Outcome of allogeneic stem cell transplantation in children with non-malignant diseases

Andre Willasch*
Walter Hoelle*
Hermann Kreyenberg
Dietrich Niethammer
Rupert Handgretinger
Peter Lang
James F. Beck
Thomas Klingebiel
Peter Bader

Background and Objectives. After allogeneic stem cell transplantation treatment failures are mostly caused by graft rejection or graft-versus-host disease (GVHD). T-cell depletion is an appropriate tool to prevent GvHD. However, it might be associated with an increased risk of graft rejection, which can be recognized by serial and quantitative characterization of chimerism. Thus, pre-emptive immunotherapy might be helpful to avoid graft rejection.

Design and Methods. We present the outcome of 56 transplants performed in 53 children with non-malignant diseases. T-cell depletion was conducted in 27/56 grafts. When increasing mixed chimerism over 30% autologous cells occurred low dose donor lymphocyte transfusions (DLT) were performed.

Results. During the course of the follow-up 29 out of 53 patients achieved complete chimerism or low mixed chimerism (0-1%) and 28/29 remained in continuous complete remission. Donor engraftment failed in 2/53 patients who died of serious infection. Increasing mixed chimerism was found in 19 out of 56 transplantations. Fifteen of these 19 patients received additional immunotherapy with DLT. Eleven out of the 15 remained in complete remission. One of the 15 patients developed GvHD grade III that turned out to extensive chronic GvHD. The 3-year overall survival was 100% for patients transplanted from matched related or unrelated donors and 75% for patients transplanted from mismatched donors.

Interpretation and Conclusions. We demonstrated that children transplanted for non-malignant diseases have an excellent overall survival. T-cell depletion is associated with an increased risk of graft rejection. Pre-emptive immunotherapy with DLT, administered on the basis of increasing mixed chimerism, is feasible and might prevent graft rejection.

Key words: allogeneic transplantation, non-malignant diseases, chimerism, donor lymphocyte transfusion, immunotherapy.

Haematologica 2006; 91:788-794

From the University Children's Hospital, Frankfurt, Germany (AW, HK, TK, PB); University Children's Hospital, Tuebingen, Germany (WH, DN, RH, PL); University Children's Hospital, Greifswald, Germany (JFB).

**A.W. and W.H. contributed equally to this manuscript.*

Correspondence:
Peter Bader, University Children's Hospital, Department of Pediatric Hematology and Oncology, Theodor-Stern-Kai 7, D-60590 Frankfurt/Main, Germany.
E-mail: peter.bader@kgu.de

Allogeneic stem cell transplantation is a treatment option for children with several acquired and inherited non-malignant diseases affecting hematopoietic stem cells and their derivates.[1-5] Besides graft-versus-host disease (GvHD), graft rejection is a common treatment complication, even years after stem cell transplantation.[6-9] T-cell depletion is an appropriate tool to prevent GvHD, however, this procedure might be associated with an increased risk of graft rejection. An early stage of graft rejection is represented by increasing autologous marrow repopulation called increasing mixed chimerism.[10] At this stage therapeutic intervention might avoid graft rejection. In theory there are two therapeutic principles: i) to increase immunosuppression, or to ii) increase the alloreactive potential of the graft e.g. by administering donor lymphocyte transfusions (DLT). Based on our experience in prospective studies in children with malignant diseases, we decided to determine whether the latter approach might also be feasible in patients with non-malignant diseases.[11-13] In order to do this we monitored hematopoietic chimerism in peripheral blood samples from children with non-malignant diseases who underwent allogeneic stem cell transplantation once a week after transplantation up to day +100 and thereafter once a month. In case of increasing mixed chimerism over 30% of autologous cells we administered DLT.

Design and Methods

Patients

Between December 1992 and July 2004, 56 allogeneic stem cell transplantations were performed in 53 children for non-malignant diseases at the University Children's Hospital Tuebingen. Peripheral blood samples from these children were analyzed for hematopoietic chimerism once a week after stem cell transplantation up to day +100 and thereafter once a month. The study protocol

was approved by the Clinical Ethics Committee of the University of Tuebingen, and the study was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from the patients and parents according to institutional guidelines. The median age at stem cell transplantation was 5.7 years (0.2-16.9 years), and the median duration of follow-up was 5.1 years (0.8-10.8 years). Data were analyzed as of July 1st 2005. Table 1 summarizes the patients' characteristics giving information about underlying diseases, types of stem cell donors, sources of stem cells, T-cell depletion and the conditioning regimens.

