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Transplantation in Children and Adolescents with Acute Lymphoblastic Leukemia from a Matched Donor versus an HLA-Identical Sibling: Is the Outcome Comparable? Results from the International BFM ALL SCT 2007 Study

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A B S T R A C T

Eligibility criteria for hematopoietic stem cell transplantation (HSCT) in acute lymphoblastic leukemia (ALL) vary according to disease characteristics, response to treatment, and type of available donor. As the risk profile of the patient worsens, a wider degree of HLA mismatching is considered acceptable. A total of 138 children and adolescents who underwent HSCT from HLA-identical sibling donors (MSDs) and 210 who underwent HSCT from matched donors (MDs) (median age, 9 years; 68% male) in 10 countries were enrolled in the International-BFM ALL SCT 2007 prospective study to assess the impact of donor type in HSCT for pediatric ALL.

The 4-year event-free survival (65 ± 5% vs 61 ± 4%; $P = .287$), overall survival (72 ± 4% versus 68 ± 4%; $P = .235$), cumulative incidence of relapse (24 ± 4% versus 25 ± 3%; $P = .658$) and nonrelapse mortality (10 ± 3% versus 14 ± 3%; $P = .212$) were not significantly different between MSD and MD graft recipients. The risk of extensive chronic (cGVHD) was lower in MD graft recipients than in MSD graft recipients (hazard ratio [HR], .38; $P = .002$), and the risks of severe acute GVHD (aGVHD) and cGVHD were higher in peripheral blood stem cell graft recipients than in bone marrow graft recipients (HR, 2.06; $P = .026$). Compared with the absence of aGVHD, grade I-II aGVHD was associated with a lower risk of graft failure (HR, .63; $P = .042$) and grade III-IV aGVHD was associated with a higher risk of graft failure (HR, 1.85; $P = .020$) and nonleukemic death (HR, 8.76; $P < .0001$), despite a lower risk of relapse (HR, .32; $P = .021$). Compared with the absence of cGVHD, extensive cGVHD was associated with a higher risk of nonleukemic death (HR, 8.12; $P < .0001$).

Because the outcomes of transplantation from a matched donor were not inferior to those of transplantation from an HLA-identical sibling, eligibility criteria for transplantation might be reviewed in pediatric ALL and possibly in other malignancies as well. Bone marrow should be the preferred stem cell source, and the addition of MTX should be considered in MSD graft recipients.

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INTRODUCTION

Chemotherapy can potentially cure >80% of the children affected with acute lymphoblastic leukemia (ALL). Currently, allogeneic hematopoietic stem cell transplantation (HSCT) is considered beneficial as the frontline treatment for <10% of pediatric patients presenting with very high-risk features, and for the majority of these patients in case of relapse [1–6].

Eligibility criteria for transplantation in ALL have varied over time, depending on the balance between the outcome of frontline and relapse chemotherapy protocols and the outcomes of transplantation, which depend mainly on the degree of compatibility within each donor-recipient pair [5,7–9]. Some patients are considered eligible for transplantation only if an HLA-identical sibling is available; as the risk profile of the patient worsens, a wider degree of HLA mismatching is considered acceptable [6–10].

According to the prospective multicenter BFM ALL SCT 2003 Study performed in Austria, Germany, and Switzerland, the outcomes of transplantation from unrelated donors were not inferior to the outcomes of transplantation from HLA-identical siblings in terms of event-free survival (EFS), overall survival (OS), and cumulative incidence of relapse (CIR), with higher nonrelapse mortality (NRM) for the former than for the latter [7].

The roles of donor type and compatibility in a broader network of countries adopting different frontline and relapse protocols in pediatric ALL have yet to be assessed.

In 2007, an international prospective study was started within the International BFM Study Group (I-BFM-SG) with the aim of assessing the impact of donor type on the outcomes of transplantation in pediatric ALL (EudraCT No. 2005-005106-23). The primary endpoint was EFS of recipients of HLA-identical siblings versus matched donor grafts. Secondary endpoints were the assessment of incidence and risk factors for OS, CIR, and NRM, as well as engraftment and acute and chronic graft-versus-host disease (GVHD) and their impact on outcome. An additional purpose of the study was to harmonize HSCT for ALL throughout Europe and create a platform for further investigations in clinical trials.

METHODS

Inclusion Criteria

All consecutive patients age ≤18 years at the time of ALL diagnosis or relapse undergoing first allogeneic HSCT in complete remission (CR), as previously defined, in the participating countries, were eligible for the study, as long as their parents or guardians provided signed informed consent [1]. Only patients with a very high-risk profile were considered eligible for transplantation; eligibility criteria for transplantation were established according to national frontline and relapse protocols [7]. The study was approved by the Ethical Committees in Vienna and in each participating center.

Donor Type

HLA compatibility with the donor, either related or unrelated, was defined by high-resolution typing for HLA-A, -B, -C, -DR, -DQ alleles [10]. As shown in Table 1, donors were defined as matched sibling donors (MSDs) if they were HLA-identical siblings who had inherited the same parental haplotypes as their recipients. Regardless of their relationship, donors were defined as HLA-matched (MDs) if they were fully matched (10/10) or had a single allelic or antigenic disparity (9/10) and as HLA-mismatched (MMDs) if they had 2 (8/10) or more allelic or antigenic disparities, up to a different haplotype [7]. Therefore, even though the vast majority of MDs were unrelated, both MDs and MMDs could be either related or unrelated donors. Only patients undergoing transplantation from MSDs or MDs were considered in this study; recipients of MMD grafts have been described elsewhere [11].

Risk Stratification

For the purpose of this analysis, patients were stratified according to BFM eligibility criteria for transplantation, as described previously, and national criteria [7]. Patients who were eligible for MSD transplant only were defined as standard risk, those eligible also for MD transplant were defined as high risk, and those eligible also for MMD transplant were defined as very high risk (Table 1) [7].

Over the last decade, indications for transplantation within BFM-oriented protocols were tailored mainly according to minimal residual disease (MRD) response to treatment during both frontline and relapse protocols. Eligibility criteria for transplantation are listed in Supplementary Tables S1 and S2.

In brief, the standard risk group included mixed-lineage leukemia (MLL) rearranged patients with good prednisone response in CR1 and patients with late relapsed B-immunophenotype ALL with MRD $<10^3$ after the first 6 weeks of chemotherapy after relapse.

The high risk group included MLL rearranged patients with poor prednisone response, BCR/ABL positive patients with good response but MRD positivity at day +33, all patients with MRD $>5 \times 10^{-4}$ but $<5 \times 10^{-3}$at day +78 in CR1, and patients with early combined or late isolated medullary relapsed B-lineage ALL with MRD $>10^{-3}$ after the first 6 weeks of chemotherapy.

Among patients in CR1, the very high risk group included patients experiencing induction failure at the end of the first induction element (no CR at day +33), BCR/ABL-positive patients with poor prednisone response, and all patients with MRD $>5 \times 10^3$–3 at the end of the second element of induction at day +78. Among patients in CR2, the very high risk group included patients with any T-lineage medullary involvement at relapse, B-lineage very early medullary or early isolated medullary involvement at relapse, and all patients achieving CR3 after second relapse.

The cutoff for defining early or late relapse was 6 months after elective discontinuation of chemotherapy, that is, approximately 30 months after initial diagnosis.

Stem Cell Source

Bone marrow (BM) was the recommended stem cell source by protocol, particularly for MSD, but the transplantation center and, in cases of MDs, the donor center, were responsible for graft selection. Granulocyte colony-stimulating factor-primed peripheral blood (PB) was also acceptable, according to transplantation or donor center preference. Target doses of $>3 \times 10^8$/kg recipient body weight of nucleated cells (NCs) and $>1.5 \times 10^6$/kg of $CD34^+$ cells were recommended. Cord blood (CB) was also acceptable and was classified as MD in cases of at least "5/6" and "6/6" compatibility with the recipient, as defined by "historical" CB typing, which is low-resolution at class I A and B loci and high-resolution at the class II HLA-DRB1 locus [10,11] (Table 1).

Transplantation Procedure

According to the protocol, the conditioning regimen was based on hyperfractionated total body irradiation (TBI; total dose 12 Gy, given as 2 Gy b.i.d. on days -7, -6, and -5) associated with etoposide (VP16; 60 mg/kg on day -4; maximum, 3600 mg) in patients age >2 years and on busulfan (BU; by body weight-adjusted dose orally or i.v. or with dose monitoring and adjustment every 6 hours according to levels on days −7 through -4, for a total of 16 doses), associated with cyclophosphamide (CY; 60 mg/kg/dose on days -3 and -2) and etoposide (VP16; 40 mg/kg on day -1), in patients age ≤2 years. BU and CY plus melphalan (MEL; 140 mg/sm single dose on day -1) was planned for patients carrying the t(4;11) translocation, irrespective of age [7,12,13]. Owing to their immature clonal phenotype, patients with MLL rearrangement were considered eligible for an acute myelogenous leukemia-oriented conditioning regimen, for which good results had already been reported in an infant-like population affected with juvenile myelomonocytic leukemia [13]. The same BU-CY-VP conditioning regimen, planned for patients age ≤2 years or younger, had been recommended in the "infant" (patients <1 year) ALL chemotherapy trial Interfant 99 and BU-CY-MEL in the Interfant 06 trial for patients eligible for HSCT [12]. The overlap between this study and the 2 subsequent infant chemotherapy trials, which had been activated with different timings across centers, and between MLL and age explains why some youngest patients received BU-CY-MEL and others received BU-CY-VP despite MLL rearrangements.

