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Allogeneic Stem Cell Transplantation from HLA-Mismatched Donors for Pediatric Patients with Acute Lymphoblastic Leukemia Treated According to the 2003 BFM and 2007 International BFM Studies: Impact of Disease Risk on Outcomes

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A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (HSCT) is beneficial for pediatric patients with relapsed or (very) high-risk acute lymphoblastic leukemia (ALL) in remission. A total of 1115 consecutive patients were included in the ALL SCT 2003 BFM study and the ALL SCT 2007 I-BFM study and were stratified according to relapse risk (standard versus high versus very high risk of relapse) and donor type (matched sibling versus matched donor versus mismatched donor). A total of 148 patients (60% boys; median age, 8.7 years; B cell precursor ALL, 75%) were transplanted from mismatched donors, which was defined as either less than 9/10 HLA-compatible donors or less than 5/6 unrelated cord blood after myeloablative conditioning regimen (total body irradiation based, 67%) for high relapse risk (HRR; n = 42) or very HRR (VHRR) disease (n = 106). The stem cell source was either bone marrow (n = 31), unmanipulated peripheral stem cells (n = 28), T cell ex vivo depleted peripheral stem cells (n = 59), or cord blood (n = 25). The median follow-up was 5.1 years. The 4-year rates of overall survival (OS) and event-free survival were 56% ± 4% and 52% ± 4%, respectively, for the entire cohort. Patients transplanted from mismatched donors for HRR disease obtained remarkable 4-year OS and event-free survival values of 82% ± 6% and 80% ± 6%, respectively, whereas VHRR patients obtained values of 45% ± 5% and 42% ± 5% ($P < .001$), respectively. The cumulative incidence of relapse was 29% ± 4% and that of nonrelapse mortality 19% ± 3%. The cumulative incidence of limited and extensive chronic graft-versus-host disease was 13% ± 3% and 15% ± 4%, respectively, among the 120 patients living beyond day 100. Multivariate

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analysis showed that OS was lower for transplanted VHRR patients ($P = .002$; hazard ratio [HR], 3.62; 95% confidence interval [CI], 1.60 to 8.20) and for patients beyond second complete remission (CR2) versus first complete remission ($P < .001$; HR, 3.68; 95% CI, 1.79 to 7.56); relapse occurred more frequently in patients with VHRR disease ($P = .026$; HR, 3.30; 95% CI, 1.16 to 9.60) and for those beyond CR2 ($P = .005$; HR, 4.16; 95% CI, 1.52 to 10.59). Nonrelapse mortality was not significantly higher for cytomegalovirus-positive recipients receiving cytomegalovirus-negative grafts ($P = .12$; HR, 1.96; 95% CI, .84 to 4.58). HSCT with a mismatched donor is feasible in pediatric ALL patients but leads to inferior results compared with HSCT with better matched donors, at least for patients transplanted for VHRR disease. The results are strongly affected by disease status. The main cause of treatment failure is still relapse, highlighting the urgent need for interventional strategies after HSCT for patients with residual leukemia before and/or after transplantation

INTRODUCTION

Approximately 80% of pediatric patients presenting with acute lymphoblastic leukemia (ALL) diagnosed over age 1 year are currently treated with conventional polychemotherapy protocols [1,2]. However, approximately 10% of patients present with poor prognostic features at initial diagnosis, and 20% of ALL pediatric patients eventually relapse after first complete remission (CR1). Additional therapy is required for patients who achieve complete remission (CR) One option is allogeneic stem cell transplantation (HSCT) [3-6].

The overall results of HSCT have consistently improved over time through reduction in nonrelapse mortality (NRM). The use of unrelated volunteer donors, unrelated cord blood (CB), or haploidentical donors makes HSCT feasible for every patient. Unfortunately, the relapse incidence has not changed and remains a substantial concern.

In many countries the number of allogeneic HSCTs from nonsibling donors has exceeded that of matched sibling donor (MSD) HSCT since the middle of 2005 [7]. In 2015 Peters et al. [8] published the results obtained by the BFM group for pediatric patients with ALL in CR. In that publication the authors demonstrated the equivalence of the results obtained with MSDs and with matched unrelated donors (MUDs). The International BFM (I-BFM) consortium reproduced these results in the I-BFM ALL HSCT 2007 study (under revision). In both studies patients were allocated to different relapse risk groups. Patients with a very high relapse risk (VHRR; Table 1) were eligible for any donor type and available stem cell source. Here, we report the outcomes of 148 patients who were transplanted in CR1 or subsequent CR from an HLA-mismatched donor (MMD) whatever the per-protocol disease risk allocation.

METHODS

Transplant centers from 3 countries (Austria, Germany, and Switzerland) participated in the 2003 BFM ALL study from 2003 to 2011, and 10 additional countries (Czech Republic, Denmark, France, Israel, Italy, the Netherlands, Poland, Sweden, Slovakia, and Turkey) participated in the I-BFM ALL SCT 2007 study from 2007 to 2011. Both studies were prospective, multicenter open trials (extended as a register studies until 2013), approved through the central and local ethics committees Informed consent was obtained from legal guardians and from patients when possible before study entry.

