Dose-reduced Conditioning and Allogeneic Hematopoietic Stem Cell Transplantation from Unrelated Donors in 42 Patients

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ABSTRACT

***Purpose*: A fludarabine-based "nonmyeloablative" preparative regimen was investigated in 42 patients with hematological malignancies receiving hematopoietic stem cell grafts from unrelated volunteer donors.**

***Experimental Design*: Recipient conditioning consisted of fludarabine 30 mg/m^2 on days −6 to −2 and i.v. busulfan 3.3 mg/kg on days −6 to −5. Antithymocyte globuline was added at 2.5 mg/kg i.v. on days −5 to −2. The patients were grafted with bone marrow ($n = 13$) or peripheral blood stem cells either unmanipulated ($n = 20$) or CD34+ selected ($n = 9$). Graft-*versus*-host disease prophylaxis was performed with cyclosporine A (CsA, $n = 12$), CsA/methotrexate ($n = 12$), or CsA/mycophenolate mofetil ($n = 18$).**

***Results*: With a median follow-up of 13 months (range, 5–26 months), the actuarial disease-free survival is 64% and 38% for patients with lymphoid malignancies and standard-risk leukemia compared with only 14% for patients with high-risk disease. The main cause of treatment failure was relapse of disease in high-risk patients ($n = 14$). An increased incidence of primary ($n = 1$) or secondary graft-failure ($n = 8$) was observed (21%). Chimerism analysis of CD56+/CD3−-sorted natural killer (NK) cells, available in 10 patients, showed an impaired increase of donor NK cell chimerism between day 10 and 30 after transplantation in three of four patients with graft failure, whereas the percentage of donor NK cells surpassed 75% in all of the six patients with stable engraftment.**

***Conclusions*: Unrelated transplants after dose-reduced conditioning are associated with a higher risk of graft-failure. Pretransplant host immunosuppression has to be optimized to overcome resistance to grafts from unrelated donors after nonmyeloablative conditioning therapy.**

INTRODUCTION

The success of allogeneic hematopoietic cell transplantation does depend not only on the intensive conditioning regimen but also on the profound antileukemic effects associated with host-reactive donor effector cells. Nevertheless, the toxicity associated with supralethal doses of TBI[3] and high-dose alkylating agents leads to a high transplant-related mortality especially in older patients with a reduced performance status.

Significant graft-*versus*-leukemia effects have been demonstrated in patients with recurrent leukemia after allogeneic BM transplantation. Prolonged remission can be achieved by donor lymphocyte infusions in 70 to 80% of patients with relapsing chronic myeloid leukemia (1, 2). Investigators in several centers have now started to explore dose-reduced conditioning using either purine analogue or low-dose TBI-based regimens to establish a mixed lympho-hematopoietic chimerism, which is the prerequisite for subsequent adoptive transfer of donor lymphocytes and their antileukemic effects.

Stable mixed donor chimerism has been demonstrated in a canine model after nonmyeloablative conditioning therapy (3). Encouraging clinical results have been achieved by using reduced doses of alkylating agents together with purine analogues for conditioning therapy (4, 5). Only recently (6), stable engraftment was described in patients after 200-rad TBI combined with immunosuppressive drugs. Other investigators (7) reached the same goal by combining cyclophosphamide at 150–200 mg/kg with thymic irradiation and antithymocyte globuline (ATG).

In most of these studies, hematopoietic stem cell grafts from HLA-identical sibling donors have been used. Taking into account the limited number of patients for whom an HLA-identical sibling donor can be identified, the use of alternative donors being either matched unrelated volunteers or mismatched family members has to be explored.

In this report, we summarize the clinical results of 42 patients receiving grafts from unrelated donors. We could show the feasibility of unrelated transplants after dose-reduced conditioning with stable remissions in standard-risk patients. A high rate of graft failure (21%) was observed. CML patients and

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[3] The abbreviations used are: TBI, total body irradiation; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; ALL, acute lymphoblastic leukemia; MM, multiple myeloma; BM, bone marrow; PBSC, peripheral blood stem cell; GvHD, graft-*versus*-host disease; CMV, cytomegalovirus; CsA, cyclosporine A; MTX, methotrexate; MMF, mycophenolate mofetil; FACS, fluorescence-activated cell sorting; MNC, mononuclear cell; CR, complete remission; CP, chronic phase; SR, standard risk; HR, high risk.

Table 1 Patient characteristics ($n = 42$)