Unfortunately, data regarding the use of the therapeutic drug monitoring-guided dosing of busulfan (TDM) and its modality (target range) have not been collected and could not be addressed by this study.

GVHD prophylaxis consisted of cyclosporine A (CSA) at a dose of 3 mg/kg/day in all patients, associated with "3-dose" methotrexate (MTX; 10 mg/sm/dose on days +1, +3, and +6, followed by leucovorin rescue 24 h later) in PB transplants from MSD and "4-dose" MTX (10 mg/sm/dose on days +1, +3, +6, and +11, followed by leucovorin rescue 24 h later) and antithymocyte globulin (ATG, Fresenius; 20 mg/kg/dose on days -3, -2, and 1) in HSCT from MDs. MTX was substituted for steroids in CB recipients [7].

After an interim analysis of the previous "BFM-only" ALL SCT 2003 had reported that aGVHD incidence and severity were unacceptably high after PBSCT, which had been performed more extensively than expected in the MSD setting, the steering committee had approved an amendment implementing MTX for MSD recipients when the stem cell source was PB [7]. The present study benefited of this amendment from the very first month of study initiation.

No graft manipulation was planned, except for ABO incompatibility.

Myeloid engraftment was considered to occur on the first of 3 consecutive days with an absolute neutrophil count (ANC) $>0.5 \times 10^9$/L. Platelet engraftment was considered to occur on the first of 5 consecutive days with a platelet count $>50 \times 10^9$/L, sustained without transfusion support.

Table 1
Definition of donor type and risk profile. HLA-identical siblings are defined matched sibling donors (MSDs) and all other matched (10 out of 10 and 9 out of 10 matched) donors, either related or unrelated, are defined MDs; donors with any lower degree of compatibility are defined mismatched donors (MMDs) and are not included in this report.
*Table displays donor type for bone marrow and peripheral blood stem cell grafts. Umbilical cord blood grafts are allocated differently, according to the historical compatibility definition: HLA-identical siblings are allocated to the MSD arm, 5/6 and 6/6 HLA matched (antigenic resolution typing for A and B loci and allelic for the DRB1 locus) to the MD arm, <5/6 matched are not included in this report.

Donor type

HLA compatibility *	HLA-identical sibling	Other family donor	Unrelated donor
10 out of 10	MSD	MD	MD
9 out of 10	-	MD	MD
less than 9/10	-	MMD	MMD

* Table displays donor type for bone marrow and peripheral blood stem cell grafts.
Umbilical cord blood grafts are allocated differently, according to the historical compatibility definition:
MSD: HLA-identical sibling
MD: 5/6 or 6/6 matching (antigenic resolution typing for A and B loci and allelic for the DRB1 locus)
MMD: less than 5/6 matching

Legenda
MSD: matched sibling donors (includes HLA identical sibling donors only)
MD: matched donors (includes related and unrelated donors)
MMD: mismatched donors (includes donors 8/10 HLA compatible or less, either related, as haploidentical, or unrelated)

Risk profile
SR: patients eligible for transplantation from MSD only
HR: patients eligible also for transplantation also from MD
VHR: patients eligible for transplantation also from MMD

Acute and chronic GVHD were graded as described previously [14,15]. Patients alive and in remission 100 days after HSCT were considered at risk for chronic GVHD.

MRD Analysis

Monitoring of MRD and chimerism before and after transplantation was potentially available for clinical decisions but was not part of this investigational protocol [16–19]. There is no reason to assume that MRD levels at the time of transplantation were differently distributed between the 2 groups.

Statistical Analysis

EFS was the primary endpoint. The null hypothesis was that SCT from MD was inferior to SCT from MSD.

Results of descriptive analyses were reported as median and range. Kaplan-Meier estimators, with their Greenwood standard errors (SE), were used to estimate EFS and OS, and the log-rank test was used for comparisons. EFS time was defined as the time from transplantation until relapse, second neoplasm or death, whichever occurred first, and OS time was defined as the time from transplantation until death due to any cause. Patients were censored at the time of last follow-up in case no event had occurred.

To assess whether there was any difference in EFS between MSD and MD HSCT, the cumulative incidence approach was used, with a 1-sided cumulative incidence for the difference of the Kaplan-Meier estimate of the 4-year EFS. With the initially planned sample of 315 patients, 80% power could be achieved to show that MD SCT was not inferior if a lower limit of the cumulative incidence of 16% was used as the margin.

Secondary endpoints besides OS included CIR, NRM, incidence of engraftment, incidence of aGVHD, and cumulative incidence of cGVHD. These endpoints were estimated using the approach of Prentice and Kalbfleisch, allowing for competing risks, which were death in remission and relapse for engraftment and cGVHD, death in remission for CIR, relapse for NRM, plus secondary malignancy for all endpoints [20]. Comparisons were made according to Gray [21].

Only patients who could be defined as high risk and very high risk, according to the BFM stratification—namely, only those who would have been eligible also for MD or MMD transplantation—were considered for assessment of the study's primary and secondary endpoints. Patients classified as standard risk—namely, those who would have been eligible for MSD transplant only and should not have been allocated to the MD cohort per protocol— were excluded from all univariate analyses but were entered into multivariable analyses, which were adjusted by risk profile.

The roles of donor type and HLA compatibility (ie, MD versus MSD) on EFS and OS were investigated using a Cox proportional hazards model after adjusting for risk stratum (standard risk, high risk, or very high risk), remission phase (CR1, CR2 after late relapse, CR2 after early relapse, or CR3), stem cell source (BM, PB, or CB), recipient age (0 to ≤2, 2 to ≤12, or >12 years), use of TBI (yes or no), and cytomegalovirus (CMV) serologic status (negative donor to positive recipient or other). The proportional subdistribution hazards model of Fine and Gray for censored data subject to competing risks was applied for the analysis of the probability of relapse (CIR) and non-leukemic death (NRM), adjusted for the aforementioned risk factors [22].

Landmark analyses were performed to assess the risk of GVHD. Complete, not censored, data (up to day +100) were used for aGVHD assessment, with percentages for univariate analyses and a logistic regression model estimating the odds ratio (OR) of aGVHD for multivariate analyses. The cumulative incidence of cGVHD was estimated using the approach of Prentice and Kalbfleisch [20], allowing for the competing risks of death in remission, relapse, and secondary malignancy; comparisons were made using the test of Gray [21]. A Fine and Gray model was used to estimate the hazard ratio (HR) of cGVHD, adjusting for donor type as well as risk profile, stem cell source, remission phase, recipient age, and TBI [22].

The impact of GVHD on outcome was assessed separately by means of Cox (for OS and EFS) or Fine and Gray (for CIR and NRM) models, including cGVHD as a time-dependent covariate, after adjustment for the aforementioned variables [22].

Countries were grouped into 3 strata according to their geographical distribution to allow for a multivariate analysis to assess the country effect on the main study endpoint—the comparison of MSD and MD transplantation—as well as on aGVHD and cGVHD occurrence and the risk of relapse or the probability of EFS and OS.

For non-time-to-event variables, the χ^2 test or, where appropriate, Fisher's exact test were used to compare groups for categorical variables, and the

Table 2
Accrual by country according to type of donor.

Country	N of patients N	%	MSD N	%	MD N	%
	348		137		211	
Czech Republic	29	*8*	4	*3*	25	*12*
Denmark	18	*5*	3	*2*	15	*7*
France	51	*15*	24	*18*	27	*13*
Israel	13	*4*	6	*4*	7	*3*
Italy	17	*5*	4	*3*	13	*6*
The Netherlands	40	*11*	20	*15*	20	*9*
Poland	116	*33*	42	*31*	74	*35*
Sweden	5	*1*	1	*1*	4	*2*
Slovakia	16	*5*	5	*4*	11	*5*
Turkey	43	*12*	28	*20*	15	*7*

Percentages are shown in italics.
MSD: matched sibling; MD: matched donor.

Wilcoxon rank-sum test (Kruskal-Wallis test for more than 2 populations) was used for continuous variables.

All *P*values <.05 were considered significant.

Patient data were collected in each institution and uploaded in digital case report forms. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Enrollment

The ALL SCT International BFM 2007 Study was carried out in 22 transplantation centers in 10 countries: Czech Republic, Denmark, France, Israel, Italy, The Netherlands, Poland, Slovakia, Sweden, and Turkey (Table 2). Patient accrual started on January 16, 2007, on each local Ethical Committee (EC) approval, and continued up to December 31, 2013 (Figure 1). In brief, of the 438 consecutive children and adolescents with ALL who were registered, 410 met the eligibility criteria for the ALL SCT 2007 Study, of whom 348 (85%) underwent transplantation with either an MSD (n = 138) or an MD (n = 210) graft and were included in this study, whereas the 62 who underwent transplantation from an MMD have been described elsewhere. The median duration of follow-up overall was 4 years and 7 months (range, .2 to 8.4 years).

Patient Characteristics

Characteristics of the patients (median age, 9 years; 68% male) are summarized in Table 3. Among the >99% of the patients with a known immunophenotype, 70% were B lineage, 25% were T lineage, and 5% were either biclonal or mixed lineage. In terms of clonal abnormalities, 15% of the patients were known to carry BCR/ABL rearrangements and 11% had MLL rearrangements, but this information was missing for 38% of the patients.

