mg/m<sup>2</sup>) was added.

#### Post BMT immunosuppression and supportive care

All patients were kept in reverse isolation until ANC exceeded  $0.5 \times 10^9/l$  for 2 consecutive days. CsA was given as GVHD prophylaxis in combination with a short course of i.v. MTX. After 3–6 months, the CsA level was tapered and withdrawal was initiated between 9–12 months in most cases. Prolonged treatment was given to patients with SAA, inborn errors of metabolism or chronic GVHD. Patients with ALL, AML M4 or M5 were given intrathecal prophylaxis with MTX or cytarabine. Antimicrobial prophylaxis included trimethoprim-sulphamethoxazole, fluconazole and acyclovir to HSV seropositive patients. Since 1992, pre-emptive treatment with ganciclovir or foscarnet has been given for 2–3 weeks to patients with positive CMV-PCR in PBL.<sup>12</sup> Chronic GVHD was classified as mild, moderate or severe (I–III) according to the judgement of the treating physician. For further details concerning conditioning and supportive care see Table 1 and Refs 3 and 10–12.

#### Statistics

Continuous variables were compared by median of the Mann–Whitney *U* test. Differences in distribution were compared by the Chi-square test or Fisher's exact test if appropriate. The cumulative time to complications and survival rates were analysed by the life-table method and differences between groups compared with the log rank (Mantel–Haenszel) method. When less than five patients were at risk all patients were censored. Patient data and outcome were analysed as of 1 June 1999.

## Results

#### Engraftment and transfusions

Engraftment occurred in 57 (97%) of the MUD and in 58 (98%) of the sibling recipients. Three children died with septicaemia during the aplastic phase. The median time to achieve ANC  $>0.5 \times 10^9/l$  was 16 days (range 11–27 days) in the MUD, vs 19 days (10–43) in the sibling recipients ( $P = 0.003$ ), to reach WBC  $>0.2 \times 10^9/l$  14 days (8–23) vs 13 days (7–28) and to reach platelets  $>30 \times 10^9/l$  22 days (8–210) vs 19 days (1–52). The dose of stem cells was higher in the MUD cases, (median  $3.8$  vs  $2.9 \times 10^8$  nucleated cells/kg in the sibling recipients, corrected,  $P = 0.018$ ) of which 34 vs 8 also were treated with G-CSF. The number of erythrocyte, platelet or granulocyte transfusions given was not significantly different between groups.

#### Rejections

In the MUD group, three patients rejected their grafts. A boy with Sanfilippo type A rejected the graft  $3\frac{1}{2}$  months post BMT and died 2 months later with pneumonia. A girl with Sanfilippo type C had a rejection episode after tapering of immunosuppression 14 months post BMT which was reversed by ATG and donor buffy coat infusions. After developing haemolytic anaemia she again rejected her graft 3 years post BMT. It was then known that BMT was not curative in this disease. She is alive with a slowly progressing disease. The third patient was a girl with ALL in CR2 in poor condition and with severe osteopenia. The graft was TCD to reduce the risk of GVHD and contained  $0.7 \times 10^8/kg$  NC. She rejected the graft at day 23 and received an autologous rescue, but died in relapse 2 months later. A 10-month-old boy with AML M5 had a life-threatening relapse and received a booster marrow without preparative treatment followed by interleukin-2. He had rapid trilineage engraftment and remains free of disease 6 years later.

No complete rejection occurred in the sibling group. A girl with ALL in CR2 had a transient rise in WBC post BMT but became pancytopenic and received a booster of PBCS from her sister on day 23. She engrafted on day 28. The episode was associated with parainfluenza type 3 and HHV-6 infection.

#### Infections

The incidence of bacteraemia in recipients of MUD (41%) or sibling (27%) grafts was not significantly different. Coagulase-negative Staphylococci predominated in the MUD group (12 vs 3 cases). In the MUD group two patients died with alpha-streptococcal septicaemia 1 and 15 days post BMT, one in combination with a capillary leak syndrome. In the sibling group one patient with acute GVHD grade III and haemorrhagic cystitis died with *Staphylococcus epidermidis* septicaemia 79 days after transplantation.