UPN[a]	Sex	Age	Disease	Interval to Tx	Status at Tx	Failed Tx/lines of therapy	Source of donor cells	CD34+ × 10e6/kg	GvHD prophylaxis	Mismatch	Donor sex
188	F	46	ALL Ph+	21	Refractory	3	CD34+	16	CsA	No	M
242	F	34	ALL	15	Refractory	3	CD34+	11,1	CsA	B/C	F
272	M	42	c-ALL	21	CR-1	3	CD34+	7,9	CsA	No	M
353	F	33	ALL	13	Refractory	3	BM	1,4	CsA/MMF	No	M
411	M	62	T-ALL	8	Refractory	2	PBSC	5,3	CsA/MMF	No	M
156	M	40	AML	18	Refractory	Auto/2	CD34+	7,5	CsA	No	M
175	F	38	AML	17	PR	2	PBSC	4,4	CsA/MTX	No	M
205	M	25	AML	12	Refractory	Allo/2	PBSC	10,6	CsA/MMF	DRB1	M
240	M	58	AML	14	PR	1	CD34+	6,5	CsA	No	M
271	M	16	AML	6	CR-1	2	PBSC	25,1	CsA/MTX	No	F
292	F	55	AML	6	Refractory	1	PBSC	11,5	CsA	No	M
297	M	56	AML	38	PR	2	PBSC	6,7	CsA/MMF	DQB1	M
322	M	42	AML	10	Refractory	2	PBSC	3,5	CsA	No	M
326	M	57	AML	4	CR-1	1	PBSC	9,6	CsA/MMF	C	M
339	M	59	AML	5	CR-1	1	BM	1.0	CsA/MMF	No	M
372	M	65	AML	64	CR-3	Auto/3	PBSC	9,7	CsA/MMF	No	M
391	F	63	AML	85	CR-2	2	PBSC	6,9	CsA/MMF	No	M
217	M	57	MDS	9	RAEB-t	2	CD34+	9,7	CsA	DQB1/C	M
219	M	32	MDS	27	RAEB	2	PBSC	3,8	CsA/MTX	No	F
223	F	50	MDS	18	RAEB-t	2	PBSC	3,75	CsA	No	M
238	M	57	MDS	18	PR	3	PBSC	4,1	CsA/MTX	No	F
287	M	56	MDS	11	RAEB-t	1	BM	1,4	CsA/MMF	B	F
216	F	47	s-AML	6	CR-1	2	CD34+	9,3	CsA	DRB1/C	M
252	M	37	s-AML	9	PR	2	BM	2,3	CsA/MTX	No	M
254	M	46	s-AML	9	Refractory	3	PBSC	3,9	CsA/MTX	No	M
344	M	59	s-AML	11	PR	1	BM	1,9	CsA/MMF	No	F
315	F	38	s-MDS	8	PR	2	PBSC	6,1	CsA/MMF	No	M
134	F	57	CML	9	2nd CP	Auto/2	PBSC	4,1	CsA/MMF	No	F
149	F	39	CML	77	1st CP	0	BM	2,3	CsA/MTX	No	F
169	M	32	CML	15	2nd CP	1	PBSC	4,6	CsA/MTX	DRB1	M
181	M	57	CML	14	AP	1	BM	3,1	CsA/MTX	No	M
206	F	51	CML	84	1st CP	0	BM	2	CsA/MTX	No	M
231	F	42	CML	72	2nd CP	Auto/2	CD34+	9	CsA	DRB1	M
300	F	47	CML	48	AP	1	BM	1,9	CsA/MMF	No	M
318	M	43	CML	73	1st CP	1	BM	1,7	CsA/MMF	A	F
201	M	41	NHL/CML	59	PR	Auto/2	BM	1,8	CsA/MMF	No	M
257	M	57	CLL	23	Richter Transf.	2	CD34+	8,3	CsA	C	M
370	F	62	CLL	68	Refractory	4	PBSC	7,9	CsA/MMF	No	M
409	M	61	CLL	45	Refractory	3	PBSC	12,7	CsA/MMF	No	M
434	M	53	CLL	27	PR	3	BM	3,13	CsA/MMF	DRB1	F
46	M	48	MM	25	Relapse	3	BM	2.0	CsA/MTX	No	F
95	M	36	NHL	22	PR	Auto/3	PBSC	7,5	CsA/MTX	No	M

[a] UPN, unit patient number; Tx, transplantation; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation; AP, accelerated phase; CD34+, CD34-positive-selected PBSCs; PR, partial remission; s-AML, secondary AML; auto, autologous Tx; allo, allogeneic Tx; Richter Transf., Richter transformation.

recipients of HLA-mismatched grafts had the highest risk for this complication.

PATIENTS AND METHODS

Eligibility Criteria. All of the patients were treated at the BM transplantation unit in Dresden. The study had been approved by the local ethical board in March 1998, and all of the patients had signed informed consent before the conditioning therapy was started. Patients with hematological malignancies could be included when they were not eligible for conventional conditioning therapy and an unrelated volunteer donor with not more than two mismatched HLA alleles was available. The reasons for dose-reduced conditioning were age >55 or reduced performance status in 18 patients, prior autologous or allogeneic stem cell transplantation ($n = 6$), CLL or NHL with intensive pretreatment ($n = 6$), prior aspergillosis ($n = 4$), long interval between diagnosis of CML and transplantation ($n = 3$), and aplastic marrow attributable to preceding chemotherapy ($n = 5$).

Patient Characteristics. The patient characteristics are summarized in Table 1. Forty-two patients were enrolled in the study. Twenty-seven were male, and 15 were female. The age range at the time of transplantation was 16 to 65 years (median, 47 years). Twenty-two patients had AML or MDS, eight had CML, five had ALL, six had CLL or indolent NHL, and one had MM. The cohort of high-risk patients had failed up to four lines of chemotherapy (median, 2). The median time interval from diagnosis to the transplant was 18 months (range, 4–85). The median Karnofsky performance score at the time of transplantation was 70% (range, 50–90%).

All of the patients and donors were tested serologically for

HLA-A, -B, and -C and with high-resolution PCR typing for DRB1 and DQB1 according to standard procedures (8). As a general rule, single allelic mismatches were accepted. More than one mismatch was only allowed in high-risk patients with acute leukemia or MDS. Complete matching was possible in 30 patients. DRB1/DQB1 subtype mismatches existed in seven transplants with an additional HLA-C mismatch in two patients. In four patient-donor pairs, a single antigen mismatch in the HLA-A, -B, or -C locus existed. In one case, a HLA-B and -C mismatch was accepted.