Inclusion Criteria

All consecutive patients up to age 18 years at the time of initial ALL diagnosis or relapse with an indication for allogeneic HSCT according to national frontline and relapse protocols were eligible for the present study. CR was defined based on bone marrow (BM) with active hematopoiesis and fewer than 5% leukemic blast cells (identified morphologically) and normal cerebrospinal fluid.

Donor Type

HLA-MMDs were defined as donors with more than 1 (>9/10) or more allelic or antigenic disparities up to a different haplotype (MMD), regardless of relationship. Unrelated CB was also accepted as a stem cell source. HLA typing was defined using low-resolution molecular techniques for HLA-A and HLA-B and high-resolution typing for HLA-DR, and less than 5/6 matches were classified as MMDs (see Supplementary Figure S1).

Risk Stratification

The patients were stratified according to BFM eligibility criteria for transplantation. Standard relapse risk patients were not eligible for any HSCT, high relapse risk (HRR) patients were eligible for either MSD or MUD HSCT, and VHRR patients also had indication for MMD transplants (see Supplementary Tables S1 and S2).

Indication for allogeneic HSCT according to BFM-frontline protocols are as follows: Risk definition and indications for allogeneic HSCT are summarized in Table 1 (second CR). Briefly, stratification was based on prednisone response, some fusion transcripts or gene abnormalities, and minimal

Table 1
Risk Definition and Indication for Allogeneic HSCT in ALL According to the BFM Criteria

Amendment 10.10.2008		PCR-MRD Results				
		MRD-SR	MRD-MR	MRD-HR		No MRD Result
				MRD-TP2 ≥ 10^{-3}	MRD-TP2 ≥ 10^{-2}	
HR by MRD only (MRD at TP2 ≥ 10^{-3})		n.a.	n.a.	MSD/MUD	MSD/MUD/MMD	n.a.
HR criteria (in hierarchical order)	No CR d33	n.a.	MSD/MUD/MMD	MSD/MUD/MMD	MSD/MUD/MMD	MSD/MUD/MMD
	PPR + (9;22)	MSD/MUD/MMD	MSD/MUD/MMD	MSD/MUD/MMD	MSD/MUD/MMD	MSD/MUD/MMD
	PPR + (4;11)	MSD/MUD	MSD/MUD	MSD/MUD	MSD/MUD/MMD	MSD/MUD
	PGR + (9;22)	No	MSD/MUD	MSD/MUD	MSD/MUD/MMD	MSD/MUD
	PGR + (4;11)	MSD	MSD	MSD/MUD	MSD/MUD/MMD	MSD
	PPR +*	No	No	MSD/MUD	MSD/MUD/MMD	MSD/MUD
	"Favorable PPR"†	No	No	MSD/MUD	MSD/MUD/MMD	No

MRD-SR: MRD negativity after 4 and 12 weeks induction treatment, measured with at least 1 target with a sensitivity of ≤10^{-4}. MRD-MR: any MRD positivity after 4 and 12 weeks induction treatment but ≤10^{-3} at week 12 (TP2). MRD-HR: MRD ≥10^{-3} at week 12 (TP2). Indications for allogeneic HSCT in ALL according to "Interfant 06": age at diagnosis below 6 months plus MLL rearrangement plus initial WBC count ≥ 300.000/μL. No indicates no SCT indicated; n.a., not applicable; PPR, poor prednisone response; GPR, good prednisone response; NRd33, no remission at day 33.

* PPR + pro-B ALL or T-ALL and/or M3 D15 and/or WBC count > 100.000/μL.

† PPR + none of the above criteria.

residual disease (MRD) level at time point 2 (ie, day 80) in CR1 and T versus B cell lineage plus delay from CR1 for patients in CR2. The MRD levels just before the transplantation procedure and at a defined time point after transplantation were not neither mandatory nor registered.

Stem Cell Source

BM was the recommended source according to the protocol, but granulocyte colony-stimulating factor–primed peripheral blood (PB) and CB stem cells were also acceptable sources, according to transplant or donor center preference. Target doses > 3×10^8 nucleated cells/kg recipient body weight and >1.5×10^6 $CD34^+$ cells/kg recipient body were recommended for both BM and PB stem cells (PBSCs). For CB the target doses were 3×10^7 nucleated cells/kg recipient body weight and >1×10^6 $CD34^+$ cells/kg recipient body.