Disease Phase

Among the 348 patients, 163 (47%) underwent transplantation in CR1, at a median of 8 months after diagnosis (range, 4 to 19 months), and 164 (44%) underwent transplantation in CR2, at a median of 5 months (range, 2 to 18 months) after relapse. Thus, 20% of the patients in CR2 had undergone transplantation after an early relapse (i.e., occurring <30 months after the initial diagnosis), and 24% did so after a late relapse. Twenty-one patients (6%) underwent transplantation in CR3 at a median of 5 months (range, 2 to 16 months) after a second relapse. These proportions were similar in MSD and MD graft recipients.

The distributions of age, sex, immunophenotype, and disease phase did not differ between MSD and MD graft recipients.

Risk Stratification

Prognostic criteria for HSCT were known for all but 2 patients. As shown in Table 3, 33 patients (10%)—namely,

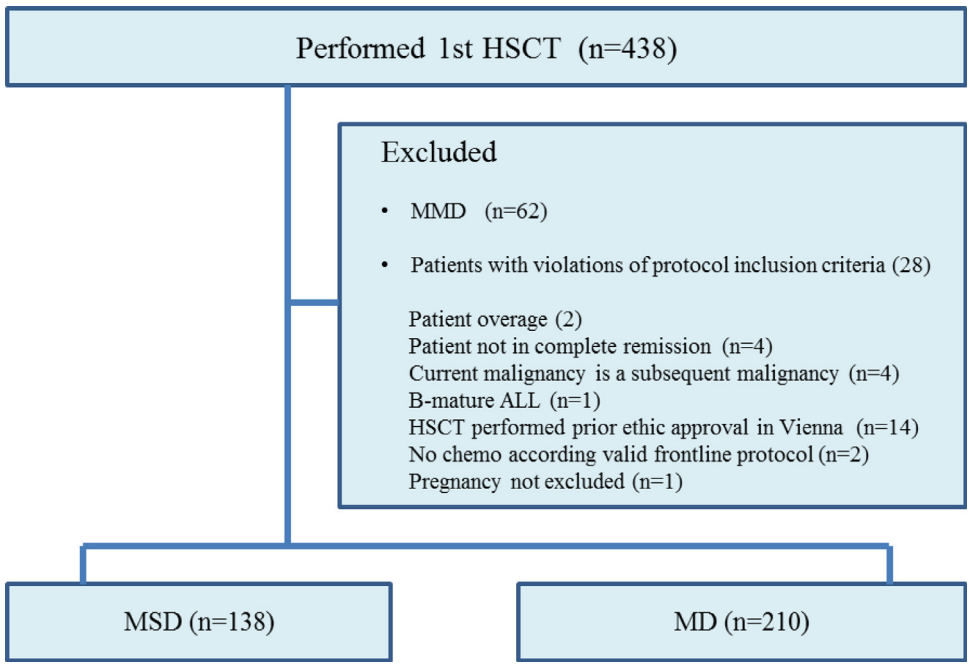

Figure 1. Patient accrual within the International BFM ALL SCT 2007 Study. Out of the 438 registered patients, 348 children and adolescents who underwent HSCT from either an MSD (n = 138) or an HLA 10/10 or 9/10 MD (n = 210) were analyzed.
MMD: mismatched donors, ALL: acute lymphoblastic leukemia, HCST:hematopoietic stem cell transplantation, MSD: matched sibling donor, MD: matched donor.

Table 3
Characteristics of the patients overall and by type of donor, matched sibling (MSD) and matched donor (MD). Percentages are shown in italics. P-values for the distribution of each characteristic between the two groups are provided.

			total	MSD	MD	*p-value*
Total			348 %	138 %	210 %	
Risk profile	standard risk		33 *10%*	17 *12%*	16 *8%*	*0,1731*
	high risk		147 *42%*	62 *45%*	85 *41%*	
	very high risk		166 *48%*	59 *43%*	107 *51%*	
	unknown		2	0	2	
Patient age at transplant		median	9,6	9,6	9,8	*0,356*
		range	(0.6-19.0)	(0.6-19.0)	(0.6-19.0)	
	≤2 years		14 *4%*	5 *4%*	9 *4%*	*0,813*
	>2, ≤12 years		218 *63%*	84 *61%*	134 *64%*	
	>12 years		116 *33%*	49 *36%*	67 *32%*	
Immunophenotype	B-lineage		242 *70%*	94 *69%*	148 *71%*	*0,2694*
	T-lineage		88 *25%*	39 *28%*	49 *23%*	
	other		17 *5%*	4 *3%*	13 *6%*	
	unknown		1	1		
Clonal abnormalities	BCR/ABL		33 *15%*	11 *14%*	22 *16%*	*0,609*
	MLL/AF4		24 *11%*	11 *14%*	13 *10%*	
	both neg		158 *73%*	58 *73%*	100 *74%*	
	unknown		133	58	75	
Remission phase	CR1		163 *47%*	66 *48%*	97 *46%*	*0,321*
	CR2 late		70 *20%*	28 *20%*	42 *20%*	
	CR2 early		82 *24%*	29 *21%*	53 *25%*	
	CR>2		21 *6%*	7 *5%*	14 *7%*	
	unknown		12	8	4	
time diagnosis - SCT		median	7,6	7,4	7,6	*0,328*
for CR1 patients (months)		range	(3.7-19.0)	(3.7-15.4)	(4.6-19.0)	
time diagnosis - relapse		median	28,1	27,8	28,2	
for CR2 patients (months)		range	(1.4-112.2)	(1,4-96.1)	(3.2-112.2)	
time relapse - SCT		median	5,2	5,2	5,1	*0,572*
for CR2 patients (months)		range	(2.2-17.6)	(2.2-13.0)	(2.7-17.6)	
time 2nd relapse - SCT		median	5,1	3,0	6,0	*0,050*
for CR3 patients (months)		range	(2.2-12.6)	(2.2-6.0)	(2.9-12.6)	
Stem cell source	bone marrow		220 *63%*	114 *83%*	106 *51%*	*<.0001*
	peripheral blood		112 *32%*	21 *15%*	91 *43%*	
	cord blood		16 *5%*	3 *2%*	13 *6%*	
TBI	overall	yes	271 *78%*	106 *77%*	165 *79%*	*0,601*
		no	77 *22%*	32 *23%*	45 *21%*	
	>2 years	yes	270 *81%*	106 *80%*	164 *82%*	
		no *	64 *19%*	27 *20%*	37 *18%*	
	≤2 years	no	13 *93%*	5 *100%*	8 *89%*	
		yes **	1 *7%*	0 *0%*	1 *11%*	
Donor/recipient gender	female/male		89 *26%*	45 *33%*	44 *21%*	*0,017*
male = M, female = F	others		212 *61%*	93 *67%*	166 *79%*	
Donor age		median	22,3	11,9	31,1	*<.0001*
		range	(0-50)	(0-44)	(2-50)	
	MSD <18 years			105 *76%*		
	≥18 years			33 *24%*		
	MD <35 years				129 *63%*	
	≥35 years				69 *35%*	
	unknown			12	12	
Donor/recipient CMV	negative/positive		96 *29%*	28 *22%*	68 *33%*	*0,0216*
IgG serological status	others		237 *71%*	102 *78%*	135 *67%*	
	unknown		15	8	7	

* deviation from the planned conditioning
** deviation from the planned conditioning except for the 10 MSD and 10 MD recipients with MLL rearrangement

12% of those grafted from an MSD and 8% of those grafted from an MD—were at standard risk of relapse and would have been eligible for MSD HSCT only; 147 patients were at high risk and would have been eligible for MD HSCT as well, and 166 were at very high risk and would have been eligible for MMD SCT as well. Therefore, 313 patients, 121 of whom underwent transplantation from an MSD and 192 who did so from an MD, were included in univariate analyses, as mentioned above, whereas all 348 patients were included in the multivariate analyses, which were adjusted for disease risk per se.

Donor and Donor-Recipient Pair Characteristics

The donor-recipient sex match was female-to-male in 33% of the MSD transplantation pairs and 21% of the MD pairs and other combinations in the remaining 67% and 79% of cases, respectively ($P = .017$). The median donor age was 12 years (range, 6 months to 44 years) for MSDs and 31 years (range, 2 to 50 years) for MDs ($P < .0001$).

In terms of donor-recipient relationships, all 138 MSDs were siblings by definition, with 3 donors and recipients monozygotic twins. Among the MDs, 96% were unrelated, 8 were parents, and 1 was a sibling phenotypically compatible with his recipient.

In terms of CMV IgG serologic status, the combination of CMV-negative donor and CMV-positive recipient was seen in 22% of the MSD and 33% of the MD donor-recipient pairs ($P = .0216$) in whom CMV serostatus was known (96%).

Transplantation Procedure

The conditioning regimen was BU-based and irradiation-free, according to the protocol, in all 14 patients age ≤2 years except 1, who underwent TBI, and in all the MLL- rearranged patients. Among the patients age ≥2 years not carrying the MLL rearrangement, 88% of the MSD graft recipients and 86% of the MD graft recipients received TBI, whereas the remaining 12% of the MSD graft recipients and 14% of the MD graft recipients deviated from the planned TBI-based conditioning and received chemoconditioning, BU-based in all of the former and in 39% of the latter. Overall, 11% of the patients deviated from the planned TBI protocol, which was given to 77% of the MSD graft recipients and 79% of the MD graft recipients ($P = .601$).