Conditioning Regimen. Conditioning therapy was performed as described before (9). Busulfan (Sigma-Aldrich, Deisenhofen, Germany) at 3.3 mg/kg/day was infused over 3 h on days −6 and −5. The area under the curve achieved after a single-dose infusion of 3.3 mg/kg busulfan had been shown to be equivalent to 4 × 1 mg/kg of the oral formulation (10). Fludarabine (Medak, Munich, Germany) was given at 30 mg/m^2/day over 30 min from day −6 to day −2. Antithymocyte globuline (Rabbit; Pasteur Mérieux, Lyon, France) was administered at a daily dose of 2.5 mg/kg over 4 h from day −5 to day −2. In four patients, ATG Fresenius (Bad Homburg, Germany) was used at a daily dose of 10 mg/kg.

Stem Cell and BM Collection. The sources of hematopoietic stem cells are provided in Table 1. BM was harvested on day 0 after informed consent of the donor in general anesthesia using conventional techniques. Mobilization of PBSCs was performed using 7.5 μg/kg lenograstim or 10 μg/kg filgrastim for 5 days and one or two aphereses on days 5 and 6. The product was cryopreserved when indicated. During the initial study period, CD34-positive selection of PBSCs from nine unrelated donors, of whom five had a HLA mismatch, was performed using an immunomagnetic device (CliniMACS; Milteny Biotec, Bergisch Gladbach, Germany) according to the manufacturer instructions (11). All of the patients who had received CD34-selected PBSCs were infused with unselected donor MNCs adjusted to contain 1 × 10^5/kg CD3-positive T cells on day 14 and 1 × 10^6/kg on day 21 when no signs of GvHD were detectable. Those T cells had been collected and frozen before granulocyte colony-stimulating factor stimulation. When <4 × 10^6 CD34-positive cells/kg were obtained with the first apheresis, unmanipulated PBSCs were infused. BM was infused without prior manipulation.

Supportive Care. The patient rooms occupied by either one or two persons at the same time from the start of conditioning until neutrophil engraftment were supplied with filtered air. All of the patients received antibacterial and antifungal prophylaxis with ciprofloxacine at 500 mg twice daily and fluconazole at 200 mg/day. In addition, acyclovir was given at 1200 mg daily. All of the patients received prophylaxis with cotrimoxazole or pentamidine against *Pneumocystis carinii* infection. Patients with negative CMV IgG titers received blood products from CMV seronegative donors. Bacterial and fungal surveillance cultures were performed every second week. Broad-spectrum antibiotics were given whenever body temperature increased beyond 38.5°C, when C-reactive protein increased significantly, or when a positive finding was made on chest X-ray. PCR for CMV DNA and pp65 antigen testing in peripheral blood were performed once weekly. Patients received filgrastim at 5 μg/kg/day from day + 6 until the neutrophil count reached 1.0 × 10^9/liter. Hemoglobin was maintained at a level of >5 mmol/liter, and the platelet count was maintained at >20 × 10^9/liter with in-line filtered and irradiated (30 Gy) blood products.

GvHD Prophylaxis. GvHD prophylaxis was performed with 5 mg/kg CsA i.v. starting 1 day before infusion of the graft. Further i.v. or oral dosage was adapted according to CsA trough blood levels. High-risk AML patients with >30% blasts in the BM (n = 3) and the patients transplanted with CD34+-selected PBSCs received only CsA (n = 9). Additional MTX (5 mg/m^2) was administered in the first 12 recipients receiving unmanipulated grafts on days +1, +3, and +6. MMF was given p.o. at 2 g from day +1 to day +40 instead of MTX to the subsequent patients (n = 18) because the rate of acute GvHD ≥ grade II with MTX still seemed to be quite high (6 of 12; 50%), and animal data supposed MMF also to be useful as graft rejection prophylaxis (3). Patients developing acute GvHD were maintained at CsA and MMF and received 2 mg/kg/day prednisolone in addition, which was tapered upon clinical response.

Analysis of Chimerism. Samples for chimerism analysis in peripheral blood were drawn twice weekly during hospital stay and weekly in the out-patient department. Chimerism analysis was performed as described recently (12). In brief, DNA was extracted from peripheral blood samples using a silica-based procedure (QiaAmp DNA blood kit; Qiagen, Hilden, Germany). Multiplex PCR was then performed using the AmpFlSTR Profiler kit (Applied Biosystems, Weiterstadt, Germany). High-resolution polyacrylamide-gel electrophoresis and four-color fluorescence detection were performed on an ABI 377 automated DNA sequencer. For each STR allele, the area under the curve for the corresponding signal was automatically processed by the GeneScan 3.1 software (PE Biosystems). The percentage of the donor chimerism was obtained by the following calculation: *area signal donor*/(*area signal donor* + *area signal recipient*). Finally, a mean value was calculated for all of the informative (different between donor and recipient) alleles. The values are given as percentage donor signal.