There were no differences in the incidence of CMV disease or a positive CMV-PCR post BMT between the groups. However, patients transplanted with MUD had an earlier onset of the first positive CMV-PCR; median 28 days (1–52) vs 44 days (5–137) for sibling recipients ( $P = 0.03$ ). Three of the five MUD recipients with CMV-disease died with CMV-pneumonitis. No deaths due to CMV occurred among the recipients of sibling grafts. For further details see transplant-related complications (TRC) in Table 2.

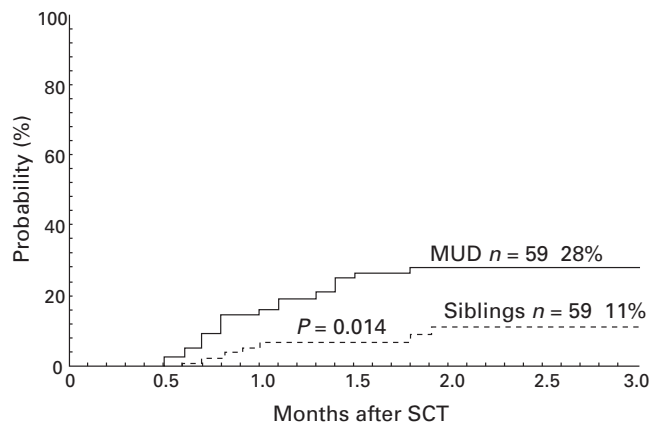
#### Graft-versus-host disease

The incidence of acute and chronic GVHD was significantly higher in recipients of unrelated donor grafts. Extensive chronic GVHD was rare in both groups, while there was a 45% cumulative risk for the MUD recipients to experience a mild course. For three MUD and two sibling recipients, GVHD was a contributing cause of death. For further details, see TRC Table 2 and Figures 1 and 2.

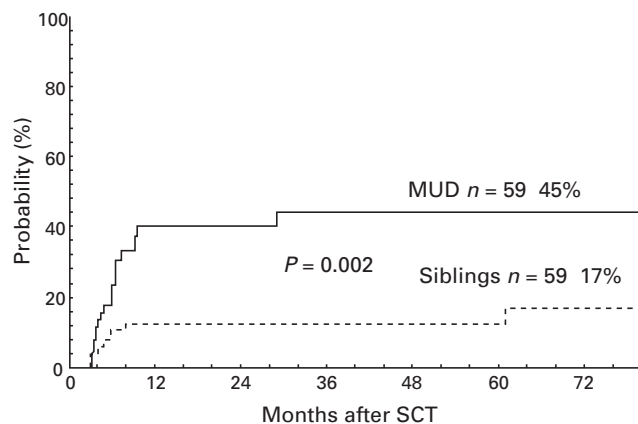
**Table 2** Transplant-related complications (TRC)

Transplant-related complications	MUD n = 59	SIB n = 59
Septicaemia (%) NS	24 (41%)	16 (27%)
CMV PCR+	23	21
CMV disease	5	5
VOD	5	3
Haemorrhagic cystitis	11	10
Obliterative bronchiolitis (BOP)	8	3
Rejection/graft failure	3/1	0/1
aGVHD		
grade 0	6	23
I/II	35/13	30/4
III/IV	2/1	1/1
cGVHD		
0	33	47
Mild	19	6
Moderate	1	1
Severe	0	1

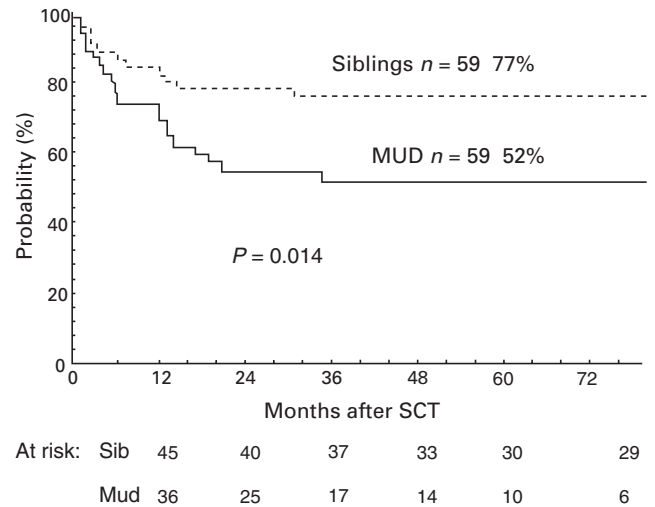
CMV = cytomegalovirus, VOD = veno-occlusive disease of the liver; aGVHD = acute graft-versus-host disease; cGVHD = chronic GVHD.