Chimerism assays

Hematopoietic chimerism was assayed in unfractionated leukocytes from peripheral blood of each patient at weekly intervals during the first 100 days and monthly afterwards. As previously described, DNA of peripheral blood samples was extracted using Qiagen Mini Kits (Qiagen, Hilden, Germany). Then a semiquantitative polymerase chain reaction (PCR) assay, based on the amplification of short tandem repeat (STR) markers, was used.[14,15] Amplified fragments were separated by capillary electrophoresis using an ABI prism 310 sequencer (Applied Biosystems). For detailed evaluation, the corresponding peak area values were exported to the Applied Biosystems Genotyper software. The percentage of donor and recipient DNA was calculated from individual proportions of donor and recipient peak areas in relation to the summation of all signals. As a quality control, a follow-up sample was analyzed together with the former sample throughout. The variability of this method for quantifying mixed chimerism was determined to be in the range of 5%.[16,11,14]

Definition of chimerism status and response

The patients were stratified individually on the basis of serial analyses by STR-PCR, within the limits of sensitivity (approximately 1-5%).[14,15,17] Patients whose samples showed no evidence of autologous cells at any time post-transplant were considered to have complete chimerism, patients whose samples constantly showed autologous signals that did not exceed 30% were specified as having stable mixed chimerism. Autologous signals immediately post-transplant that did not further increase but spontaneously decreased during follow-up were categorized as signifying decreasing mixed chimerism. A significant increase (5% or more) in the proportion of autologous cells between two consecutive assessments, which, furthermore, exceeded 30%, was defined as increasing mixed chimerism. Patients whose samples showed only autologous signals and no donor signals were categorized as having autologous reconstitution. Response was defined as a decreasing proportion of the autologous signal or a return to complete chimerism.

Strategy for additional immunotherapy

The cut-off level of 30% autologous cells for pre-emptive immunotherapy was chosen somewhat arbitrarily partly based on the finding of Nesci *et al*. in 1992 who reported a significantly increased risk of graft rejection in patients transplanted for thalassemia after autologous cells had exceeded more than 30%.[18] Our own observational experience at the beginning of the study confirmed this high risk of graft rejection in children with acquired anemias and increasing mixed chimerism with more than 30% autologous cells. On the other hand broad experience in pre-emptive treatment of patients with advanced hematologic malignancies has revealed that low starting doses of DLT are unlikely to induce severe GvHD when treatment is initiated at a stage in which patients have high levels of autologous cells in contrast to the situation in patients who are complete chimeras at the time of pre-emptive immunotherapy.[11] In addition, various previous studies in patients with severe aplastic anemia indicate that lower levels of stable mixed chimerism are not correlated with an

Table 1. Patients' characteristics.

	n
Patients	53
Transplants	56
Age[a]	5.7 years (0.2-16.9)
Follow up[b]	5.1 years (0.8-10.8)
Disease	
Severe aplastic anemia	21
Wiscott-Aldrich syndrome	7
Adreno-leukodystrophy	6
Blackfan-Diamond anemia	5
Osteopetrosis	5
Thalassemia	3
Histiocytosis	3
Polycythemia vera	1
Schwachman-Diamond syndrome	1
Sickle cell disease	1
Donor	
Matched family donor	24
Mismatched family donor	9
Matched unrelated donor	18
Mismatched unrelated donor	5
Stem cell source	
Bone marrow	32
Peripheral blood	24
T-cell depletion	
Yes	27
No	29
Conditioning regimen	
Busulfan based	33
Myeloablative	
Bus+Cyc (±ATG)	23
Bus+Cyc+Eto (±ATG)	2
Bus+Cyc+Flu±ATG/OKT3	6
Bus+Cyc+Thio+ATG	2
Not busulfan based	23
Non-myeloablative	
Cyc+ATG	10
Flu+Cyc+ATG	1
Thio+Flu+Mel+OKT3	1
TBI+Cyc+ATG	1
TBI+Flu+Cyc+OKT3	1
TBI+Thio+Cyc (±ATG)	4
TLI+Cyc (±ATG/OKT3)	5

[a]Median (range); [b]of patients alive and in complete remission independently of type of chimerism or immunological intervention. ATG: anti-thymocyte globulin; Bus: busulfan; Cyc: cyclophosphamide; Eto: etoposide; Flu: fludarabine; Mel: melphalan; OKT3, orthoclone; TBI, total body irradiation; Thio, thiotepa; TLI; total lymphoid irradiation.