Cell Sorting for Subset Analysis. FACS was performed on a FACS Vantages cell sorter (Becton Dickinson, San Jose, California). MNCs were enriched by density gradient centrifugation (Lymphoprep; Pharmacia, Freiburg, Germany) from 30 to 60 ml of peripheral blood, depending on the actual cell counts. Erythrocytes were removed by hypotonic lysis. MNCs were then incubated with the following monoclonal fluorophore-conjugated antibodies: anti-CD3 PECy5, anti-CD3 PE, and anti-CD15 PE (Coulter Immunotech Diagnostics, Hamburg, Germany), anti-CD4 PE (PharMingen, Hamburg, Germany), anti-CD8 FITC and anti-CD56 FITC (DAKO Diagnostika, Hamburg, Germany). After washing in PBS solution, cells were sorted into the following populations: CD3+/CD4+ and CD3+/CD8+, CD3−/CD56+. The sample containing the granulocytes was incubated with anti-CD15 PE (Coulter Immunotech) and sorted as described above. Whenever possible, between 1,500–10,000 cells were sorted for each population. Samples were also collected to assess the purity of the sort for at least three populations whenever possible. The median purity, as measured by repeated FACS analysis, was 98% (range, 92–100%).

Table 2 Clinical course, graft failure, and GvHD

UPN	Disease	Graft failure (days)	Neutrophils $> 0.5 \times 10e9/1$	Platelets $> 50 \times 10e9/1$	Acute GvHD	Chronic GvHD	Current status (months)	Cause of death
188	ALL Ph+	d 45[a]	14		0	No	Dead 6	Disease
242	ALL	No	15	24	0	No	Dead 4	Aspergillus pneumonia
272	c-ALL	No	11	17	0	Extensive	Dead 12	Heart failure
353	ALL	No	14	31	0	No	Dead 4	Sepsis
411	T-ALL	No	17	15	I skin		Dead 2	Disease
156	AML	No	13	15	0	No	Dead 6	Disease
175	AML	No	13	21	III liver/GI	Extensive	Dead 12	Disease
205	AML	No	17	11	II skin/GI	Extensive	Dead 6	Disease
240	AML	No	12	15	0	Extensive	CCR 22+	
271	AML	No	12	16	II GI/liver	No	CCR 18+	
292	AML	No	12	14	IV skin/liver/GI		Dead 2	GvHD
297	AML	No	10	13	0	No	Dead 9	Disease
322	AML	No	14	24	I skin	No	Dead 4	Disease
326	AML	No	17	12	I skin	No	CCR 14+	
339	AML	No	18	16	II skin/liver	No	Alive, Relapse 12+	
372	AML	No	13	11	I skin	No	CCR 11+	
391	AML	No	13	13	I skin	No	Dead 6	Herpes encephalitis
217	MDS	No	11	25	0	No	Dead 8	Disease
219	MDS	d 58	12	16	0	No	Dead 8	*Pneumocystis carinii* pneumonia
223	MDS	No	11	13	I	Limited	CCR 23+	
238	MDS	No	16	20	II skin	No	Dead 6	Disease
287	MDS	d 100	19	22	I skin	No	Dead 4	Disease
216	s-AML	d 65	15	16	0	No	Dead 7	Disease
252	s-AML	No	12	10	II skin	Limited	Dead 14	Aspergillus pneumonia
254	s-AML	No	16	24	II skin	No	Dead 4	Disease
344	s-AML	No	21	39	I skin	No	Dead 6	Disease
315	s-MDS	No	11	NA	0	No	Dead 3	Disease
134	CML	No	10	13	II skin	Limited	CCR 18+	
149	CML	No	13	17	I skin	Extensive	Dead 16	GvHD
169	CML	d 28	13	15	0	No	Dead 2	Pneumonitis
181	CML	d 130	15	32	0	Limited	Dead 5	Aplasia/infection
206	CML	No	24	24	II skin	Limited	CCR 25+	
231	CML	No	11	11	0	No	Dead 4	Pneumonia
300	CML	d 56	11	23	0	No	Alive, CP 16+	
318	CML	Primary	NA	NA			Alive, CP 15+	
201	NHL/CML	No	15	21	I skin/liver	Limited	Dead 11	Aspergillus pneumonia
257	CLL	d 37	12	14	0	Extensive	Alive, stable disease 19+	
370	CLL	No	15	NA	III skin/liver	Extensive	CCR 11+	
409	CLL	No	13	22	I skin	Limited	CCR 8+	
434	CLL	No	19	19	0	Limited	CCR 7+	
46	MM	No	15	18	0	Limited	Dead 15	Toxoplasmosis
95	NHL	No	14	17	I skin	No	CCR 26+	

[a] d, day; NA, not achieved; CCR, continuous complete remission; GI, gastrointestinal.

Study End Points. Engraftment defined as $>0.5 \times 10^9$/liter neutrophils for 3 days, $>50 \times 10^9$/liter platelets without transfusion and treatment-related mortality in this patient cohort were the primary end points. Primary graft failure was defined as no hematological recovery until day +21. Secondary graft failure occurred when patients who had recovered in the early post-transplant period experienced cytopenia and decreasing chimerism not associated with relapsing disease. Secondary end points were the rate of acute GvHD observed and disease-free survival. Organ toxicity was documented according to the toxicity scales developed by the cancer therapy evaluation program of the National Cancer Institute. Acute and chronic GvHD were graded according to consensus criteria (13, 14).

Statistical Methods. Most quantitative parameters are provided as median or mean with range or SD. The estimated overall and disease-free survival was calculated as of November 1st, 2000 from the day of transplantation and depicted according to the methods of Kaplan and Meier (15). The probability of survival of different groups were compared using the method of Kaplan-Meier with a log-rank test (Mantel-Haenszel). Univariate analysis was performed with the Fisher's exact test. Cox's regression model was used for multivariate analysis. All of the analyses were performed with the SPSS soft ware package 6.0 (SPSS Software, Munich, Germany).