Transplant Procedure

A consistent myeloablative conditioning regimen was performed depending on both recipient age and donor type and did not depend on disease risk group. For patients in the present study (ie, those transplanted from MMDs and older than 2 years of age [except for 8 patients < 2 years old]), the conditioning regimen was based on hyperfractionated total body irradiation (TBI; total dose 1200 cGy i.e. 200 cGy b.i.d. on days –10 to –8), fludarabine (40 mg/m^2/day for 4 days from days –7 to –4), and etoposide (40 mg/kg at day –3). Patients younger than 2 years of age received body weight–adjusted doses of either i.v. or oral busulfan from days –11 to –8, followed by fludarabine (40 mg/m^2/day from days –7 to –4) and cyclophosphamide (60 mg/kg for 2 days, days –3 and –2).

Graft-versus-host disease (GVHD) prophylaxis comprised cyclosporine A, methotrexate, and antithymocyte globulin (Fresenius, Bad Homburg vor der Höhe, Germany; 20 mg/kg/dose, on days –4, –3, and –2). Methotrexate was substituted with steroids in CB recipients. Some patients received ex vivo T cell–depleted grafts either by $CD34^+$ selection or CD3/CD19 depletion according to protocols and physician/center decision .

Acute and chronic GVHD (aGVHD and cGVHD, respectively) were graded as previously described. Patients who were alive and in remission 100 days after HSCT were considered at risk for cGVHD. The discontinuation of immunosuppression was considered the absence of GVHD.

Statistical Analysis

For non–time to event variables, chi-square tests, or where appropriate Fisher's exact test, were used to compare groups for categorical variables, and the Wilcoxon rank-sum test (Kruskal-Wallis test for more than 2 populations) was used for continuous variables. The overall survival (OS) and event-free survival (EFS) probabilities were calculated using the Kaplan-Meier method, and the groups were compared using the log-rank test. For OS death resulting from any cause was defined as an event, and for EFS the events included relapse, secondary malignancy, and death of any cause. The starting point for survival analysis was the date of the first HSCT. Survivors were censored at the last follow-up.

The cumulative incidence of cGVHD, cumulative incidence of relapse (CIR) and death in remission, defined as NRM, were estimated using the Kalbfleisch and Prentice approach [9], considering competing risks, which included death in remission and relapse for GVHD, death in remission for CIR, relapse for NRM, and secondary malignancy for all case types described above. Comparisons were made according to the Gray test [10]. Variables included in the multivariate models were patient disease and donor characteristics possibly associated with the defined outcomes according current knowledge (ie, patient age, gender match, stratification group according the risk of relapse, remission status at time of SCT, disease cytomegalovirus (CMV) serostatus, stem cell source, and conditioning regimen).

For multivariable analyses we used logistic regression to model the impact of risk factors on the incidence of aGVHD (data not censored until 100 days after HSCT). The impact of prognostic factors on EFS and OS was evaluated using the Cox proportional hazards model with time-dependent covariates, and the impact on cGVHD, CIR, and NRM was evaluated using the proportional subdistribution hazards model of Fine and Gray for censored data subject to competing risks [11]. The impact of aGVHD and cGVHD on OS, EFS, CIR, and NRM was assessed by means of separate Cox (OS, EFS) and Fine and Gray (CIR, NRM) models, including GVHD as a time-dependent covariate and adjusting for the variables mentioned above.

All $P < .05$ were considered significant. The statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

All transplant patients were subjected to general analyses, but only patients transplanted from MMDs are reported here. Between 2003 and 2013, 1115 patients up to age 18 years, presenting with ALL in CR1 or subsequent CR, were enrolled in either the ALL SCT 2003 BFM Study (n = 705) or the ALL SCT 2007 I-BFM study (n = 410). Among these patients, 148 underwent HSCT from MMDs, 86 were enrolled in the 2003 BFM study, and the 62 remaining patients were enrolled in the international protocol.

Data regarding patients treated on the ALL SCT 2003 BFM study and ALL SCT 2007 I-BFM study and transplanted from named MSDs and MUDs (as defined per protocol) were published by Peters et al. [8] in 2015. We did not perform statistical comparisons between the cohort reported by Peters et al. and the cohort described here. Follow-up was updated as of May 25, 2016 for patients enrolled in the 2003 BFM ALL study and as of November 15, 2016 for patients enrolled in the I-BFM ALL SCT 2007 study.

The median observation time was 5.1 years for the 148 patients evaluated in the present study. According to the protocol, 106 patients belonged to the VHRR group (71 patients in the 2003 study and 35 patients in the 2007 study), whereas 42 patients were transplanted from MMDs because of HRR disease, meaning that 106 patients received HSCT as per protocol, whereas 42 patients were transplanted per physician decision.