For GVHD prophylaxis, all MSD graft recipients were given CSA only, except for 10 of 21 who received PB grafts, in accordance with protocol, and 13 of 114 who received BM grafts, deviating from the protocol, who also received MTX. All MD graft recipients received MTX and serotherapy, in accordance with the protocol, except for 10 and 24 patients for whom MTX and ATG use were not reported, respectively. Serotherapy consisted of ATG-Fresenius (Neovii, Rapperswil, Switzerland) in all but the Italian patients, who received Thymoglobulin (Sanofi-Genzyme, Cambridge, MA), 2.5 mg/kg/dose on days -3, -2, and -1. The 13 CB recipients received prednisolone instead of MTX, in accordance with the protocol.

Stem Cell Source

The stem cell source was BM in 83%, PB in 15%, and CB in 2% of the 138 MSD graft recipients and BM in 51%, PB in 43%, and CB in 6% of the MD graft recipients ($P < .0001$).

Engraftment

All patients achieved an absolute neutrophil count $>.5 \times 10^9$/L at a median of 19 days (range, 6 to 78 days), but 3 patients experienced early death, and 2 patients failed to engraft. Engraftment details are reported in Table 4. The cumulative incidence of myeloid engraftment at 30 days was 92% (± standard error [SE] 3) for MSD and 86 ± 2% for MD recipients ($P < .0001$). By stem cell source (Supplementary Table S3), the cumulative incidence of engraftment at 30 days among MSD graft recipients was 93 ± 3% for BM and 90 ± 7% for PB recipients ($P = .31$), and that among MD graft recipients was 80 ± 4% for BM recipients and 98 ± 2% for PB recipients ($P < .0001$).

The cumulative incidence of achieving a platelet count of $\geq 50 \times 10^9$/L was 56 ± 5% for MSD graft recipients and 41 ± 4% for MD graft recipients at 30 days and 88 ± 3% for MSD graft recipients and 75 ± 3% for MD graft recipients at 100 days ($P < .0001$) (Table 4).

aGVHD

aGVHD occurred in 68% of the MSD graft recipients and in 65% of the MD graft recipients at a median of 17 and 19 days after transplantation, respectively, with data missing in 12 patients. Grade III-IV aGVHD occurred in 15% of the MSD recipients and in 15% of the MD recipients ($P = .984$) (Table 4).

By donor type and stem cell source, 14% of the MSD BM graft recipients and 10% of the MD BM graft recipients ($P = .358$) and 20% of the MSD PB graft recipients and 23% of the MD PB recipients ($P = .768$) experienced grade III-IV aGVHD (Supplementary Table S4). There was only a trend toward a higher risk of grade III-IV aGVHD in PB compared with BM in MD graft recipients ($P = .103$).

In the subgroup of MSD BM recipients, only 1 of the 10 patients who deviated from the protocol and were given additional MTX for GVHD prophylaxis experienced grade I-IV aGVHD, compared with 42% of those who did not receive additional MTX.

Table 4
Incidence and cumulative incidence of engraftment and acute and chronic GVHD overall and by type of donor.
The 313 patients at high (147) or very high (166) risk of relapse were included for the purpose of this univariate analysis to allow comparability between matched sibling (MSD) and matched (MD) donor recipients (standard-risk patients would not have been eligible for MD transplantation). Percentages are shown in italics. P-values for the distribution of each characteristic between the two groups are provided.

			Total (N=313)	MSD (N=121)			MD (N=192)			
			Nr. of events	Nr. of events	%	30-day CI	Nr. of events	%	30-day CI	*p-value*
Engraftment	Absolute neutrophil count >0.5x10⁹/L	N	306	118		0.92±0.03	188		0.86±0.03	*<0.001*
	graft failure	N	1	0			1			
	early death with no engraftment	N	3	1			2			
	unknown	N	3	2			1			
	Platelets>50x10⁹/L	N	272	114		0.56±0.05	158		0.41±0.04	*<0.001*
	graft failure	N	12	0			12			
	early death with no engraftment	N	21	3			18			
	unknown		8	4			4			
Acute GVHD	0	N	102	38	*32%*		64	*35%*		*0,508*
	1	N	68	35	*29%*		33	*18%*		*0,027*
	2	N	77	27	*23%*		50	*28%*		*0,319*
	3	N	32	14	*12%*		18	*10%*		*0,636*
	4	N	13	4	*3%*		9	*5%*		*0,494*
	Grade 3 or 4		45	18	*15%*		27	*15%*		*0,984*
	early death without aGVHD	N	9	2			7			
	missing data	N	12	1			11			
		N	193	79			114			
	aGVHD time of onset (days after transplant)	Median	19	17			19			
		Min	5	6			5			
Chronic GVHD	Patients at risk	N	269	108		2-years CI	161		2-years CI	
	Limited+extensive	N	80	41		0.37±0.05	39		0.25±0.03	*0,021*
	Limited	N	33	13		0.13±0.03	20		0.13±0.03	*0,947*
	Extensive	N	47	28		0.26±0.04	19		0.12±0.03	*0,005*
	death without cGVHD	N	55	18		0.18±0.04	37		0.23±0.03	*0,201*
		N	77	39			38			
	cGVHD time of onset (days after transplant)	Median	143	170			138			

Table 5
Risk of aGVHD. An OR as an estimate of the relative risk assesses the impact of each variable on the risk of developing severe (grade III-IV) aGVHD as a result of a logistic regression (multivariate analysis).

		Odds ratio *	95% Confidence Limits Lower	Upper	*p-value*
Donor type	MD vs MSD	0,63	0,33	1,20	*0,159*
Risk profile	high risk vs standard risk	0,14	0,05	0,41	*0,0003*
	very high risk versus standard risk	0,32	0,11	0,93	*0,668*
Remission phase	CR2 early relapse + CR3 vs CR1	0,56	0,26	1,21	*0,786*
	CR2 late relapse vs CR1	0,39	0,15	1,04	*0,190*
Stem cell source	cord blood vs bone marrow	0,25	0,01	4,42	*0,211*
	peripheral blood vs bone marrow	2,36	1,22	4,59	*0,045*
TBI	no TBI vs TBI	0,85	0,40	1,77	*0,657*
Age of patient	age > 12 years vs ≤ 12 years	0,85	0,44	1,62	*0,614*

* An odds ratio is provided to assess the impact of each risk factors for aGVHD estimates are calculated with the logit analysis:

According to the multivariate analysis, as shown in Table 5, there was only a trend toward a higher risk of grade III-IV aGVHD in MSD graft recipients compared with MD graft recipients (OR, 0.63, 95% confidence interval [CI], 0.33 to 1.20; $P = .120$). Furthermore, the risk of grade III-IV aGVHD was significantly associated with disease risk and stem cell source, being more than double in PB recipients compared with BM recipients (OR, 2.36, 95% CI, 1.22 to 4.59; $P = .045$). The associations between recipient age >12 years and disease recurrence with the risk of severe aGVHD were not significant in our series.

cGVHD

cGVHD data are reported in Table 4. The 2-year cumulative incidence of any cGVHD was 37 ± 5% for MSD graft recipients and 25 ± 3% for MD graft recipients ($P = .021$), and that of extensive cGVHD was 26 ± 4% for MSD graft recipients and 12 ± 3% for MD graft recipients ($P = .005$) (Figure 2A).

By stem cell source, within MSD graft recipients at risk, the 2-year cumulative incidence of extensive cGVHD was 22 ± 4% in MSD BM graft recipients and 11 ± 3% in MD BM graft recipients ($P = .079$), compared with 50 ± 12% in MSD PB graft recipients and 13 ± 4% in MD PB graft recipients ($P = .001$) (Figure 2B and C). The cumulative incidence of cGVHD was also significantly higher in MSD PB graft recipients compared with MSD BM graft recipient ($P = .011$) (Supplementary Table S4).

The 2-year cumulative incidence of extensive cGVHD according to recipient age ≤2 years, 2 to ≤12 years, and >12 years was 0, 14 ± 4%, and 48 ± 8%, respectively, among MSD graft recipients ($P < .001$) and 0, 10 ± 3%, and 16 ± 6%, respectively, among MD graft recipients ($P = .423$) (Supplementary Figure S1A and B).

Among BM graft recipients, the 2-year cumulative incidence of extensive cGVHD was 14 ± 3% in patients age ≤12 years and 39 ± 9% in older patients for MSD recipients ($P = .007$) and 10 ± 4% and 14 ± 7%, respectively, for MD recipients ($P = .615$) (Supplementary Figure S1C and D).

Within the subgroup of MSD BM graft recipients, the difference in the 2-year cumulative incidence of extensive cGVHD between those who received CSA only in accordance with protocol (24 ± 5%) and those who deviated and received additional MTX (10 ± 9%) was not statistically significant ($P = .376$).

According to the multivariate analyses, as shown in Table 6, the risk of developing extensive cGVHD was significantly associated with donor type, being almost 3-fold lower in MD graft recipients than in MSD graft recipients (HR, 0.38; 95% CI, .21 to .71; $P = .002$). Furthermore, the stem cell source was associated with the risk of cGVHD, which was higher in the cohort of patients who received PB compared with those who received BM (HR, 2.06; 95% CI, 1.09 to 3.90; $P = .026$) and with recipient age, being more than double in patients age >12 years than in patients age ≤12 years (HR, 2.35; 95% CI, 1.36 to 4.08; $P = .002$).