enhanced risk of graft rejection and vice versa.[19,20] These findings supported our adoption of the policy of pre-emptive immunotherapy when autologous cells exceeded 30% also in patients with non-malignant diseases. Taken together, we planned a stratified immunological intervention for patients with increasing mixed chimerism. If the patient was receiving cyclosporine A this immunosuppressive agent was to be immediately withdrawn. Chimerism was to be assayed weekly until complete chimerism was restored. If mixed chimerism continued to increase after cessation of cyclosporine A, a DLT was to be given. Immunotherapy for patients not receiving cyclosporine A consisted of DLT as front-line treatment. Patients who showed further increasing mixed chimerism, despite the first DLT, were to receive additional DLT after at least four weeks had elapsed. The cell dose to administer was based on the number and potential severity of HLA mismatches between the donor and recipient, and ranged from 2.5×10^4 to 7×10^6/kg body weight (BW). Patients with a matched family donor were to receive starting doses of 5×10^4/kg BW. Patients with a mismatched family donor, matched unrelated donor or mismatched unrelated donor were to receive starting doses of 2.5×10^4/kg BW. The CD3-positive cells were collected from the donor's peripheral blood by positive selection using the magnetic activated cell sorting (MACS) technique provided by Miltenyi Biotech (Bergisch-Gladbach, Germany). The CD3-positive cells were counted by flow cytometry and frozen in aliquots of 2.5×10^4/kg body weight of the recipient.[21,22] Since no patient was on cyclosporine A when mixed chimerism increased, immunotherapy consisted of DLT as frontline treatment (Table 2).

Graft-versus-host disease

GVHD was graded according to previously described clinical criteria.[23,24]

Statistical methods

The probability of event-free survival (EFS) and overall survival was estimated by the method of Kaplan and Meier.[25] An event was defined as disease progression or transplant-related death. Univariate analyses of prognostic factors were performed using the log-rank test and Fisher's exact test. Values were considered statistically significant when the p value <0.05.

Results

Patients

Fifty-three patients underwent allogeneic stem cell transplantation and three of them received a second transplant after graft rejection. Patients were transplanted due to severe aplastic anemia (n=21), Wiscott-Aldrich syndrome (n=7), adreno-leukodystrophy (n=6) and other diseases (n=19) (Table 1).

Chimerism and response

During the course of follow up 29/53 patients showed complete chimerism in all consecutive blood samples or showed only low mixed chimerism (0-1%) immediately after the stem cell transplantation which then converted to complete chimerism (Table 3). Of these 29 patients, only one, who suffered from adreno-leukodystrophy (ALD), showed progressive disease, whereas 28/29 are in continuous complete remission. Donor engraftment failed in two out of the 53 patients, who recovered an autologous status and died of serious infection. In the follow-up of the 56 transplants, mixed chimerism was found in 25. Of these 25 cases, five showed a stable form of mixed chimerism never exceeding 30% of autologous cells. Four of the five patients remained in continuous complete remission and one died of transplant-related causes. One of the 25 later had decreasing mixed chimerism and is in complete remission.

Increasing mixed chimerism was found in the follow-up of 19 of the 56 transplants (Table 2). Fifteen of these 19 cases received additional immunotherapy with DLT. No patient was on cyclosporine A when mixed chimerism increased. Eleven of the 15 remained in complete remission, two were in partial remission and one patient with ALD had progressive disease. Only one patient rejected his graft, received a second transplant and is a complete chimera in complete remission. In the follow-up of four out these 19 transplants increasing mixed chimerism was detected but no additional therapy was administered. In one patient with thalassemia in complete remission the autologous signal spontaneously decreased. Two of the four with increasing mixed chimerism rejected their grafts, received a second transplantation and achieved complete remission with complete chimerism. One patient with ALD showed progressive disease. Rejection occurred exclusively in the group of children with increasing mixed chimerism (Table 3).

Survival

The probability of event-free survival (EFS) at 3 years was 0.90 for all patients (Figure 1). Progression of the underlying disease and transplant-related mortality were defined as events. The probability of 3-year EFS was 1.00 for patients transplanted from a matched family donor (n=23), 0.86 for patients transplanted from a matched unrelated donor (n=18) and 0.75 for patients transplanted from a mismatched donor (n=12) (log rank test $p<0.05$; Figure 2). The probability of overall survival at 3 years was 1.00 for patients transplanted from a matched family donor or matched unrelated donor and 0.75 for patients transplanted from a mismatched donor (log rank test $p<0.05$; Figure 3). The reduced overall survival of patients transplanted from a mismatched donor is due to three patients who died from transplant-related causes. The probability of 3-year EFS was 0.91 for patients with increasing mixed chimerism who therefore received DLT (n=14). Increasing mixed chimerism and autologous reconstitution occurred more frequently in patients who received T-cell depleted transplants ($p<0.05$; Table 4).

Graft-versus-host disease and toxicity

Severe acute GvHD (grade II-IV) occurred more frequently in patients with complete chimerism than in

Table 2. Characteristics of patients with increasing mixed chimerism or autologous reconstitution.