Patient characteristics are shown in Table 2. Briefly, there were 89 boys and 59 girls (sex ratio, 60.1%). A total of 107 patients presented with B cell lineage ALL, and 12 and 6 patients suffered from either Philadelphia chromosome–positive or Mixed Lineage Leukemia gene (MLL)-AF4 ALL, respectively. The median age at HSCT was 8.7 years (range, .8 to 20.6), and 88 patients below the age of 10 years were transplanted. A total of 59 patients were transplanted in CR1, 62 patients were transplanted in CR2, and 27 patients were transplanted in CR > 2. Ninety-six patients received a TBI-based conditioning regimen, and 97 patients received grafts from unrelated donor. Thirty-three male recipients were transplanted from a female donor. The stem cell sources were ex vivo T cell–depleted PBSCs for 59 patients, unmanipulated PBSCs for 28 patients, unmanipulated BM for 31 patients, and CB for 25 patients. Twenty-seven CMV serologic-positive patients received transplants from CMV serologic-negative donors. GVHD prophylaxis was performed according to protocols for patients transplanted with unmanipulated grafts. Ten patients received cyclosporine A ± methotrexate in addition to ex vivo T cell depletion (Table 2).

A comparison of patients transplanted for VHRR disease with those transplanted for HRR disease revealed no statistically significant difference in donor age, sex match, stem cell ex vivo T cell depletion, ALL phenotype (T cell lineage versus B cell lineage), CMV status match, or TBI- versus non–TBI-based conditioning regimen. There were more patients with advanced disease in the VHRR group ($P = .007$) (Table 2).

Engraftment

One hundred thirty-five patients reached neutrophil counts above $.5 \times 10^9$/L within a median time of 16 (range, 8 to 70), 26 (range, 12 to 41), and 27 (range, 12 to 84) days for PBSCs, BM, and CB, respectively. A total of 114 and 100 patients reached platelet counts above 20×10^9 and 50×10^9/L, respectively. The median time to reach more than 50×10^9/L platelets was 22 (range, 10 to 78), 34 (range, 18 to 54), and 62 (range, 11 to 142) days for PBSCs, BM, and CB, respectively. Nine patients died before leukocyte engraftment.

Graft-versus-Host Disease

Seventy-nine patients did not develop any aGVHD above grade I, 32 patients experienced grade II aGVHD, and 15

Table 2
Patient Characteristics

	Patients (N = 148)		HRR (n = 42)		VHRR (n = 106)		*P*
Median age of patient at SCT (range)	8.7 (.8-20.6)		7.5 (.8-17.7)		8.9 (1.0-20.6)		
≤4 yr	24	16%	7	17%	17	16%	.758
>4 and ≤10 yr	64	43%	20	48%	44	42%	
>10 yr	60	41%	15	36%	45	42%	
Age of donor			0				
≤18 yr	27	20%	8	20%	19	19%	.207
>18 and ≤35 yr	38	28%	15	38%	23	23%	
>35 yr	73	53%	17	43%	56	57%	
Missing	10		2		8		
Gender donor/patient							
Female/male	33	23%	9	21%	24	24%	.8238
Others	111	77%	33	79%	78	76%	
Missing	4				4		
Remission status at SCT			0				
CR1	59	40%	23	55%	36	34%	.007
CR2	62	42%	17	40%	45	42%	
CR > 2	27	18%	2	5%	25	24%	
Phenotype of patient			0				
B cell	107	75%	30	71%	77	77%	.859
T cell	32	23%	11	26%	21	21%	
Other	3	2%	1	2%	2	2%	
Not available	2		0		2		
Missing	4		0		4		
Graft source/manipulation			0				
BM unmanipulated	31	22%	11	26%	20	20%	.216
PB unmanipulated	28	20%	11	26%	17	17%	
CB unmanipulated	25	17%	8	19%	17	17%	
Ex vivo manipulated PB	59	41%	12	29%	47	47%	
Graft manipulation data missing	5		0		5		
CMV status donor/patient							
Negative/positive	27	19%	7	17%	20	20%	.6321
Others	113	81%	34	83%	79	80%	
Not tested	2		1		1		
Missing	6				6		
TBI			0				
No	47	33%	12	29%	35	35%	.694
Yes	96	67%	30	71%	66	65%	
Missing	5		0		5		

exhibited grades III to IV aGVHD. The cumulative incidence of grades II to IV aGVHD was 33%. Multivariate analysis revealed that only remission status had a significantly negative impact on the occurrence of grade III to IV aGVHD. Neither donor age, sex match, GVHD prophylaxis, patient age, nor TBI showed any statistically significant impact (see Supplementary Table S3).

Among the 120 patients who survived after day 100 and were subsequently evaluated for cGVHD, 16 experienced extensive cGVHD. The 2-year cumulative incidence of limited and extensive cGVHD was 13% ± 3% and 15% ± 4%, respectively. In multivariate analysis none of the risk factors (HSCT indication, age and gender of donor, disease status at HSCT, GVHD prophylaxis, TBI in conditioning regimen, and patient age) had statistically significant impact on cGVHD (limited and extensive) or on extensive cGVHD alone.