Overall Outcome

The 4-year EFS of the cohort of 313 patients was 62 ± 3% and 4-year OS was 70 ± 3%. Of the 313 patients, 72 relapsed and 37 died in CR at a median of 5 months after HSCT (range, 2 days to 3.0 years). The outcomes overall and for subgroups of patients assessed by univariate analysis are shown in Supplementary Table S5. The main causes of death were infection (in 28 patients: 5 bacterial, 5 viral, 5 fungal, and 13 undefined) and liver failure (in 6 patients, including venous occlusive disease in 3). All but 1 nonleukemic deaths occurred in patients who had experienced some degree of GVHD. Two cases of second malignancies (myelodysplasia and melanoma) were reported in year 4 and year 6 after transplantation, respectively, with the first one occurring after relapse.

Outcomes by donor type are shown in Figure 3.

EFS

EFS at 4 years was 65 ± 5% for MSD graft recipients and 61 ± 4% graft recipients for MD ($P = .287$), as shown in Figure 3A. The upper limit of the 95% CI for the difference in 4-year EFS was 14%.

According to the risk profile, among the high risk patients, the 4-year EFS was 76 ± 6% for MSD graft recipients and 65 ± 5% for MD graft recipients ($P = .123$). Among the very high risk patients, these values were 53 ± 7% for the former and 57 ± 5% for the latter ($P = .869$).

In the multivariate analysis, no significant association could be detected between the risk of any failure (1-EFS) and the type of donor and compatibility, or with immunophenotype or stem cell source (Table 6). The probability of any failure was significantly associated with the remission phase, being higher for patients in CR3 or CR2 after early relapse compared with those in CR1 (HR, 1.81; 95% CI, 1.09 to 3.01; $P = .023$) but similar for patients in CR2 and those in CR1 after late relapse. Moreover, the risk of failure was associated with the lack of TBI in the conditioning, being lower for patients treated with TBI compared with those treated with chemoconditioning (HR, 2.17; 95% CI, 1.39 to 3.38; $P = .007$).

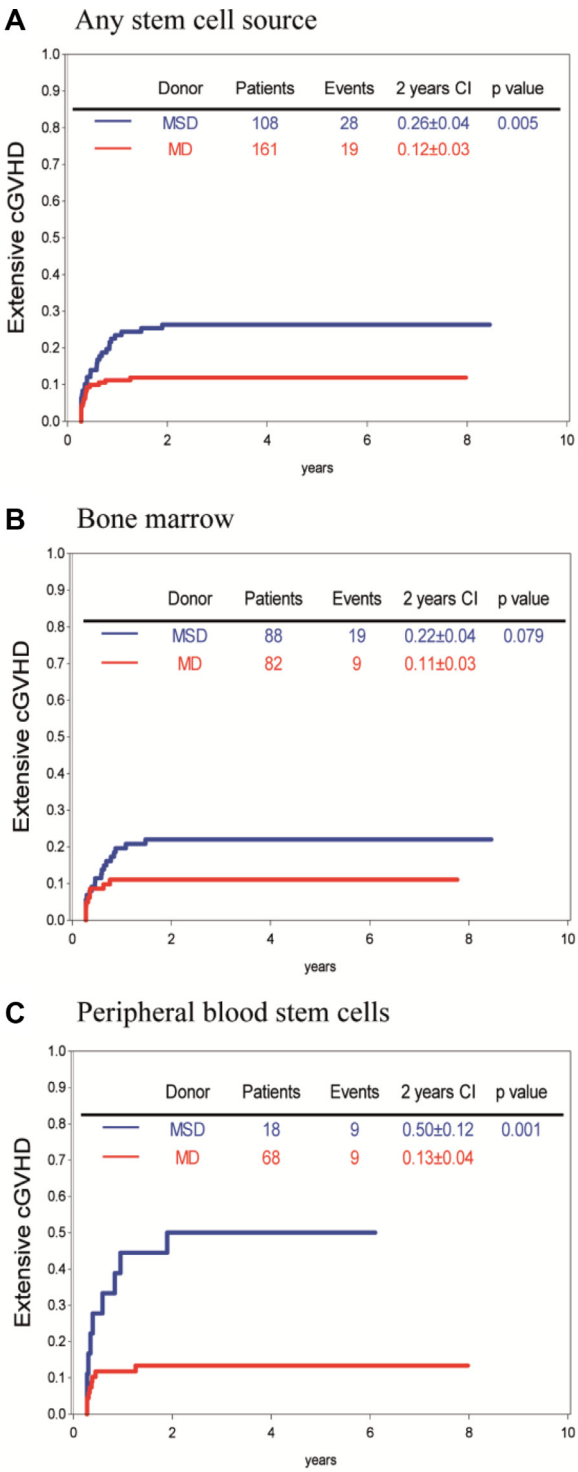

Figure 2. CI of extensive cGVHD for MSD (blue curve) and MD (red curve) graft recipients. Estimates at 2 years after transplantation with their standard errors are provided. The 269 patients evaluable for cGVHD among the 313 patients at high or very high risk are included in this univariate analysis to allow for the comparability between matched sibling and matched donor recipients. The cumulative incidence of cGVHD is shown by type of donor, overall (A), as well as for BM recipients (B) and PBSC recipients (C).

Patients transplanted from MSD had higher probability of extensive cGVHD, compared with those transplanted from MD; moreover the probability was higher in PB recipients.

OS

OS at 4 years was 72 ± 4% for MSD graft recipients and 68 ± 4% for MD graft recipients ($P = .235$) (Figure 3B). In the multivariate analysis, as reported in Table 6, no significant association was detected between the risk of death (1-OS) and donor type and compatibility, or with risk profile, remission phase, or stem cell source. The risk of death was higher in patients treated with TBI compared with those treated with chemoconditioning (HR, 2.06; 95% CI, 1.25 to 3.93; $P = .005$), in CMV-seropositive recipients from a seronegative donor compared with other combinations (HR, 1.63; 95% CI, 1.05 to 2.52; $P = .030$) and in patients age >12 years compared with those age 2 to ≤12 years (HR, 1.62; 95% CI, 1.04 to 2.53; $P = .034$), with only a trend toward a greater risk of failure in infant recipients compared with patients age 2 to 12 years (HR, 2.21; 95% CI, .92 to 5.29; $P = .075$).

CIR

CIR at 4 years was 24 ± 4% for MSD graft recipients and 25 ± 3% for MD graft recipients ($P = .658$) (Figure 3C). In the multivariate analysis, no significant association could be detected between the risk of relapse and the type of donor and compatibility, or with recipient age, stem cell source or CMV status (Table 6). The risk of relapse was higher in very high risk patients compared with standard risk patients (HR, 4.34; 95% CI, 1.11 to 16.97; $P = .035$), in patients in CR2 after early relapse and in patients in CR3 compared with those in CR1 (HR, 2.70; 95% CI, 1.35 to 5.40; $P = .005$), as well as in patients treated with chemoconditioning compared with those treated with TBI (HR, 2.26; 95% CI, 1.33 to 3.85; $P = .003$).

NRM

NRM at 4 years was 10 ± 3% for MSD graft recipients and 14 ± 3% for MD graft recipients ($P = .212$) (Figure 3D). In the multivariate analysis, no significant association could be detected between NRM and type of donor and compatibility, as well as with risk profile or remission phase (Table 6). The risk of nonleukemic death was almost 3-fold higher in patients age >12 years compared with those age ≤12 years (HR, 2.91; 95% CI, 1.50 to 5.65; $P = .001$). Furthermore, there was a trend toward higher NRM in CMV-seropositive recipients who underwent transplantation from a CMV-seronegative donor compared with other combinations (HR, 1.79; 95% CI, .91 to 3.55; $P = .093$) and for PB recipients compared with BM recipients (HR, 1.84; 95% CI, .89 to 3.81; $P = .099$).

Impact of GVHD on Outcome

The impact of GVHD on outcome was analyzed by multivariate analysis, after adjustment for donor type, risk profile, remission phase, stem cell source, CMV serostatus, patient age, and use of TBI (Table 7).

The occurrence of grade I-II aGVHD, compared with the absence of aGVHD, was associated with a lower risk of any failure (HR, .63; 95% CI, .40 to .98; $P = .042$). The occurrence of grade III-IV aGVHD compared with the absence of aGVHD was associated with a higher risk of any failure (HR, 1.85; 95% CI, 1.00 to 3.09; $P = .020$), death (HR, 2.35; 95% CI, 1.35 to 4.10; $P = .002$), and nonleukemic death (HR, 8.76; 95% CI, 3.65 to 21.05; $P < .0001$), despite a lower risk of relapse (HR, .32; 95% CI, .12 to .84; $P = .021$).