No.	Diagnosis	Sex	Age (y)	Donor	Regimen	T-Depl.	DLT	No. DLT	Cells/kg	Time to MC (d)	max. MC (%)	last chim. (%)	GVHD II-IV	Outcome	Status	Follow-up (years)
1	ALD	m	5.9	MUD	Bus+Cyc+ATG	yes	yes	7	$1\times2.5\times10^4$ $2\times5\times10^4$ $2\times1\times10^5$ $1\times2\times10^5$ $1\times4\times10^5$	99	>80	60-80	no	PR	alive	7.4
2	ALD	m	11	MMUD	TBI+Cyc+ATG	yes	no	–	–	56	AR	AR	no	Progression	alive	4.0
3	ALD	m	11.5	MUD	Bus+Cyc+ATG	yes	yes	9	$4\times2.5\times10^4$ $4\times5\times10^4$ $1\times1\times10^5$	28	20-30	5-10	no	Progression	alive	2.7
4	Osteopetrosis	m	0.3	MUD	Bus+Cyc+Flu+ATG	yes	yes	16	$3\times5\times10^4$ $1\times2.5\times10^5$ $1\times1\times10^5$, $1\times5\times10^5$ $1\times1\times10^6$,$1\times2\times10^6$ $1\times4\times10^6$, $1\times5,8\times10^6$ $1\times7\times10^6$, $5\times1\times10^7$	37	60-80	40-60	no	CR	alive	3.1
5	Osteopetrosis	f	0.3	MMFD	Bus+Cyc+Thio+ATG	yes	yes	3	$2\times5\times10^4$ $1\times1\times10^5$	118	AR	AR	no	Rejection*	alive	2.7
6	PV	m	12.1	MUD	Bus+Cyc+ATG	yes	yes	1	$1\times2.5\times10^4$	74	30-40	CC	no	CR	alive	5.0
7	SAA	f	10.2	MFD	Cyc+ATG	no	no	–	–	15	AR	AR	no	Rejection*	alive	0.3
8	SAA	m	3.6	MFD	Cyc+ATG	yes	yes	2	$2\times2.5\times10^4$	22	>80	10-20	no	CR	alive	5.0
9	SAA	m	12.3	MUD	Flu+Cyc+ATG	yes	yes	4	$2\times2.5\times10^4$ $1\times5\times10^4$ $1\times6\times10^5$	21	20-30	CC	yes**	CR	alive	2.4
10	SAA	m	7.8	MUD	TLI+Cyc+ATG	yes	yes	5	$5\times2.5\times10^4$	20	30-40	15-25	no	CR	alive	7.0
11	SAA	f	4.8	MMUD	TLI+Cyc+OKT3	yes	no	–	–	24	>80	>80	no	Rejection*	alive	0.1
12	SCD	f	15.6	MFD	Bus+Cyc+Flu+OKT3	yes	yes	5	$2\times2.5\times10^4$ $1\times5\times10^4$ $2\times1\times10^5$	27	>80	CC	no	CR	alive	5.5
13	Thalassemia	m	1.9	MFD	Bus+CYC+ATG	no	no	–	–	826	30-40	10-20	yes	CR	alive	7.0
14	Thalassemia	m	3.1	MFD	Bus+CYC+ATG	no	yes	13	$2\times5\times10^4$ $1\times7.5\times10^5$ $2\times7\times10^5$, $8\times6\times10^5$	12	>80	80	no	PR	alive	6.7
15	Thalassemia	f	16.9	MFD	Bus+CYC+ATG	yes	yes	3	$1\times2.5\times10^4$ $1\times1\times10^5$ $1\times5\times10^4$	19	>80	1-5	no	CR	alive	3.0
16	WAS	m	2	MMFD	Bus+Cyc	no	yes	1 (AC133)	$1\times16,6\times10^6$	1541***	>80	40-60	no	CR	alive	10.8
17	WAS	m	1.3	MUD	Bus+Cyc+ATG	no	yes	6	$2\times2.5\times10^4$ $1\times5\times10^4$ $3\times7.7\times10^4$	74	20-30	5-10	no	CR	alive	3.0
18	WAS	m	0.5	MUD	Bus+Cyc+ATG	no	yes	14	$2\times2.5\times10^4$ $3\times5\times10^4$, $3\times1\times10^5$ $5\times5\times10^5$, $1\times2\times10^6$	73	60-80	40-60	no	CR	alive	1.7
19	WAS	m	3.3	MUD	Bus+Cyc+ATG	yes	yes	14	$13\times2.5\times10^4$ $1\times5\times10^4$	2	60-80	10-20	no	CR	alive	5.7