Overall Survival

The 4-year rate of OS was 56% ± 4% for the entire cohort of 148 patients, with a trend for better results in the I-BFM 2007 study compared with in the 2003 BFM study (64% ± 6% versus 50% ± 6%, not significant; Supplementary Figure S2). Univariate analysis revealed that HSCT for VHRR versus HRR disease resulted in worse survival (45% ± 5% versus 82% ± 6%, $P < .001$). Similarly, HSCT in CR1 or CR2 versus CR > 2 was a favorable prognostic factor (71% ± 6% versus 53% ± 6% versus 27% ± 9%, $P < .0001$) (Figure 1). In a multivariate analysis considering indication, disease status at HSCT, donor and recipient CMV status, GVHD prophylaxis, TBI in the conditioning regimen, and patient age as risk factors for OS, VHRR disease was associated with a statistically significantly worse OS ($P = .002$; hazard ratio [HR], 3.62; 95% confidence interval [CI], 1.60 to 8.20). HSCT for advanced disease (CR > 2 versus CR1) ($P < .001$; HR, 3.68; 95% CI, 1.79 to 7.56) and transplantation from a CMV-negative donor to a CMV-positive patient ($P = .028$; HR, 1.99; 95% CI, 1.08 to 3.66) were also negative prognostic factors (see Supplementary Table S4). An additional multivariate analysis did not reveal statistically significant impact of aGVHD of any grade or aGVHD of grades III and IV on OS (Supplementary Table S5, Figure 2).

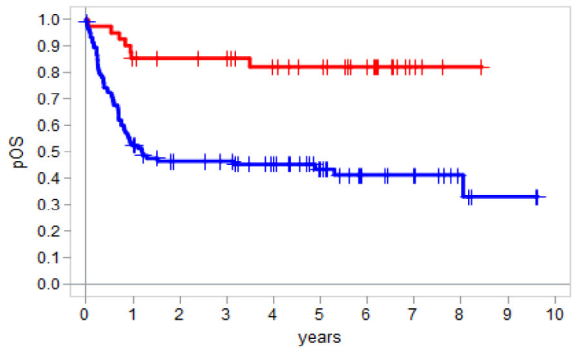
Event-Free Survival

The 4-year rate of EFS was 52% ± 4% for the entire cohort of 148 patients. One patient developed a secondary myelodysplastic syndrome. No other secondary malignancies were reported up to the date of point.

As for OS, univariate analysis revealed that HSCT for VHRR versus HRR disease resulted in worse outcomes (42% ± 5% versus 80% ± 6%, $P < .001$). Similarly, transplantation in CR1 or CR2 versus CR > 2 was a favorable prognostic factor (66% ± 6% versus 50% ± 6% versus 27% ± 9%, $P < .001$).

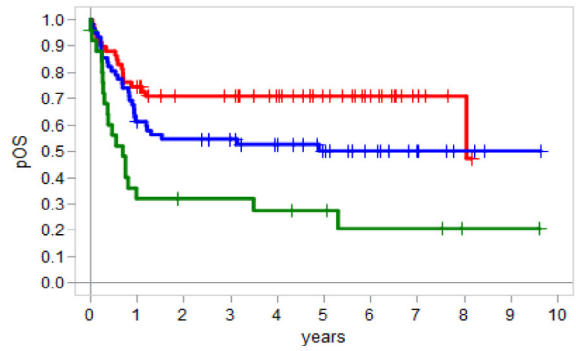
In a multivariate analysis adjusted for indication, disease status at HSCT, donor and recipient CMV status, GVHD prophylaxis, TBI in the conditioning regimen, and patient

A



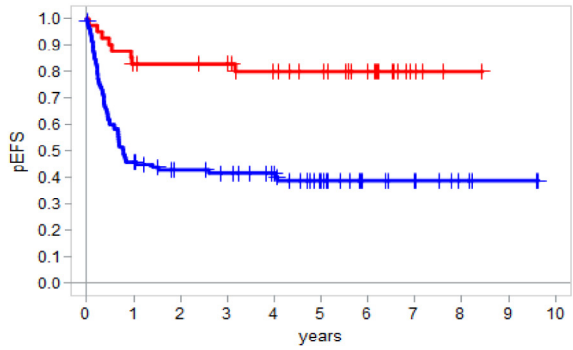
	Patients	Events	4 years - pOS	p-value
High Relapse Risk (HRR)	42	7	0.82±0.06	<0.001
Very High Relapse Risk (VHRR)	106	60	0.45±0.05	

B



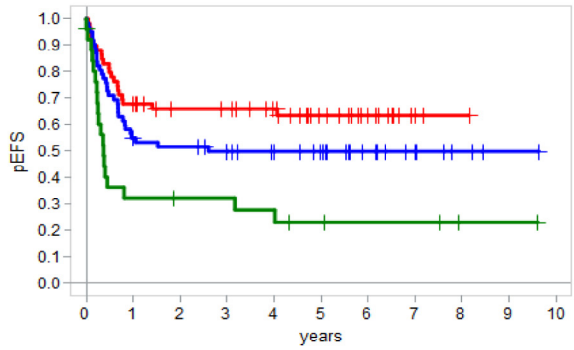
	Patients	Events	4 years - pOS	p-value
CR1	59	18	0.71±0.06	<0.0001
CR2	62	30	0.53±0.06	
CR>2	27	19	0.27±0.09	

C



	Patients	Events	4 years - pEFS	p-value
High Relapse Risk (HRR)	42	8	0.80±0.06	<0.001
Very High Relapse Risk (VHRR)	106	63	0.42±0.05	

D



	Patients	Events	4 years - pEFS	p-value
CR1	59	21	0.66±0.06	<0.0001
CR2	62	31	0.50±0.06	
CR>2	27	19	0.27±0.09	.