Table 6
Outcome overall and by donor type: results of the multivariate analysis. The hazard ratio (HR) associated with the listed features estimates the risk of any event (1 - event-free survival), death (1 - overall survival), relapse, and non-leukemic death of all patients, patients transplanted from HLA-identical siblings (MSD) and from matched donors (MD). All 348 patients were included in the multivariate analysis, which was adjusted for disease risk *per se*. If "1" is included in each confidence interval, differences in the risk of failure associated with the listed characteristics are not statistically significant, whereas HR<1 (>1) indicates that the former listed feature has a lower (greater) risk of failure compared with the latter feature (p-values <.05).
Legenda. MSD: matched sibling; MD matched donor; CR: complete remission; ys: years

		Risk of any failure (1 - event-free survival)			Risk of death (1 - overall survival)		
Variable	**variant versus referral category**	Hazard ratio	95% Confidence Interval	*p-value*	Hazard ratio	95% Confidence Interval	*p-value*
Donor type	MD vs MSD	0,904	0,598 1,365	*0,6299*	1,003	0,636 1,580	*0,990*
Risk profile	high-risk vs standard risk	1,23	0,52 2,909	*0,6377*	1,177	0,426 3,253	*0,753*
	very high-risk vs standard risk	1,515	0,626 3,671	*0,3572*	1,715	0,613 4,796	*0,304*
Remission phase	CR2 early relapse + CR3 vs CR1	1,809	1,086 3,012	*0,0227*	1,464	0,857 2,502	*0,163*
	CR2 late relapse vs CR1	1,166	0,646 2,105	*0,6102*	0,948	0,473 1,897	*0,879*
Stem cell source	cord blood vs bone marrow	1,486	0,698 3,161	*0,3039*	1,060	0,432 2,601	*0,898*
	peripheral blood vs bone marrow	1,129	0,735 1,736	*0,5795*	1,085	0,679 1,735	*0,732*
CMV donor/recipient IgG status	negative/positive vs others	1,367	0,91 2,055	*0,1325*	1,626	1,048 2,522	*0,030*
Total body irradiation	no vs yes	2,166	1,387 3,383	*0,0007*	2,060	1,250 3,393	*0,005*
Patient age at transplant	patient ≤2 y vs >2,≤12y	1,665	0,71 3,904	*0,2412*	2,211	0,924 5,292	*0,075*
	patient > 12 y vs >2,≤12 y	1,293	0,861 1,942	*0,2159*	1,619	1,037 2,529	*0,034*

		Risk of relapse			Risk of non-leukemic death		
Variable	**variant versus referral category**	Hazard ratio	95% Confidence Interval	*p-value*	Hazard ratio	95% Confidence Interval	*p-value*
Donor type	MD vs MSD	0,912	0,546 1,523	*0,724*	1,037	0,514 2,094	*0,9182*
Risk profile	high-risk vs standard risk	2,821	0,721 11,042	*0,1363*	0,547	0,145 2,063	*0,3731*
	very high-risk vs standard risk	4,336	1,108 16,971	*0,0351*	0,506	0,139 1,839	*0,3006*
Remission phase	CR2 early relapse + CR3 vs CR1	2,697	1,346 5,403	*0,0051*	0,727	0,28 1,886	*0,5122*
	CR2 late relapse vs CR1	1,673	0,819 3,418	*0,1576*	0,796	0,262 2,421	*0,6877*
Stem cell source	cord blood vs bone marrow	1,261	0,525 3,026	*0,6037*	2,449	0,579 10,364	*0,2238*
	peripheral blood vs bone marrow	0,822	0,46 1,47	*0,509*	1,842	0,892 3,806	*0,0989*
CMV donor/recipient IgG status	negative/positive vs others	0,968	0,561 1,67	*0,9073*	1,794	0,907 3,548	*0,093*
Total body irradiation	no vs yes	2,26	1,326 3,851	*0,0027*	1,711	0,674 4,349	*0,2587*
Patient age at transplant	patient ≤2 y vs >2,≤12y	1,256	0,342 4,614	*0,7316*	2,372	0,475 11,851	*0,2928*
	patient > 12 y vs >2,≤12 y	0,708	0,403 1,245	*0,2308*	2,91	1,499 5,648	*0,0016*

		Risk of Extensive Chronic GVHD			Risk of Limited+Extensive Chronic GVHD		
Parameter		Hazard ratio	95% Hazard Ratio Confidence	*p-value*	Hazard ratio	95% Hazard Ratio Confidence	*p-value*
Donor type	MD vs MSD	0,384	0,207 0,712	*0,002*	0,534	0,336 0,849	*0,008*
Risk profile	high-risk vs standard risk	0,907	0,257 3,195	*0,879*	1,366	0,484 3,856	*0,556*
	very high-risk vs standard risk	1,966	0,479 8,071	*0,348*	2,437	0,775 7,670	*0,128*
Remission phase	CR2 early relapse + CR3 vs CR1	1,034	0,466 2,295	*0,934*	1,031	0,570 1,866	*0,920*
	CR2 late relapse vs CR1	1,820	0,798 4,151	*0,155*	1,540	0,820 2,892	*0,179*
Stem cell source	cord blood vs bone marrow	0,791	0,098 6,409	*0,826*	1,023	0,290 3,602	*0,972*
	peripheral blood vs bone marrow	2,063	1,091 3,899	*0,026*	1,639	1,005 2,674	*0,048*
Total body irradiation	no vs yes	0,610	0,268 1,387	*0,238*	0,881	0,510 1,520	*0,648*
Patient age at transplant	patient > 12 y vs ≤12y	2,351	1,356 4,076	*0,002*	1,599	1,052 2,430	*0,028*

The occurrence of extensive cGVHD, compared with the absence of any cGVHD, was associated with a higher risk of nonleukemic death (HR, 8.12; 95% CI, 3.21 to 20.57; $P < .0001$) but had no significant impact on EFS and OS. Moreover, in patients experiencing extensive cGVHD, there was a trend toward a lower probability of relapse, which did not achieve significance. The occurrence of limited cGVHD did not significantly affect the risk of nonleukemic death or relapse, or the probability of being alive and disease-free.

Outcome by Donor Type According to Remission Phase

Outcomes according to remission phase are detailed in the Supplementary text, Supplementary Table S5, and Supplementary Figure S2. In brief, among the 157 patients who underwent transplantation in CR1, for MSD graft recipients and MD graft recipients, the 4-year OS was 83 ±5% and 69 ± 5%, respectively ($P = .025$); 4-year EFS was 82 ± 5% and 66 ± 5%, respectively ($P = .030$); CIR was 9 ± 4% and 17 ± 4%, respectively ($P = .126$); and NRM was 7 ± 3% and 17 ± 4%, respectively ($P = .067$). EFS and OS were significantly higher for MSD graft recipients compared with MD graft recipients in the subgroup of patients who underwent transplantation in CR1.

Among the 146 patients who underwent transplantation in CR2 or CR3, for MSD graft recipients and MD graft recipients, 4-year EFS was 47 ± 7% and 55 ± 5%, respectively ($P = .460$), CIR was 39 ± 7% and 33 ± 5%, respectively ($P = .470$), and NRM was 14 ± 5% and 12 ± 3%, respectively ($P = .884$).

Outcome by Donor Type According to Recipient Age

Results according to recipient age are reported in Supplementary Table S5. In brief, among patients age ≤ 2 years, 4-year EFS was 67 ± 27% for MSD graft recipients and 33 ± 16% for MD graft recipients ($P = .214$), whereas 4-year NRM was 0 for the former and 33% for the latter ($P < .0001$). Among patients aged 2 to ≤12 years, 4-year EFS was 68 ± 6% for MSD

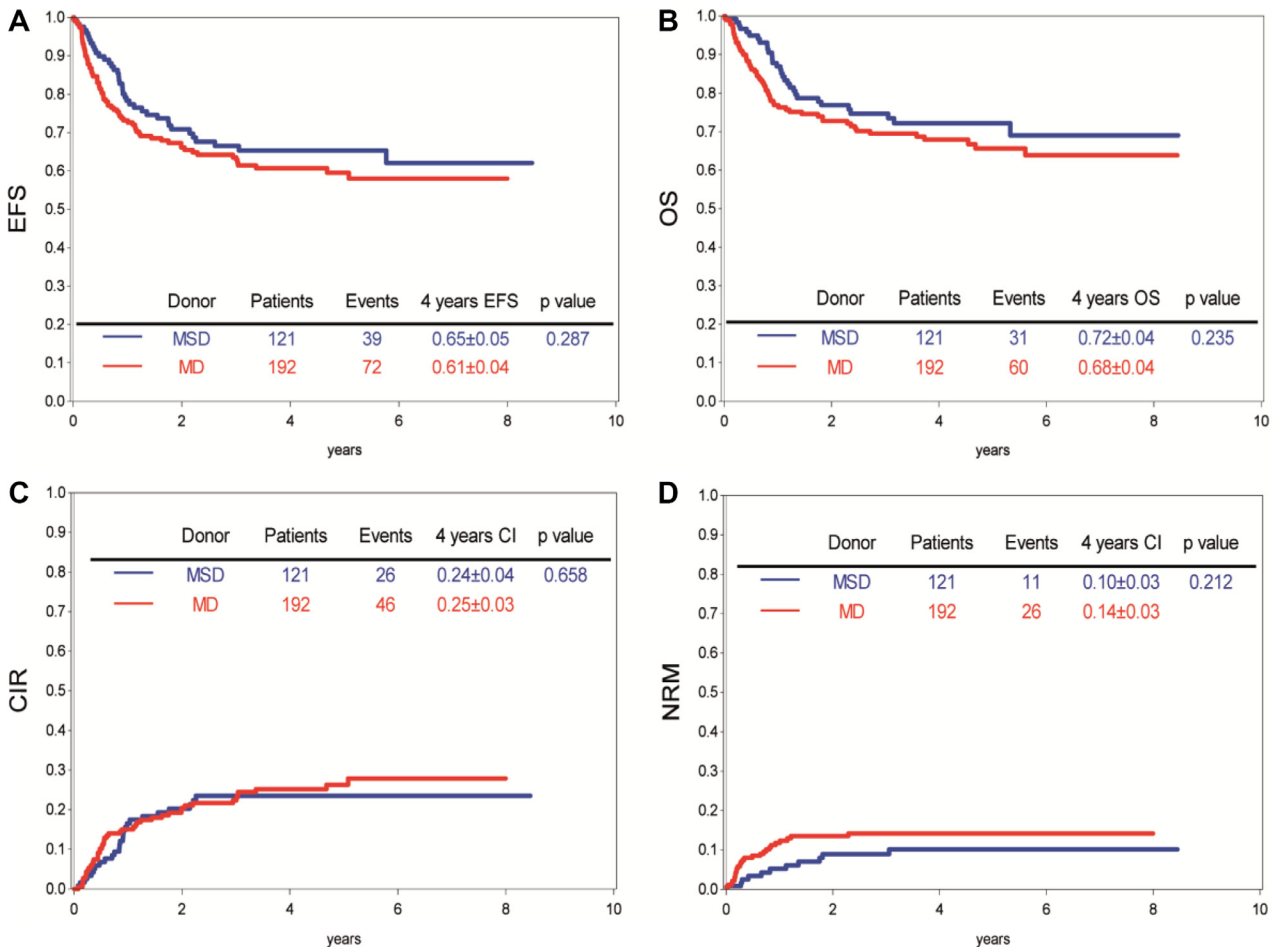

Figure 3. Outcome by type of donor. The probability of EFS (A), OS (B), CIR (C), and NRM (D) for MSD (blue curve) and MD (red curve) graft recipients. The 313 patients at high or very high risk are included in this univariate analysis to allow for the comparability between MSD and MD graft recipients. (Standard-risk patients would not have been eligible for MD transplantation). Estimates at 4 years after transplantation with their standard errors are provided in the tables.