*ALD: adreno-leukodystrophy; ATG: anti-thymocyte globulin; AR: autologous reconstitution; Bus: busulfan; CC: complete chimerism; CR: complete remission; Cyc: cyclophosphamide; d: days; DLT: donor lymphocyte transfusion; f: female; Flu: fludarabine; m: male; MC: mixed chimerism; Mel: melphalan; MFD: matched family donor; MMFD, mismatched family donor; MMUD: mismatched unrelated donor; MUD: matched unrelated donor; IT, immunotherapy; OKT3, orthoclone; PR, partial remission; SAA, severe aplastic anemia; SCD: sickle cell disease; TBI: total body irradiation; Thio: thiotepa; TRM: transplant-related mortality; WAS: Wiscott-Aldrich syndrome; y: years. *patients received a second allogeneic stem cell transplant and are in CR; **GVHD occurred after immunotherapy; ***no earlier post-transplant sample available.*

Table 3. Outcome according to chimerism status.

			Stable/		Increasing MC	
	Patients/Tx n=53/56	Complete chim. n=29	Decreasing MC n=6	AR n=2	All patients/Tx n=16/19	DLI n=15
Complete remission	45	28	5	0	12	11
Partial remission	2	0	0	0	2	2
Progression	3	1	0	0	2	1
Transplant-related mortality	3	0	1	2	0	0
Rejection	3*	0	0	0	3	1

**these patients received a second transplant and are in CR with complete chimerism. AR: autologous reconstitution; CR: continuous remission; MC: mixed chimerism; PR: partial remission; TRM: transplant-related mortality; Tx: transplantations.*

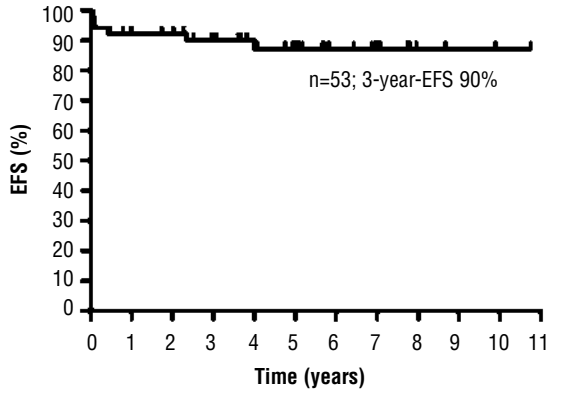

Figure 1. Kaplan-Meier analysis of event-free survival (EFS) for all study patients (n=53).

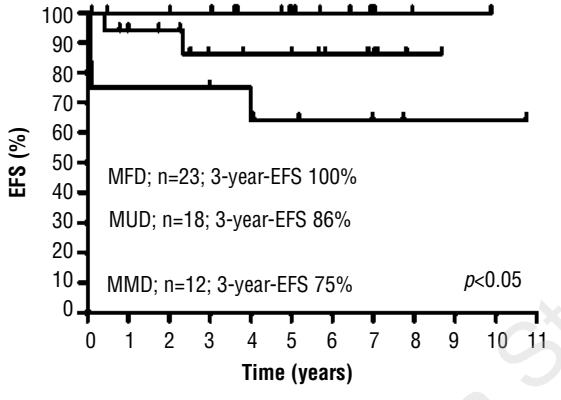

Figure 2. Kaplan-Meier analysis of event-free survival (EFS) according to the donor. *MFD, matched family donor; MUD, matched unrelated donor; MMD, mismatched donor.*

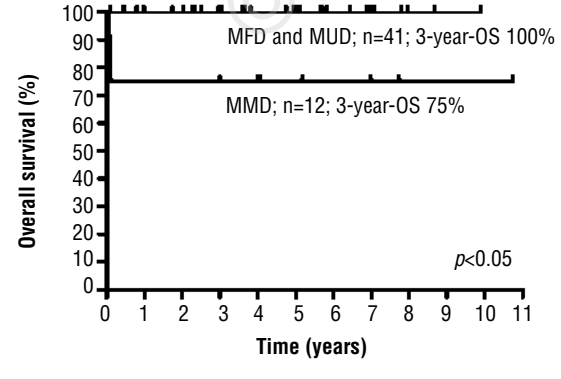

Figure 3. Kaplan-Meier analysis of overall survival (OS) according to the donor. *MFD, matched family donor; MUD, matched unrelated donor; MMD, mismatched donor.*

Table 4. Stem cell source and chimerism status.