RESULTS

Toxicity. The clinical course for all of the patients is summarized in Table 2. Extramedullary toxicity with a toxicity score of greater than 2 was observed in four patients only. One patient experienced a hemorrhagic cystitis requiring local therapy. Reversible jaundice with bilirubine levels elevated more

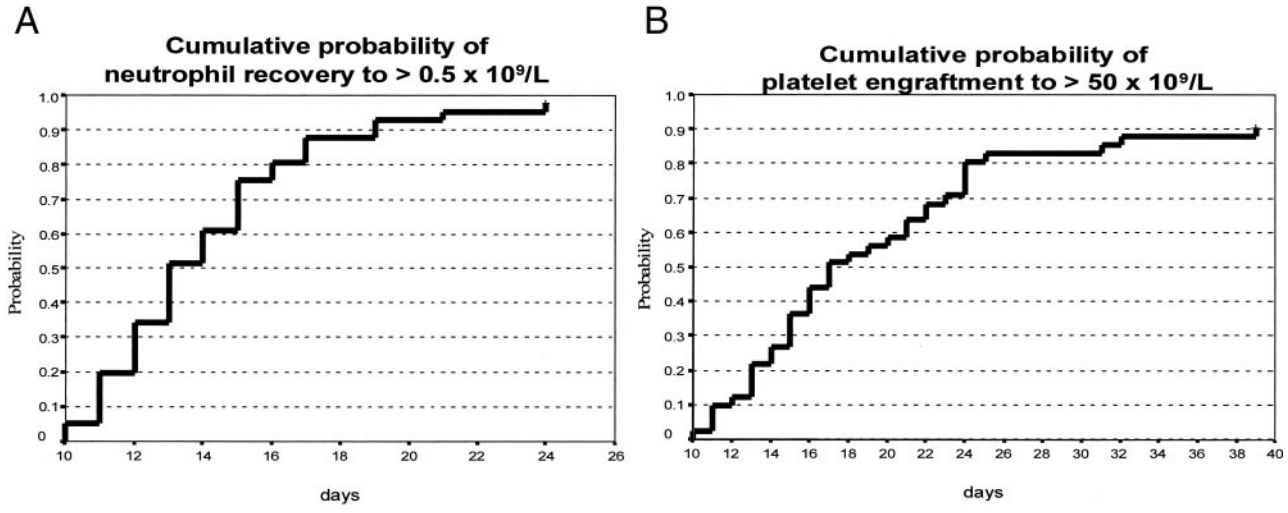

Fig. 1 Engraftment. *A*, days to an absolute neutrophil count greater than 0.5×10^9/liter after transplantation. *B*, days to a platelet count greater than 50×10^9/liter.

than 10-fold was observed in two patients. One patient had a pneumonitis on day 21 that was attributed to a CMV reactivation. Fever of unknown origin (>38.5°C) occurred in 15 patients and lasted between 1 and 6 days. The median time with less than 0.5×10^9/liter neutrophils was 8 days (2–29). Therefore, we did not observe early infectious complications. The overall day-100 treatment-related mortality was 12%. Nevertheless, seven patients died from opportunistic infections after a longer time of immunosuppressive treatment and one patient from heart failure 1 year after transplantation. CMV antigenemia was treated with ganciclovir in 16 patients (37%).

Engraftment and Graft Failure. The median dose of CD34+ cells $\times 10^6$/kg transplanted was 5.3 (1.0–25.1). Forty-one patients achieved primary neutrophil engraftment, whereas only 38 patients reached more than 50×10^9/liter platelets. The probability of neutrophil and platelet recovery is depicted in Fig. 1. The median time to recover an absolute neutrophil count of 0.5×10^9/liter was 13 days (range, 10–24 days) and of platelets above 50×10^9/liter, it was 16.5 days (range, 11–39 days). The median transfusion requirements were six packed RBCs (range, 0–20 units) and four thrombapheresis products (range, 0–23 units). Platelet engraftment was not different in patients with ABO-matched grafts compared to those with mismatched grafts. One patient with CML did not recover with his blood counts, and no donor signals could be detected until day 28 when autologous PBSCs were reinfused to rescue the patient. Eight patients (three CMLs, two MDSs, 1 secondary-AML, 1 ALL, and 1 CLL) experienced late loss of graft function and decreasing donor chimerism. In six of those eight cases, an autologous PBSC graft could be reinfused to reconstitute hemopoiesis. The two other patients (UPN 169 and 181) remained aplastic and died from infectious complications.

Statistical analysis showed a trend toward more graft failure in patients with CML ($P = 0.08$) and in recipients of HLA-mismatched grafts ($P = 0.09$). No impact of CD34+ cell dose, CD3+ cell dose, donor/recipient sex mismatch, source of donor cells, and use of MMF could be found (Table 3). Cox regression analysis with the mentioned risk factors for graft failure confirmed HLA-mismatch to be of borderline significance ($P = 0.065$).