**Figure 1** Probability of and time to acute GVHD grade II-IV in children transplanted with a MUD (—) or with a sibling donor (---).



**Figure 2** Probability of and time to chronic GVHD in children transplanted with a MUD (—) or with a sibling donor (---).



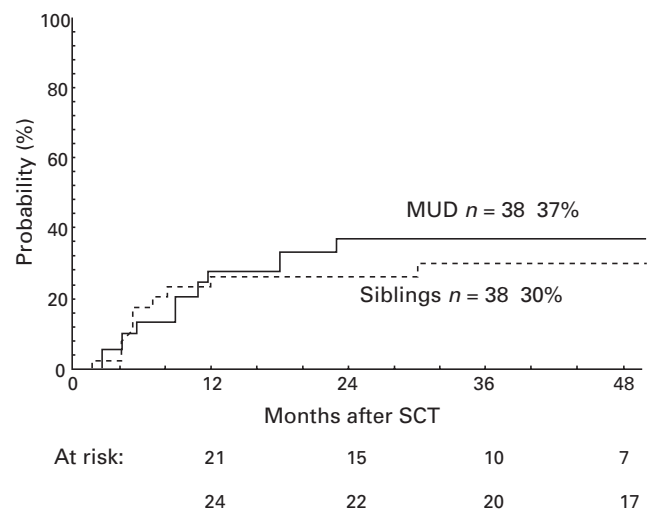
**Figure 3** Probability of survival in children transplanted for haematological malignancies, SAA, immunodeficiencies and inborn errors of metabolism, with a MUD (—) and with a sibling donor (---).

*Immunohaematological problems*

Haemolytic anaemia or Evans syndrome exclusively affected MUD recipients and were contributing causes of death in two SAA patients. Three other children with nonmalignant disorders developed Evans syndrome,<sup>3</sup> while one patient with AGU and one with WAS had haemolytic anaemia associated with mixed chimaerism in the myeloid and B cell lines.

*Relapses, survival and mortality*

At the end of follow-up, 34 recipients (58%) of MUD grafts and 46 (78%) recipients of sibling grafts were alive. The 5-year probability of survival for the MUD group was 52% vs 77% for the sibling group ( $P = 0.014$ ) (see Figure 3). Disease-free survival is shown in Figure 5. The probability of relapse is presented in Figure 4 and the distribution is



**Figure 4** Probability of and time to relapse in children transplanted with a MUD (—) or with a sibling donor (---). Three children in the MUD group and two in the sibling group are alive after relapse.

shown in Table 1 according to diagnoses and remission status. Transplant-related mortality (TRM) was 27% in the MUD group *vs* 8% in the sibling group. While relapse was the dominating cause of death for the sibling recipients (8/13), various lung complications (9/25); pneumonia (2), CMV-pneumonitis (3) and respiratory insufficiency with obliterative bronchiolitis (4), were the major cause of death in the MUD recipients along with relapse (8/25).

When relapses were related to chronic GVHD in the 36 MUD and 35 sibling patients at risk, the majority of relapses occurred in children without chronic GVHD,  $P = 0.03$ .

The 4-year probability of survival for recipients with malignant diseases ( $n = 38$ ) was 52% for MUD *vs* 67% for siblings with a relapse-free survival (RFS) of 49% *vs* 62%. For the ALL patients the 4-year probability of survival was 77% *vs* 74% and for AML 28% *vs* 53%. These differences were not significant in contrast to the better outcome for the sibling recipients transplanted for metabolic disorders 63% *vs* 89% ( $P = 0.025$ ). For aplastic anaemia, the 4-year probability of survival was 43% *vs* 86% ( $P = 0.09$ ). All four deaths in children receiving unrelated grafts for SAA occurred between 1 and 53 days post BMT. One patient with SAA and two patients with amegakaryocytic anaemia are alive.