	All transplants	*Stem cell characteristics*				
	n=56	*MFD n=24*	*MUD n=18*	*MMD n=14*	*T-cell depletion n=27*	*No T-cell depletion n=29*
Complete chimerism	29	16 (67%)	7 (39%)	6 (43%)	9 (33%)	20 (67%)
Stable/decreasing MC	6	2 (8%)	2 (11%)	2 (14%)	3 (11%)	3 (10%)
Increasing MC*	19	6 (25%)	9 (50%)	4 (29%)	13 (48%)	6 (23%)
Autologous reconstitution*	2	0 (0%)	0 (0%)	2 (14%)	2 (8%)	0 (0%)

**Increasing mixed chimerism/autologous reconstitution occurred more frequently in patients who received T-cell depleted transplants (p<0.05). MC: mixed chimerism; MFD: matched family donor; MMD: mismatched donor (9 mismatched family donors and 5 mismatched unrelated donors); MUD: matched unrelated donor.*

Table 5. Status of chimerism and acute GvHD prior to DLT.

	Transplants (n=56)	*Grade 0-I*	*Grade II-IV*
CC	29	21 (72%)	8 (28%)*
MC	25	24 (96%)	1 (4%)
AR	2	2 (100%)	0 (0%)

Table 6. Status of chimerism and acute GvHD after DLT.

	n	*Grade 0-I*	*Grade II-IV*
inc-MC	15	14 (93%)	1 (7%)

**acute GvHD grade II-IV occurred more frequently in patients with CC (p<0.05). AR: autologous reconstitution; CC: complete chimerism; DLT: donor lymphocyte transfusion; MC: mixed chimerism.*

patients with mixed chimerism or autologous reconstitution. In all, 8/29 patients with complete chimerism developed acute GvHD grade II-IV (28%), compared to 1/27 patients with mixed chimerism or autologous reconstitution (4%) ($p<0.05$; Table 5). Among the 15 patients who received immunotherapy because of increasing mixed chimerism, one developed severe GvHD afterwards (7%) (Table 6). This patient with severe aplastic anemia who faced clinical graft rejection and therefore received repeated DLT developed GvHD grade III that evolved to extensive chronic GvHD. Three out of the 53 patients died from transplant-related causes (Table 3). Among these patients one showed stable mixed chimerism and two failed to achieve permanent donor engraftment. They recovered an autologous status and died of infection.

Discussion

Allogeneic stem cell transplantation is the only curative treatment option for patients with inherited and acquired non-malignant diseases affecting hematopoietic stem cells or their derivates.[26] The aim of transplanta-

tion in these diseases is to achieve sustained engraftment in order to improve hematopoietic function, to provide immune competence or to increase or normalize enzyme shortage. Therefore it is not necessary to replace the recipient hematopoietic system completely. The achievement of a state of stable mixed chimerism is usually sufficient to improve the patient's well being substantially. Thus, to reduce toxic side effects, most conditioning regimens are less myeloablative and mixed chimerism is more likely.[27,20] Furthermore since children with non-malignant diseases do not benefit from a graft-versus-leukemia effect, T-cell depletion is often performed in these patients. As a consequence, graft rejection or non-engraftment remain the major causes of treatment failures in these diseases.[28,29] Pre-emptive immunotherapy on the basis of serial and frequent analyses of chimerism might be a valuable tool to avoid graft rejection.

In this study we administered additional immunotherapy, consisting of low dose DLT, which might explain the lower rate of rejections in this trial than in previous reports.[31,32,33] Indeed, 87% of the patients with increasing mixed chimerism who received immunotherapy are in complete or partial clinical remission. Only one patient with ALD showed progressive disease. This favorable outcome of immunotherapy in 15 cases in which increasing mixed chimerism was detected was not associated with an increased risk of developing GvHD. Only one patient with severe aplastic anemia who faced a clinical graft rejection, and, therefore, received repeated DLT, developed GvHD grade III that evolved to extensive chronic GvHD.

It is known from treatment of adult patients with chronic myeloid leukemia that immunotherapy can cause serious pancytopenia. This is most likely if immunotherapy starts when hematopoiesis is dominated by autologous cells. To avoid this complication immunotherapy should be initiated when donor hematopoiesis still predominates. Since we adopted this strategy in the present study, pancytopenia was not observed. In 21% of the cases with increasing mixed chimerism no additional therapy was administered. In two patients with severe aplastic anemia rejection occurred too fast to initiate immunotherapy.

In one patient with thalassemia in complete remission the autologous signal decreased spontaneously without any immunotherapy.

One patient with ALD and progressive disease showed increasing mixed chimerism with an autologous signal of 30% during follow-up. No immunotherapy was initiated because of the poor general condition of the patient. Three months later autologous recovery had occurred.