Table 3 Risk for graft failure[a]

Risk factor	Percentage	*P*
HLA-mismatch (Y/N)	41.7% *vs.* 13.8%	0.09[b]
Sex mismatch D/R[c] (Y/N)	28.6% *vs.* 15.0%	0.45
CML (Y/N)	44.4% *vs.* 15.6%	0.08
Unmanipulated PBSC (Y/N)	10% *vs.* 33%	0.13
CD34+ > *vs.* < 5.3×10^6/kg	15% *vs.* 28.6%	0.45
MMF (Y/N)	17.6% *vs.* 25%	0.71

[a] Risk factor analysis for graft failure. *P*s were calculated by the Fisher's exact test (two-sided).
[b] Multivariate regression analysis shows a *P* of 0.065 for HLA-mismatch.
[c] D, donor; R, recipient.

Chimerism. All of the patients who did not relapse developed a complete donor chimerism in peripheral blood leukocytes. The sequential analysis of the sorted CD56+/CD3− and CD15+ cell subsets is summarized in Fig. 2. The analysis could be performed for 10 patients between day 10 and 30 after transplantation. In this subgroup, all of the six recipients with stable engraftment obtained more than 75% donor NK cell chimerism, whereas three of four patients with graft failure did not surpass the 75% level ($P = 0.03$; Fisher's exact test). The median absolute number of NK cells between day 10 and 30 was similar for patients with durable engraftment (9.8×10^6/liter; range 1.3–31) or with graft failure (6.8×10^6/liter; range 3.1–19). Because subset chimerism was available only for a limited number of patients, no multivariate statistical analysis was performed. The CD15+ myeloid compartment showed early complete donor chimerism in most patients. As expected, a low percentage of donor T cells was observed, especially in recipients of CD34+-selected PBSCs. Given the low number of patients with complete data for the CD4+ and CD8+ subsets

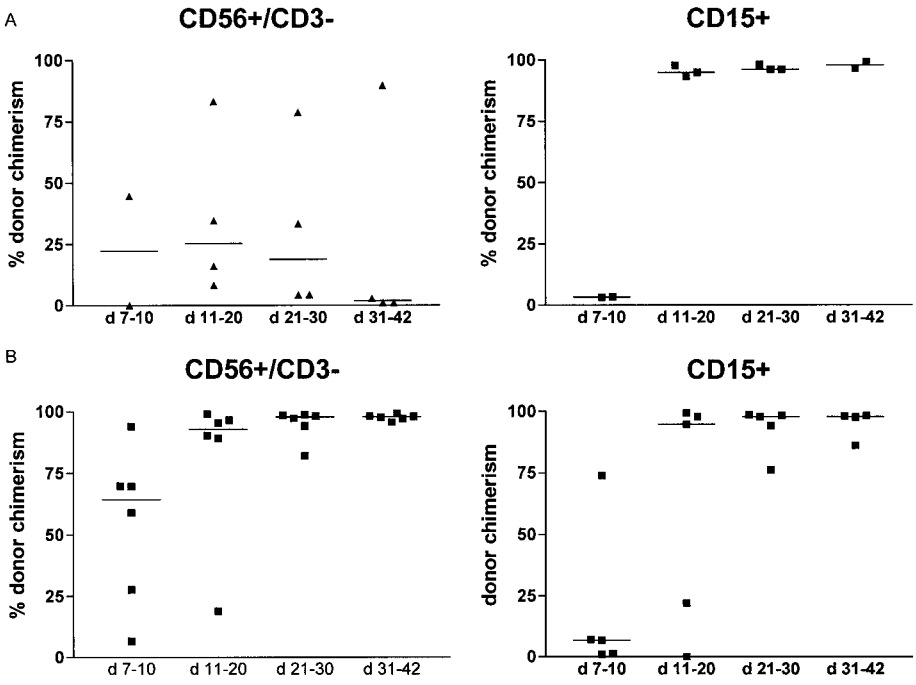

Fig. 2 Chimerism analysis in cell subsets. The percentage of donor NK (CD56+/CD3−) cells and myeloid (CD15+) cells are depicted at different time points after the transplant for 7–10 patients for whom cell sorting could be performed. In the *top row* (*A*), four patients with graft failure are summarized. In three cases, no increase of donor NK cells was observed early after transplantation, whereas the CD15+ subset showed no difference to the course seen in six patients with stable engraftment (*B*).

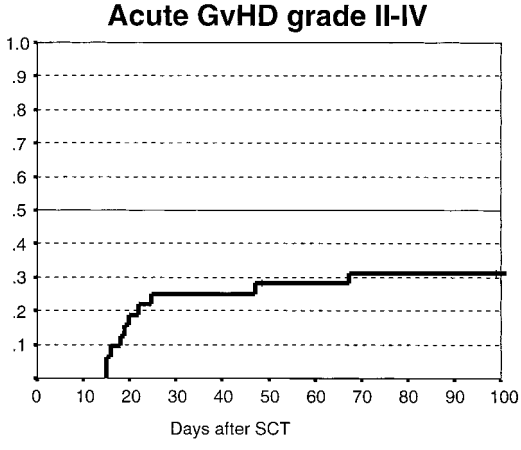

Fig. 3 Acute GvHD grade II-IV. Probability of acute GvHD grade II-IV after dose-reduced conditioning and stem cell transplantation from an unrelated donor until day 100. Only patients with stable engraftment ($n = 33$) were analyzed. *SCT*, stem cell transplantation.

and the low absolute lymphocyte counts early after transplantation (range, 0.06. to 0.46 $\times$ 10^9/liter), the graphs for CD4+ and CD8+ are not provided.