## Discussion

Children are only referred for BMT if they have a very low chance of survival with other treatment options.<sup>13–15</sup> In this study, however, more than half of the children who underwent an allogeneic BMT, are likely to be alive 5 years later, even when transplanted with a MUD.

Our aims were to report the outcome of all paediatric patients transplanted with a MUD by our team and compare course and outcome after MUD *vs* sibling grafting, since such case control studies are lacking. The difference in outcome, a 5-year probability of survival of 52% after MUD *vs* 77% after sibling BMT is in line with the higher incidence of GVHD and the higher TRM (16 *vs* 5 cases) seen in the MUD group. It is consistent with previous adult case

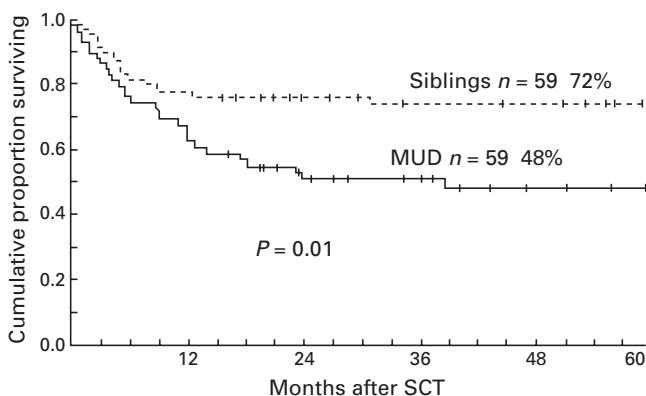
control studies.<sup>4,8,16,17</sup> Yet, the outcome for our MUD recipients is in agreement with several previous reports on sibling grafting.<sup>13,14</sup>

There was a tendency towards more morbidity (Table 2), repeated CMV treatments, IHA and also a higher TRM in the MUD group. The overall incidence of positive CMV-PCR (39% *vs* 36%) and the incidence of CMV disease (8.5%) in this material is somewhat lower than reported<sup>18</sup> and partly due to a low proportion of pre-transplant CMV seropositive patients and donors. The pre-emptive treatment strategies have led to a later onset and lower incidence of CMV disease but the effect on survival has been more controversial.<sup>18–20</sup> Infections and a higher TRM in recipients of URD are associated with the higher incidence of GVHD.<sup>2,5–8,16</sup> In the study by Casper *et al*<sup>6</sup> infections were the major source of morbidity and mortality. Secondary EBV-associated lymphomas are a problem if a MMUD or TCD graft are used.<sup>6,21,22</sup> Complications involving the lungs contributed importantly to TRM and morbidity for our MUD recipients.

The incidence of acute GVHD grade II–IV in this study (28%) can be compared with 33% observed by Casper *et al*<sup>6</sup> who applied TCD but who also reported that 80% of their patients with acute GVHD grade II–IV died. Balduzzi *et al*<sup>2</sup> found acute GVHD grade II–IV in 83% of their paediatric MUD recipients transplanted with unmanipulated MUD grafts. It is possible that the inclusion of ATG during conditioning has contributed to the comparatively low incidence of moderate and severe acute GVHD, as well as severe chronic GVHD in our MUD patients.<sup>11</sup>

The relapse frequency in recipients of MUD and sibling grafts was not significantly different. A slight bias towards more advanced disease in the MUD group after matching of all controls cannot be ruled out (Table 1). Nevertheless more than half of the patients who were in relapse or partial relapse before BMT did not get a subsequent relapse. Relapse was the major cause of death in both groups. The relapse rates are in accordance with previous reports on URD SCT for leukaemia in children.<sup>2,5,6,21</sup> In an earlier report from our centre<sup>3</sup> after a median follow-up time of 1.8 years, fewer relapses were observed in the MUD group than in the sibling group. In the present study, follow-up is longer than in the studies mentioned. The survival curve (Figure 3) does not reach a plateau until more than 2 years after MUD BMT, while recipients of sibling grafts who survive the first year, have a good chance of continued survival. A trend towards a later occurrence of relapses in the MUD group can also be discerned in Figure 4.