Figure 1. (A) OS according to indication. (B) OS according to remission status at HSCT. (C) EFS according to indication. (D) EFS according to remission status at HSCT.

age, HSCT for VHRR disease ($P = .002$; HR, 3.33; 95% CI, 1.55 to 7.16) and HSCT for advanced disease ($P < .001$; HR, 3.55; 95% CI, 1.76 to 7.16) were statistically significant negative prognostic factors (see Supplementary Table S6). An additional multivariate analysis did not reveal statistically significant impact of aGVHD of any grade or aGVHD of grades III and IV on EFS (Supplementary Table S4).

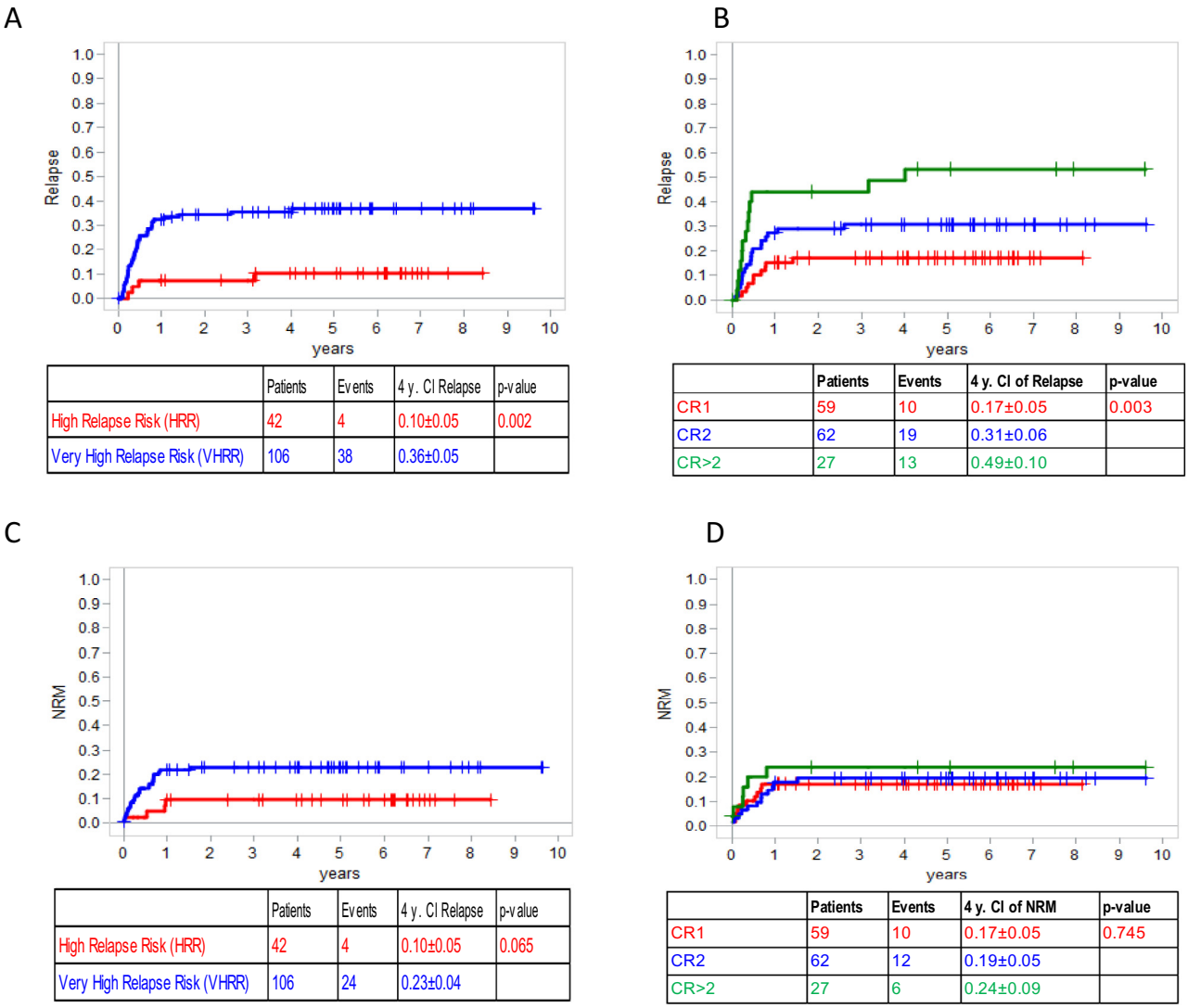
Relapse Incidence

In univariate analysis, remission status appeared to be a strong prognostic factor for relapse with 4-year CIR rates of 17% ± 6%, 31% ± 6%, and 49% ± 10% for patients transplanted in CR1, CR2, and CR > 2, respectively ($P = .003$). In multivariate analysis adjusted for HSCT indication, disease status at HSCT, GVHD prophylaxis, donor and recipient CMV status, TBI in the conditioning regimen, and patient age, HSCT for VHRR disease appeared to be a statistically significant negative prognostic factor ($P = .026$; HR, 3.33; 95% CI, 1.16 to 9.60), and HSCT for more advanced disease was also a negative prognostic factor (CR > 2 versus CR1; $P = .005$; HR, 4.02; 95% CI, 1.52 to 10.59).

In an additional multivariate analysis no statistically significant impact of aGVHD of any grade or aGVHD of grades III and IV on the relapse incidence was reviled. There was a significant positive impact of cGVHD on relapse incidence when limited and extensive cGVHD were considered all together, but this impact disappeared when limited or extensive cGVHD were considered separately.

Nonrelapse Mortality

The 4-year cumulative incidence of NRM was 19% ± 3% without any difference between both studies. Univariate analysis revealed that the combination of CMV-negative donors with CMV-positive recipients had a negative impact on NRM of borderline significance: 4-year cumulative incidence of NRM was 30% ± 9% versus 15% ± 3% ($P = .053$). Remission status



	Patients	Events	4 y. CI Relapse	p-value
High Relapse Risk (HRR)	42	4	0.10±0.05	0.002
Very High Relapse Risk (VHRR)	106	38	0.36±0.05	

	Patients	Events	4 y. CI of Relapse	p-value
CR1	59	10	0.17±0.05	0.003
CR2	62	19	0.31±0.06	
CR>2	27	13	0.49±0.10	

	Patients	Events	4 y. CI Relapse	p-value
High Relapse Risk (HRR)	42	4	0.10±0.05	0.065