Acute and Chronic GvHD. For this analysis, only patients with durable engraftment ($n = 33$) were analyzed. As shown in Fig. 3 for grade II-IV GvHD, several patients had a late occurrence of GvHD symptoms. The probability of acute GvHD grade II-IV was 32% with one patient dying from acute GvHD grade IV after immunosuppressive prophylaxis had been stopped early. Regression analysis showed no significant impact of disease, donor-recipient sex, graft-source, GvHD prophylaxis, and number of CD34+ and CD3+ cells in the graft on the risk of acute GvHD. The organ involvement, as well as the grade of acute and chronic GvHD, is summarized in Table 2. Chronic GvHD beyond day 100 was documented in 16 patients (9 limited, 7 extensive).

Survival. Relapsing disease was the main cause for treatment failure in this high-risk cohort. Because the patient group was very heterogeneous in terms of underlying disease and disease status before inclusion, we decided to analyze the outcome according to risk categories as follows: the standard-risk group contains all of the patients with AML, ALL in CR, and CML patients in CP ($n = 12$). All of the patients having no CR or MDS patients with more than 10% blasts in the BM by the time of transplantation are grouped as high-risk ($n = 23$). Patients with lymphoma, MM, and CLL are analyzed separately as lymphoid malignancies. The overall and disease-free survival with a median follow-up of 13 months is shown for the different risk groups in Fig. 4. The actuarial disease-free survival was significantly better in patients with SR (38%) and lymphoid malignancies (64%) compared with the HR group (14%; $P = 0.04$ for SR *versus* HR and 0.0025 for lymphoid *versus* HR). There was no significant difference for overall and disease-free survival between patients who received completely HLA-matched grafts compared with recipients of mismatched grafts. Fourteen patients died because of relapsing disease, 7 from opportunistic infections, 3 from early toxicity (sepsis, GvHD, and pneumonitis), and 1 from heart failure 1 year after transplantation.

DISCUSSION

Low intensity immunosuppressive conditioning has been shown to significantly reduce the early toxicity of allogeneic blood stem cell transplantation and has even been used with success in patients who relapsed after conventional autologous

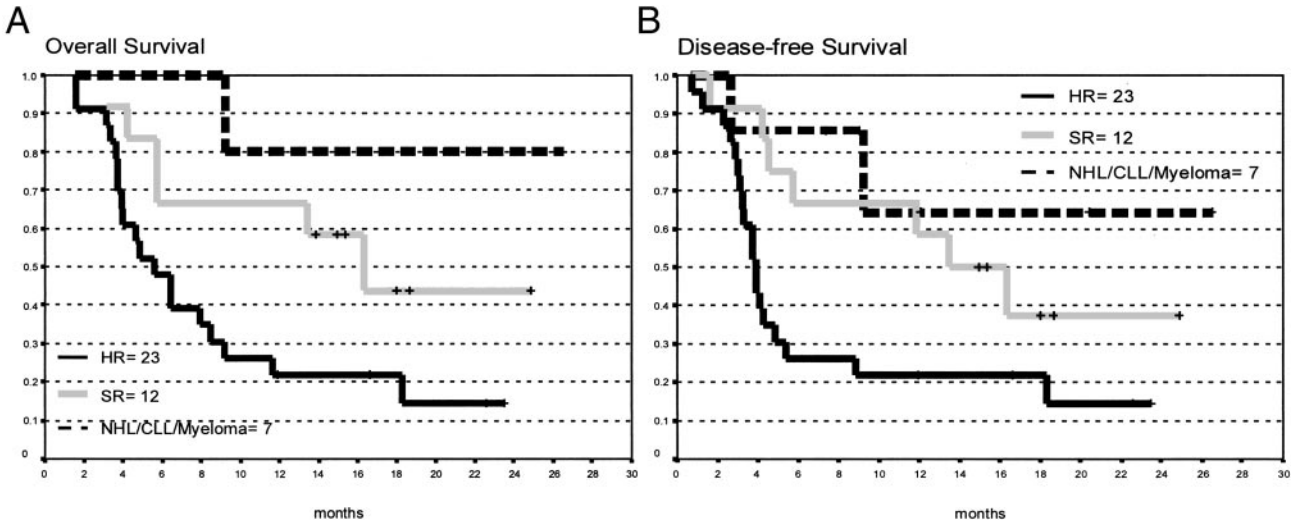

Fig. 4 Kaplan-Meier curves according to risk groups. *A*, overall survival in patients with SR (SR, AML, ALL in complete remission before transplant, and CML in chronic phase), HR (HR, AML, ALL not in complete remission before transplant, CML in accelerated phase and after blast crisis, and MDS with more than 10% blasts) and patients with lymphoid malignancies (CLL, NHL, and MM). *B*, disease-free survival for the same subgroups.

or allogeneic stem cell transplantation (16, 17). Although this kind of transplant procedure seems to be an attractive approach in patients ineligible for conventional preparative regimen, the problems of acute and chronic GvHD and continuous immunosuppression with infectious complications have not been solved. Most studies (18) on purine analogue-based conditioning therapy have used fully or partially matched sibling donors. The experience with unrelated donors is still limited (19). This is especially true for the use of granulocyte colony-stimulating factor mobilized PBSCs from unrelated donors after nonmyeloablative conditioning.