In 1979, Weiden *et al*<sup>23</sup> proposed the theory of a graft-versus-leukaemia effect (GVL), and acute and chronic GVHD have been presented as favourable when evaluating leukaemia-free survival,<sup>17</sup> particularly in CML.<sup>24</sup> Hence it has been suggested that fewer relapses would be seen after MUD than after related BMT.<sup>8,16,25</sup> In the present study, relating acute GVHD and relapses, 8/12 MUD and 7/10 sibling recipients with relapse had acute GVHD grade I, which suggests that diagnosed acute GVHD does not protect against relapse nor is the best marker for an expected GVL effect. On the other hand patient numbers are limited and only two of all relapses occurred in patients having acute GVHD grade II–IV. Relating chronic GVHD and



**Figure 5** Disease-free survival for all 59 MUD recipients (—) and 59 sibling recipients (---). The cumulative proportion surviving 5 years post BMT is 48% in the MUD group (—) and 72% in the sibling group (---).

relapses, we found that all relapses in the sibling group and eight in the MUD group occurred in patients without chronic GVHD suggesting it conveys a GVL effect, while type of donor was of minor importance.

#### *Survival of subgroups of patients*

In ALL MUD recipients, the probability of survival was on a par with the sibling group. This includes patients in CR1–4, partial remission, relapse or with Ph+ ALL and compares favourably with previous reports.<sup>2,5,21</sup> Three of four patients in relapse immediately pre-BMT are alive and all five Ph+ are alive in the MUD group. After conventional chemotherapy the outcome for this category of patients is very poor.<sup>26</sup> The advantage of allogeneic BMT for Ph+ ALL is in accordance with previous proposals.<sup>15</sup> Improved pretransplant chemotherapy and the use of unmanipulated grafts could explain the favourable outcome in the ALL patients. Balduzzi *et al*,<sup>2</sup> who also used non-TCD grafts presented similar outcome for all stages of ALL, but with a 3-year DFS of 47% for low risk and 52% for high risk, while three groups using T cell depletion (TCD) techniques present an EFS in children ranging from 15–30% in the high risk groups and up to 50–70% in low risk groups.<sup>5,6,21</sup> The ALL patients are the only group in our study who have an equal outcome whether receiving sibling or unrelated grafts.

The results for the small group of AML patients seem less favourable, with high relapse and TRM rates as reported by others.<sup>1,4</sup> Patients with MDS/AML and several with advanced disease were included in the AML group, with a slight bias for the MUD group. The outcome for the CML patients, one of four survivors in the MUD group and three of four in the sibling group, is similar to the outcome reported by Locatelli *et al*<sup>27</sup> where one of three MUD recipients and four of four sibling recipients were alive at follow-up.<sup>27</sup>

The survival of the MUD recipients with nonmalignant diseases was significantly lower than in case-matched recipients of sibling marrow. A survival above 60% in MUD patients with inborn errors is however encouraging.<sup>8</sup> In SAA, our data and EBMT recommendations, as well as largely unpublished information on the poor outcome after MUD BMT, eg in Diamond–Blackfan anaemia or thalassaemia, presently advise against such transplants in patients with transfusion-dependent anaemias unless immunosuppressive therapy or supportive care have failed. However, with improved donor matching, conditioning and support, URD SCT in SAA currently may have a better prognosis, especially in the early stage of disease.<sup>28</sup>

When interpreting these results, the limited number of patients has to be taken into consideration. In summary, the outcome after MUD SCT in the present study is encouraging. Today MUD SCT can be recommended for most children with malignant diseases and inborn errors who need SCT but lack a sibling donor. Added caution is however still necessary for the transfusion-dependent anaemias. New techniques for diagnosis of infections, relapse and chimaerism, application of virus and leukaemia-specific CTLs, the mini-transplant concept, *in vivo* or *ex vivo* manipulation of the graft may enable a more precise and active inter-

vention policy to reduce GVHD and infections while not compromising the curative potential of BMT.

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