graft recipients and 62 ± 5% for MD graft recipients ($P = .434$), whereas 4-year NRM was 6 ± 3% for the former and 10 ± 3% for the latter ($P = .369$). Among patients age >12 years, 4-year EFS was 59 ± 8% for MSD graft recipients and 62 ± 6% for MD graft recipients ($P = .806$), whereas 4-year NRM was 19 ± 7% for the former and 20 ± 5% for the latter ($P = .577$).

Country Effect

The country effect was analyzed by means of a multivariate analysis after adjustment for donor type, risk profile, remission phase, stem cell source, CMV serostatus, patient age, and use of TBI; the endpoints of the analyses were risk of aGVHD, risk of cGVHD, EFS, OS, CIR, and NRM. No significant association was detected between country strata and the risk of events in MSD graft recipients versus MD graft recipients, including relapse and nonleukemic death (data not shown). Furthermore, no significant associations were detected between country strata and the risk of relapse or the probability of EFS or OS.

DISCUSSION

This International BFM ALL SCT 2007 Study met its aim of assessing the impact of donor type in children and adolescents with ALL in morphological remission eligible for allogeneic HSCT. This study showed that transplantation from a 10/10 or 9/10 allele-matched donor, either related or unrelated, was not inferior to transplantation from an HLA-identical sibling in terms of EFS, OS, CIR and NRM, in both univariate and multivariate analyses.

Compared with the previous BFM ALL SCT 2003 Study, the present study confirms the conclusion of noninferiority of MD versus MSD HSCT in terms of EFS, OS, and CIR [7]. The BFM 2003 study was conducted in Austria, Germany, and Switzerland, where frontline and relapse protocols, eligibility criteria for HSCT, and donor search procedures were homogeneous [7]. Whether the concept of noninferiority of transplantation from MDs versus MSDs held in a broader international setting remained to be assessed.

As a consequence of the findings of the previous study and the present study, eligibility criteria for MD HSCT might be reviewed and extended to those for HLA-identical sibling HSCT, at least in patients with ALL in CR2, and possibly considered for other malignant diseases as well.

The significantly higher NRM of 10% previously reported in MD graft recipients compared with 3% in MSD graft recipients in the BFM 2003 Study could not be confirmed here [7]. Even though the difference in NRM (14% versus 10%) was not statistically significant in this I-BFM 2007 Study, NRM per se was higher in both the MSD and MD groups compared with the BFM 2003 study. A moderately higher NRM was expected in

Table 7
Impact of acute and chronic graft-versus-host disease (GVHD) on outcome. A multivariate analysis, including all of the 348 patients, assessed the impact of aGVHD and cGVHD on event-free survival, overall survival, relapse and non-relapse mortality, after adjustment for donor type, risk stratification, remission phase, stem cell source, CMV donor-recipient serologic status and total body irradiation according to age. * Odds ratio is provided for aGVHD and hazard ratio for cGVHD estimates.

Risk	Hazard/ Odds Ratio*	95% Confidence Limits	*p-value*
Any failure (1 - event-free survival)			
aGVHD I-II vs 0	0,630	0,404 0,983	*0,042*
aGVHD III-IV vs 0	1,846	1,103 3,088	*0,020*
cGVHD vs none	1,511	0,932 2,452	*0,094*
cGVHD limited vs none	1,419	0,723 2,785	*0,308*
cGVHD extensive vs none	1,478	0,812 2,689	*0,201*
Death (1 - survival)			
aGVHD I-II vs 0	0,635	0,384 1,049	*0,076*
aGVHD III-IV vs 0	2,354	1,353 4,096	*0,002*
cGVHD vs none	1,530	0,880 2,660	*0,132*
cGVHD limited vs none	1,199	0,529 2,720	*0,664*
cGVHD extensive vs none	1,729	0,890 3,358	*0,106*
Relapse			
aGVHD I-II vs 0	0,757	0,459 1,247	*0,275*
aGVHD III-IV vs 0	0,323	0,124 0,843	*0,021*
cGVHD vs none	0,731	0,374 1,428	*0,359*
cGVHD limited vs none	1,065	0,477 2,378	*0,878*
cGVHD extensive vs none	0,388	0,141 1,064	*0,066*
Non-leukemic death			
aGVHD I-II vs 0	0,507	0,168 1,532	*0,229*
aGVHD III-IV vs 0	8,761	3,646 21,051	*<.0001*
cGVHD vs none	5,666	2,429 13,215	*<.0001*
cGVHD limited vs none	2,988	0,781 11,435	*0,110*
cGVHD extensive vs none	8,120	3,206 20,566	*<.0001*

an international platform including 22 centers in 10 countries, where diagnostics and supportive care could not be completely homogeneous [23]. Nevertheless, the variability in NRM among countries did not affect the core comparison of MSD HSCT and MD HSCT as assessed by multivariate analysis (data not shown).

The distribution between the MSD and MD arms was well balanced in terms of biological characteristics as well as in terms of risk stratification and remission phase, except for a borderline significantly longer duration of CR1 in MSD graft recipients. As expected, donors were significantly older and a PB stem cell source was more often used in MD HSCT compared with MSD HSCT, and the CMV negative donor to CMV-positive recipient combination was more frequent in the MD arm, because CMV serostatus is more often consistent among siblings and more often positive among adult donors. Nevertheless, this imbalance in distribution might have potentially favored the outcomes in the MSD arm. In contrast, the female donor to male recipient combination was more frequent in MSD HSCT compared with MD HSCT, for which an upfront selection could be made when multiple donors were available.

Overall, the adherence to the planned conditioning regimens and GVHD prophylaxis was satisfactory, with only 11% deviations in the former and 4% in the latter, limited to those patients for whom the information was available.

The occurrence of rejection in only 4 patients confirms that rejection is a rare complication in pediatric patients in the HLA-matched and myeloablative setting in ALL, as reported previously, even with a lower VP16 dose [7,24].

Both myeloid and platelet engraftment occurred significantly earlier after MSD HSCT compared with after MD HSCT, in which the lower degree of HLA compatibility and the addition of MTX possibly could have played a role in delaying engraftment. Furthermore, myeloid engraftment was similar in MSD graft recipients, regardless of the source, but faster after PB compared with after BM in MD graft recipients [25].

The use of PB was exceedingly more frequent than expected, given that BM was the recommended stem cell source and thus PB should have been used only in cases of donor/donor center refusal, which apparently was not the case. Unfortunately, the reasons for the use of PB grafts were not investigated further. The proportion of PB grafts was higher in the unrelated donor setting, where the choice is based mainly on the preference of donors and donor centers, but not limited to it, given that PB mobilization in minor donors is not allowed in some of the participating countries [26]. Along with unfavorable weight disparities within donor-

recipient pairs, a higher risk of relapse, mainly MRD-based, might have led to the choice of PB. Nevertheless, the stem cell source had no role in preventing relapse in our series, after adjusting for other variables.

The same results between MSD and MD graft recipients held across different prognostic risk groups, but not across different disease phases. In patients who underwent transplantation in CR1, MSD HSCT was associated with significantly higher EFS (83%) compared with MD HSCT (69%), despite similar OS and CIR, and there was only a trend toward a lower NRM in MSD HSCT (7% versus 17%). It can be hypothesized that a lower degree of alloreactivity is sufficient to eventually cure the disease in patients in CR1 but not in those in CR2. The outcomes of patients in CR2 were not significantly different between MSD and MD graft recipients but were better after late relapse compared with after early relapse [7].

In terms of disease risk, very high-risk patients had a significantly higher probability of relapse compared with standard risk patients. It has been suggested that MSDs might not be the best donors for HSCT to treat most aggressive leukemias, owing to the high degree of HLA compatibility. Nevertheless, no significant interactions between donor type and disease risk were detected on multivariate analysis (data not shown).

The Center for International Blood & Marrow Transplant Research (CIBMTR) retrospectively compared the outcomes of HSCT (94 MSD graft recipients and 168 MD graft recipients) in a cohort of children and adolescents with ALL in CR2 after early relapse. In this very high-risk patient series, MSD-MD differences in EFS (50% versus 44%), OS (54% versus 49%), NRM (13% versus 21%), and CIR (37% versus 35%) also were not statistically significant [27].