Severe acute GvHD (grade II-IV) was observed more frequently in patients with complete chimerism than in patients with mixed chimerism or autologous reconstitution. This is consistent with previous findings.[34,35,36] The probability of event-free survival at 3 years was 0.90 for all patients. The probability of overall survival at 3 years was 1.00 for patients transplanted from a matched family donor or matched unrelated donor and 0.75 for patients transplanted from a mismatched donor ($p<0.05$). This reduced survival of patients transplanted from a mismatched donor was due to three patients who died from transplant-related causes.

In this single center trial we demonstrate that children with non-malignant diseases transplanted from a matched family donor, matched unrelated donor or mismatched donor have an excellent overall survival. T-cell depletion is associated with a low risk of inducing GvHD but with an increased risk of graft rejection. Pre-emptive immunotherapy with infusions of low doses of donor T-cells, administered on the basis of increasing mixed chimerism, is, however, feasible and is able to prevent graft rejection. The potential risk of GvHD should be kept in mind.

AW and WH contributed equally to the work reported in this paper. DN, JB, TK and PB conceived and designed the study. RH and PL were responsible for graft manipulation. TK and PB interpreted the data. AW and PB wrote the manuscript. HK, WH, AW and PB performed the chimerism analyses. The final version of this manuscript was approved by all the authors. The authors declare that they have no potential conflicts of interest. This work was supported by the "Deutsche Krebshilfe", Bonn, Germany and the "Deutsche José Carreras Leukämie Stiftung e.V.", Muenchen, Germany.

Manuscript received November 10, 2005. Accepted April 5, 2006.

References

1. Ballen KK, Becker PS, Emmons RV, Fitzgerald TJ, Hsieh CC, Liu Q, et al. Low-dose total body irradiation followed by allogeneic lymphocyte infusion may induce remission in patients with refractory hematologic malignancy. Blood 2002;100:442-50.
2. Ball SE. The modern management of severe aplastic anaemia. Br J Haematol 2000;110:41-53.
3. Filipovich AH. Unrelated donor bone marrow transplantation for correction of lethal congenital immunodeficiencies. Transfus Sci 1991;12:135-42.
4. Peters C Steward CG. Hematopoietic cell transplantation for inherited metabolic diseases: an overview of outcomes and practice guidelines. Bone Marrow Transplant 2003; 31:229-39.
5. Shapiro E, Krivit W, Lockman L, Jambaqué I, Peters C, Cowan M, et al. Long-term effect of bone-marrow transplantation for childhood-onset cerebral X-linked adrenoleukodystrophy. Lancet 2000;356:713-8.
6. Bacigalupo A, Brand R, Oneto R, Bruno B, Socie G, Passweg J, et al. Treatment of acquired severe aplastic anemia: bone marrow transplantation compared with immunosuppressive therapy--The European Group for Blood and Marrow Transplantation experience. Semin Hematol 2000;37:69-80.
7. McCann SR, Bacigalupo A, Gluckman E, Hinterberger W, Hows J, Ljungman P, et al. Graft rejection and second bone marrow transplants for acquired aplastic anaemia: a report from the Aplastic Anaemia Working Party of the European Bone Marrow Transplant Group. Bone Marrow Transplant 1994; 13:233-7.
8. Peters C, Charnas L, Tan Y, Ziegler R, Shapiro E, DeFor T, et al. Cerebral X-linked adrenoleukodystrophy: the international hematopoietic cell transplantation experience from 1982 to 1999. Blood 2004;104:881-8.
9. Krivit W. Allogeneic stem cell transplantation for the treatment of lysosomal and peroxisomal metabolic diseases. Springer Semin Immun 2004; 26: 119-32.
10. Hoelle W, Beck JF, Dueckers G, Kreyenberg H, Lang P, Gruhn B, et al. Clinical relevance of serial quantitative analysis of hematopoietic chimerism after allogeneic stem cell transplantation in children for severe aplastic anemia. Bone Marrow Transplant 2004;