The effectiveness of allogeneic cell therapy after dose-reduced conditioning relies mainly on graft-*versus*-leukemia effects of alloreactive and minor-histocompatibility antigen-specific T cells (20). The experience in CML patients suggests that these effects are even more pronounced after allogeneic transplantation from unrelated donors (21). Therefore, we decided to incorporate the use of unrelated matched donors in this protocol evaluating a dose-reduced preparative regimen. Given the increased risk of GvHD and graft failure after transplantation from unrelated donors, we used antithymocyte globuline to achieve suppression of host-*versus*-graft and graft-*versus*-host reactions. Because the rate of acute GvHD grade II-III was still 50% with the use of CsA and MTX, the GvHD prophylaxis was adapted to a combination of CsA and MMF for patients with unselected grafts during the study period. The latter compound has shown to decrease the rate of acute GvHD in a dog leukocyte antigen (DLA)-mismatched dog model and has shown to be effective to support engraftment after conditioning with 200-cGy total body irradiation (22, 3). Although the rate of acute GvHD ≥grade II appeared to be lower (22%) with the combination of CsA and MMF, matched-pair comparison was not possible because of the heterogeneous patient population.

In a previous study (9) we had shown that ATG is not necessary for stable engraftment in the related setting after conditioning therapy with 150 mg/m^2 fludarabine and 50% of the usual busulfan dose. Nevertheless, the dose of ATG used could have been too low. On the other hand, the long half-life time of ATG might have led to an *in vivo* depletion of donor T cells that are important mediators of engraftment, especially after low-dose conditioning. We have omitted ATG from the preparative regimen in a second cohort of seven patients with unrelated donors and have not seen any graft failure since then (data not shown). Other forms of *in vivo* T-cell depletion might be advantageous to decrease the risk of acute GvHD without hampering engraftment. Encouraging results with a very low rate of acute GvHD and no graft failure have been achieved with the use of CAMPATH-1H by a study group from the United Kingdom (17). Because the eight recipients of unrelated transplants in their series received BM grafts after a regimen containing melphalan and fludarabine, the data cannot be compared directly with ours. Nevertheless, the use of *in vivo* CAMPATH-1H should be studied prospectively in nonmyeloablative transplants from unrelated donors.

The higher rate of graft failure in patients with CML can be seen in relation with the less intensive pretreatment or prior IFN application (23). Higher rates of graft failure in CML patients have also been published for conventional transplants from unrelated donors in a retrospective registry analysis (24).

The HLA match grade is an accepted risk factor for graft failure after allogeneic stem cell transplantation (25). Especially HLA-C mismatching has been shown to be highly predictive for this complication after transplants from unrelated donors (26). The patient cohort in our study is too small and heterogeneous to perform multivariate analysis for these and other risk factors like CD34+ cell dose and source of donor cells, and many patients had early treatment failure because of disease relapse.

The rationale to use mobilized PBSCs whenever possible in our study was to overcome the risk of graft failure after less intensive conditioning by a higher dose of CD34+ cells and the

knowledge that higher BM cell doses have been related to a better outcome for patients with high-risk leukemia (27). After having seen graft failure in two patients who had received CD34-positive-selected PBSCs, we decided to use unmodified PBSCs thereafter. Nevertheless, this policy did not prevent graft failure in three cases receiving unselected PBSCs where the number of CD34+ cells infused was adequate. The course of the hematological parameters in the patients with graft failure strongly suggests that 50% busulfan cannot be called "nonmyeloablative" in the context of allogeneic stem cell transplantation because all of the patients experienced aplasia and had to be rescued with autologous stem cells.

The observation of a decreased donor NK cell chimerism in three patients with graft failure is interesting. Donor NK cells that normally come up early after allogeneic stem cell transplantation could hardly be detected, suggesting that the residual fraction of host NK cells mediated graft failure. The importance of donor NK cells for engraftment after conventional conditioning therapy has been shown previously (28, 29). Targeting NK cell function using antibodies against CD44 can facilitate engraftment in mismatched transplants after nonmyeloablative conditioning in a large animal model (30). Because comparable antibodies are currently developed in the human system, this approach might become an attractive strategy for future clinical trials.

The prolonged aplasia after graft failure might result from the additive effects of busulfan administration and temporary competition between recipient and donor hematopoiesis (31). In contrast to low-dose TBI-based regimen (3), mixed chimerism could only be detected in the T-cell compartment for a limited time period.

Although the early extramedullary toxicity of the preparative regimen was moderate with only 12% toxic deaths until day 100, the nonrelapse mortality associated with chronic GvHD and opportunistic infections is considerable. This has to be kept in mind when the age limits for studies including allogeneic cell therapy after modified conditioning therapy are set up. The use of a dose-reduced conditioning regimen was not helpful in patients with acute leukemia or MDS who had elevated blast counts by the time of inclusion. Early relapse was the cause of treatment failure in one-third of all of the patients. This reflects the progressed disease status of most patients included. Many years of allogeneic BM transplantation and studies on the use of adoptive immunotherapy with donor lymphocytes have shown that immunotherapy is not sufficient to control rapidly proliferating acute leukemia (2, 32).

Despite the inclusion of older patients and patients with intensive pretreatment, this study shows encouraging results in patients with standard risk diseases, indolent lymphoma, and CLL. In these diseases, the competition between antigen-dependent cellular toxicity and tumor proliferation seems to favor effective graft-*versus*-tumor effects (5).

In summary, our results show the feasibility of allogeneic transplantation of BM and PBSCs from unrelated donors after a preparative regimen containing a purine analogue and 50% of the common busulfan dose. The rate of graft failure is significantly higher than after transplants from matched sibling donors. Prolonged remission can only be achieved in patients with a limited burden of disease by the time of the transplant, and GvHD and opportunistic infections still remain unsolved problems.

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