Very High Relapse Risk (VHRR)	106	24	0.23±0.04	

	Patients	Events	4 y. CI of NRM	p-value
CR1	59	10	0.17±0.05	0.745
CR2	62	12	0.19±0.05	
CR>2	27	6	0.24±0.09	

Figure 2. (A) CIR according to indication. (B) CIR according to remission status at HSCT. (C) NRM according to indication. (D) NRM according to remission status at HSCT.

appeared to be a strong prognostic factor for NRM with 4-year cumulative incidences of 17% ± 6% %, 19% ± 5%, and 24% ± 9% for patients transplanted in CR1, CR2, and CR > 2, respectively.

Multivariate analysis adjusted for HSCT indication, disease status at HSCT, GVHD prophylaxis, TBI in conditioning regimen, and age of patient showed that none of these factors was significant. Similarly, the negative impact of the combination of CMV-negative donors with CMV-positive recipients reached only borderline significance ($P = .12$; HR, 1.96; 95% CI, .84 to 4.58). In an additional multivariate analysis aGVHD of any grade was associated with higher NRM ($P = .002$; HR, 3.35; 95% CI, 1.19 to 9.43).

DISCUSSION

Allogeneic HSCT has represented the best available treatment for pediatric patients suffering from poor-risk malignant hematologic diseases in general and particularly those with poor-risk ALL [3-5,8,12-15]. The progress achieved in both conventional chemotherapy and SCT techniques has augmented the potential for a cure, particularly after MSD and MUD HSCT.

High molecular class 1 and 2 HLA typing completely changed the ability to identify full-matched donors defined as 8/8 (HLA-A, -B, -C, and -DR) or 10/10 (same + DQB1) HLA-compatible donors in North America and Europe, respectively. Moreover, at least in pediatric patients with malignant diseases, the use of unrelated compatible donors was integrated into treatment algorithms for poor-risk conditions. The ability to use partially matched unrelated CB completed the repertoire of potential stem cell sources. However, HSCT from alternative donors—whether related or unrelated—remained associated with high treatment failure rates, reflecting both NRM and relapse. Thus, alternative HSCT is often limited to high-risk patients and to some experienced centers able to perform ex vivo graft manipulations, such as T cell depletion through either positive $CD34^+$ cell selection or $CD3^+ \pm CD19^+$ cell depletion [14,16-19]. However, these disappointing results were mainly described from retrospective and single-center studies and should be cautiously interpreted.