33:219-23.
11. Bader P, Kreyenberg H, Hoelle W, Dueckers G, Handgretinger R, Lang P, et al. Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukemia after allogeneic stem-cell transplantation: possible role for pre-emptive immunotherapy? J Clin Oncol 2004; 22: 1696-706.
12. Bader P, Kreyenberg H, Hoelle W, Dueckers G, Kremens B, Dilloo D, et al. Increasing mixed chimerism defines a high-risk group of childhood acute myelogenous leukemia patients after allogeneic stem cell transplantation where pre-emptive immunotherapy may be effective. Bone Marrow Transplant 2004;33:815-21.
13. Bader P, Niemeyer C, Willasch A, Kreyenberg H, Strahm B, Kremens B, et al. Children with myelodysplastic syndrome (MDS) and increasing mixed chimaerism after allogeneic stem cell transplantation have a poor outcome which can be improved by pre-emptive immunotherapy. Br J Haematol 2005; 128:649-58.
14. Bader P, Hoelle W, Klingebiel T, Handgretinger R, Niethammer D, Beck J. Quantitative assessment of mixed hematopoietic chimerism by polymerase chain reaction after allogeneic BMT. Anticancer Res 1996;16:1759-63.
15. Kreyenberg H, Holle W, Mohrle S, Niethammer D, Bader P. Quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection: the Tuebingen experience. Leukemia 2003;17:237-40.
16. Bader P, Niethammer D, Willasch A, Kreyenberg H, Klingebiel T. How and when should we monitor chimerism after allogeneic stem cell transplantation? Bone Marrow Transplant 2005; 35:107-19.
17. Bader P, Klingebiel T, Schaudt A, Theurer-Mainka U, Handgretinger R, Lang P, et al. Prevention of relapse in pediatric patients with acute leukemias and MDS after allogeneic SCT by early immunotherapy initiated on the basis of increasing mixed chimerism: a single center experience of 12 children. Leukemia 1999; 13:2079-86.
18. Nesci S, Manna M, Andreani M, Fattorini P, Graziosi G, Lucarelli G. Mixed chimerism in thalassemic patients after bone marrow transplantation. Bone Marrow Transplant 1992; 10:143-6.
19. Hill RS, Petersen FB, Storb R, Appelbaum FR, Doney K, Dahlberg S, et al. Mixed hematologic chimerism after allogeneic marrow transplantation for severe aplastic anemia is associated with a higher risk of graft rejection and a lessened incidence of acute graft-versus-host disease. Blood 1986; 67:811-6.
20. Lawler M, McCann S, Gardiner N, Marsh J, Ljungman P, Locasciulli A, et al. Mixed chimerism predicts graft rejection following BMT for severe aplastic anemia. Bone Marrow Transplant 1995;15:64[abstract].
21. Lang P, Schumm M, Taylor G, Klingebiel T, Neu S, Geiselhart A, et al. Clinical scale isolation of highly purified peripheral CD34$^+$progenitors for autologous and allogeneic transplantation in children. Bone Marrow Transplant 1999;24:583-9.
22. Schumm M, Lang P, Taylor G, Kuci S, Klingebiel T, Buhring HJ, et al. Isolation of highly purified autologous and allogeneic peripheral CD34$^+$ cells using the CliniMACS device. J Hematother 1999;8:209-18.
23. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974;18:295-304.
24. Klingebiel T and Schlegel PG. GVHD: overview on pathophysiology, incidence, clinical and biological features. Bone Marrow Transplant 1998; 21: S45-S9.
25. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Stat Assoc 1958; 58:457-81.
26. Thomas ED, Blume KG, Forman SJ. Hematopoietic cell transplantation. 1999; 2nd edition.
27. Ortega M, Escudero T, Caballin MR, Olive T, Ortega JJ, Coll MD. Follow-up of chimerism in children with hematological diseases after allogeneic hematopoietic progenitor cell transplants. Bone Marrow Transplant 1999; 24:81-7.
28. Gyger M, Baron C, Forest L, Lussier P, Lagace F, Bissonnette I, et al. Quantitative assessment of hematopoietic chimerism after allogeneic bone marrow transplantation has predictive value for the occurrence of irreversible graft failure and graft-vs.-host disease. Exp Hematol 1998; 26:426-34.
29. Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S, et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. Blood 1999;94:3234-41.
30. Marmont AM, Horowitz MM, Gale RP, Sobocinski K, Ash RC, Van Bekkum DW, et al. T-cell depletion of HLA-identical transplants in leukemia. Blood 1991; 78:2120-30.
31. Lucarelli G, Andreani M, Angelucci E. The cure of thalassemia by bone marrow transplantation. Blood Rev 2002; 16:81-5.
32. Walters MC, Storb R, Patience M, Leisenring W, Taylor T, Sanders JE, et al. Impact of bone marrow transplantation for symptomatic sickle cell disease: an interim report. Multicenter investigation of bone marrow transplantation for sickle cell disease. Blood 2000;95:1918-24.
33. Vermylen C. Hematopoietic stem cell transplantation in sickle cell disease. Blood Rev 2003;17:163-6.
34. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood 1990;75:555-62.
35. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N Engl J Med 1979;300:1068-73.
36. Passweg JR, Tiberghien P, Cahn JY, Vowels MR, Camitta BM, Gale RP, et al. Graft-versus-leukemia effects in T lineage and B lineage acute lymphoblastic leukemia. Bone Marrow Transplant 1998;21:153-8.