In 2003 the Austrian-German-Swiss BFM protocol initiated a prospective study to evaluate the feasibility of the

systematic use of either 9 or 10/10 unrelated donors for patients < 18 years old with an indication of allogeneic HSCT for ALL in CR1 or subsequent CR. The results demonstrated the equivalence between MSDs and MUDs, regardless of the disease relapse risk [8]. The 4-year OS rates were 79% ± 4% and 73% ± 3% for patients transplanted from MSDs and MUDs, respectively (not significant). Both 4-year EFS and CIR were also similar in both groups. NRM was statistically better in the MSD group. This initial study was followed by an international study of 10 countries, and the global results were similar (Balduzzi et al., under revision). Both studies also included patients without an MSD or MUD to provide a common platform of therapy.

If MRD levels at the end of induction and consolidation in first- and second-line therapy were part of the disease risk stratification, MRD levels were not registered just before and after HSCT. However, both protocols BFM 2003 and I-BFM 2007 led to better descriptions of this specific pediatric population.

Here, we report the results of these 2 prospective studies on 148 HRR and VHRR patients transplanted from MMDs for ALL in CR1 or subsequent CR. Both 4-year OS and EFS rates (56% ± 4% and 52% ± 4%) were inferior to the results reported within the same studies for patients with better-matched donors. However, the results remained remarkable and satisfying for HRR patients transplanted from MMDs, with 4-year rates of OS and EFS of 82% ± 6% and 80% ± 6%, respectively. These results seem to be comparable with those reported by Peters et al. [8] among HRR patients transplanted from either MSDs or MUDs along the same protocols. The overall results were better than those recently published by a French group on the comparisons between transplantation from 1 and 2 CB units in patients with acute leukemia [20]. However, in that study it was not possible to depict patients transplanted with either MUDs or MMDs for HRR or VHRR disease as defined in our current study. Indeed, a strong comparison appears as hazardous.

In the present study relapse was the main cause of failure, higher than NRM, consistent with the literature. In a 25-year retrospective study, Mateos et al. [21] showed a static relapse incidence over this period, whereas treatment-related mortality was reduced significantly during the same time. In the SCT-BFM 2003 trial Peters et al. [8] reported a CIR from 22% to 24% in patients transplanted from either MSDs or MUDs. Mo et al. [22] reported the same results when describing transplantation from either haploidentical donor or unrelated CB transplantation. Finally, Michel et al. [23] reported as well 14.9% to 23.4% CIR in a French randomized trial comparing transplantation from either 1 or 2 CB unit in patients younger than 35 years with leukemia. However, 2-fold higher transplant-associated deaths were observed in patients transplanted from MMDs compared with those transplanted from MSDs and MUDs.

Relapse represents the most important challenge to address. The options to reduce post-transplant relapse incidence currently include the better determination of peritransplant measurable residual disease, tailored chemotherapy, and immunomodulation. We were able to analyze the impact of relapse risk and disease status on the end points. Both factors appeared statistically adverse for OS, EFS, and relapse incidence, indicating the need for developing new approaches for VHRR. Based on these results it is likely mandatory and safer to perform HSCT in CR1 or CR2 and not wait for further relapse. Here, we presented our experiences with MMD HSCT for patients with VHRR and HRR who were uniformly pretreated with BFM/I-BFM frontline protocols and a harmonized transplant procedure. These findings showed a good outcome for CR1 + CR2 and HRR patients. Nevertheless, there was a high rate of treatment failure for patients beyond CR2 or patients with VHRR, and the source of mismatched stem cells did not influence this outcome.

For VHRR patients, the introduction of new agents and techniques, such as bispecific antibodies or chimeric antigen receptor T cells, may represent progress in decreasing relapse rates and NRM if they allow HSCT to be performed at an earlier stage [24-28]. The optimal timing for using these new tools, before or after transplantation, for positive MRD and before overt relapse remains undefined. Obtaining significantly less transplant-associated early and late toxicity is also desired.

Thus, we avoided TBI-based conditioning regimen for patients younger than age 2 years with MMDs. To further investigate the possibility of TBI-free conditioning regimen, we initiated a prospective randomized trial (Eudract no. 2012-003032-22, see also www.clinicaltrials.gov) to evaluate the outcome after TBI- versus chemotherapy-based conditioning regimens in patients over 4 years of age with ALL and MSDs or MUDs. All other patients (younger than 4 years and those with only MMDs, including T cell–replete post-transplant cyclophosphamide haploidentical HSCT patients) receive TBI-free conditioning regimens.

In the present study we demonstrated the feasibility of allogeneic HSCT from alternative donors in both HRR and VHRR pediatric patients with ALL in CR after myeloablative conditioning. Our findings show good outcomes for HRR patients in CR1 or CR2. These results demonstrate the feasibility of using MMDs in HRR patients with acceptable results. In HHR patients, MMDs seem to offer the same chance of success as using better HLA-matched donors. However, further progress is needed to decrease overall treatment failure (ie, both relapse rate and treatment-related mortality) in VHRR patients.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.05.009.

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