Secondary endpoints of the study included the incidence and severity of GVHD.

The overall probability of GVHD, especially cGVHD in MSD graft recipients, was generally higher in this study compared with that reported in the literature. The possibility that earlier MRD-driven immunosuppression tapering could have occurred and contributed to the increased GVHD incidence and severity cannot be ruled out.

Because less alloreactivity was expected in MSDs compared with MDs, GVHD prophylaxis differed between the 2 arms, in accordance with protocol, with MTX and serotherapy added in the MD arm to CSA, which was the only immunosuppressive drug in the MSD arm in BM graft recipients or associated with MTX in PB graft recipients [7].

Nevertheless, there was a trend toward a higher risk of aGVHD in MSD graft recipients compared with MD graft recipients in multivariate analysis. Moreover, the addition of MTX was not sufficient to prevent an elevated risk of severe aGVHD in PB recipients, which was more than double that seen in BM recipients in the multivariate analysis, after adjusting for donor type and recipient age.

The probability of any cGVHD was significantly higher in MSD graft recipients compared with MD graft recipients (37% versus 25%), as was the probability of extensive cGVHD (26% versus 12%). The risk of extensive cGVHD was 3-fold lower in the MD arm compared with the MSD arm, also after adjusting for confounding factors.

In the aforementioned CIBMTR study, aGVHD (22% versus 46%) and cGVHD (10% versus 36%) were significantly lower in the MSD arm compared with the MD arm. Nevertheless, GVHD prophylaxis was not necessarily reinforced in MD graft recipients [27].

Intensified GVHD prophylaxis is the logical explanation for the reduced cGVHD in MD graft recipients compared with MSD graft recipients [28]. These findings might suggest that intensifying GVHD prophylaxis in MSD graft recipients could be of benefit, even though Locatelli et al. [29] demonstrated that monoprophylaxis with even low doses of i.v. CSA (1 mg/kg versus standard 3 mg/kg) was feasible and reduced the risk of relapse without affecting EFS, despite higher aGVHD and NRM. Elgarten et al. [30] retrospectively reported a 32% probability of grade II-IV aGVHD, a 9% probability of grade III-IV aGVHD, and a 9% probability of any cGVHD in 32 children age $\leq$14 years after MSD HSCT for malignant diseases with a calcineurin inhibitor as a single-agent prophylaxis.

Moreover Weiss et al. [31] reported that 19 children who underwent MSD HSCT and had received CSA alone as GVHD prophylaxis had a significantly lower CIR (5% versus 40%; $P = .002$) and a higher 5-year EFS (84% versus 35%; $P = .001$) compared with an historical control of 44 children who had received CSA and MTX. The incidence of grade II-IV aGVHD and cGVHD in the CSA group was equivalent to that of the CSA + MTX group (26% versus 19% [$P = .440$] and 32% versus 23% [$P = .428$]) [31].

Whether the addition of MTX might reduce the occurrence of GVHD in MSD BM graft recipients could not be assessed in the present study, even though it might be suggested by the lower incidence of aGVHD and cGVHD in the 10 MSD BM graft recipients who received MTX in addition to CSA. The possibility that a more intensive GVHD prophylaxis regimen might increase the risk of relapse cannot be ruled out.

The incidences of any grade aGVHD (63%), grade II-IV aGVHD (40%), grade III-IV aGVHD (15%), any cGVHD (30%), and extensive cGVHD (17%) detected in this study were higher than those of the standard arm (49%, 31%, 13%, 29%, and 6%, respectively) certainly exceeded those of the experimental arm of the COG ASCT0431/PBMTC ONC051 trial (29%, 18%, 10%, 22%, and 12%, respectively), which assessed the impact of the addition of sirolimus to tacrolimus and MTX for GVHD prophylaxis [19,27]. In that study, the reinforced GVHD prophylaxis did not translate into statistically significant differences between the standard arm and the experimental arm in 2-year EFS (56% versus 46%; $P = .28$), 2-year OS (65% versus 55%; $P = .23$), and relapse (2-year CIR, 32% versus 36%; $P = .45$) [19,28].

Moreover, in our series, PB recipients had greater than double the risk of extensive cGVHD compared with BM recipients, as did patients age >12 years compared with those age $\leq$12 years. Even if older patients were more likely to receive PB, age per se was strongly associated with the risk of cGVHD in both univariate and multivariate analyses and also after adjustment for stem cell source.

Even though BM was the recommended stem cell source, particularly for MSD HSCT, many PB grafts occurred and led to higher cGVHD. The reinforcement of CSA as GVHD prophylaxis with MTX might not have been sufficient to properly prevent cGVHD in MSD PB recipients. Nevertheless, there was only a trend toward a better final outcome after BM HSCT compared with PB HSCT that was not statistically significant, and so the superiority of BM grafting could not be directly demonstrated in this series.

A higher risk of GVHD was actually expected in PB recipients compared with BM recipients, as has been reported previously in both the adult and pediatric settings, even if not consistently through the literature [32–34]. In 2004, Eapen et al. [34] reported a retrospective pediatric study that found a higher risk of cGVHD in the PB HSCT group, with no advantage in survival or relapse reduction. A more recent study from the Pediatric Diseases Working Party of the European Society for Blood and Marrow Transplantation reported a significantly lower 3-year EFS

(54% versus 59%; *P*= .0007) and higher NRM (12% versus 20% [*P* = .0002]; HR, 1.91 [*P* = .001]) and cGVHD (HR, 1.91; *P* = .001) after PB HSCT compared with BM HSCT [25].

In our series, the higher incidence and severity of cGVHD in MSD graft recipients did not translate into a significant graft-versus-leukemia effect able to lower the probability of relapse compared with MD graft recipients, because CIR was superimposable between the 2 arms. Such a finding does not allow ruling out the possibility that potentiating GVHD prophylaxis in MSD HSCT might increase the probability of relapse. A minimum change would consist of the addition of MTX for all MSD graft recipients, not only for PB recipients, whereas a deeper change would consist of the addition of another immunosuppressive drug, like sirolimus, or serotherapy [25,28,35].

Few randomized trials have addressed the role of the addition of serotherapy in the MSD setting. A prospective multicenter randomized international European Society for Blood and Marrow Transplantation Phase III trial reported that the use of ATG reduced the likelihood of cGVHD and improved GVHD-free, relapse-free survival after HLA-identical sibling PBSCT in adults for malignant diseases [34]. The intensification of GVHD prophylaxis might not necessarily improve the final outcome, as has been reported previously [28,33].

Data on the discontinuation of immunosuppression as well as MRD-driven immunomodulation were not available, so an estimate of the probability of being GVHD-free could not be calculated, and neither could the proportion of patients still on immunosuppression at 1, 2, or more years after transplantation. Moreover, the morbidity of the survivors could not be assessed, because an analysis of quality of life after transplantation was beyond the purpose of this study.

GVHD was more frequent and of greater severity in the present I-BFM 2007 study compared with the previous BFM 2003 study [7]. A Cox model, adjusted for donor type, risk stratum, remission phase, stem cell source, CMV serostatus, and TBI by age, found elevated risks of grade III-IV aGVHD (HR, 1.80; *P* = .005) and extensive cGVHD (HR, 1.66; *P* = .015) in the 2007 patients compared with the 2003 patients (data not shown).

Assessment of the impact of GVHD on outcome showed that grade I-II aGVHD did not affect the risk of nonleukemic death in this cohort and had a protective effect on the risk of any failure and a borderline protective effect on the probability of death; however, its effect was not statistically significant for relapse prevention. In contrast, grade III-IV aGVHD was associated with almost double the risk of any failure, reduced the risk of relapse by two-third, but also led to a 9-fold higher risk of nonleukemic death. Similarly, extensive cGVHD led to an 8-fold higher risk of nonleukemic death.

In the aforementioned COG ASCT0431/PBMTC ONC051 trial, grade I-III aGVHD was associated with higher EFS (HR, .5; *P* = .02), likely due to a lower risk of relapse (HR, .4; *P*= .04), with no statistically detectable effect on NRM (HR, .6; *P*= .42). On the other hand, grade IV aGVHD markedly increased NRM (HR, 6.4; *P*= .003) and thus decreased both EFS (HR, 2.6; *P* = .06) and OS (HR, 3.0; *P*= .03) [19,28].

As expected, age >12 years was associated with a higher risk of cGVHD, as well as with a higher risk of non-leukemic death, even though none of these measures of outcome translated into an inferior final outcome, as EFS and OS were not significantly associated with age [26].

These findings could not be confirmed by data for adolescents and young adults with ALL who underwent transplantation within the Japanese Registry, who experienced lower OS, higher NRM, and similar CIR compared with children [36].

The only age group associated with a lower probability of survival was the group of children age ≤2 years, for whom aGVHD was not negligible, but no extensive cGVHD occurred. It is well known that infants often suffer from poor responsive diseases and are extremely fragile [12,37].

The impact of donor age was difficult to analyze in our series, because MSDs are usually much younger than MDs. Nevertheless, when donor type and age were analyzed as a joint variable, receipt of a graft from an MSD either older or younger than 18 years was associated with an increased risk of extensive cGVHD compared with receipt of a graft from an MD age ≤35 years (data not shown).

The use of a CMV-seronegative donor for a CMV-seropositive recipient might jeopardize the ultimate outcome by increasing the risk of nonleukemic death, but the effect was borderline in this study [38]. This risk factor for NRM was not detected in the BFM 2003 Study; possibly the inclusion of many countries and centers with different monitoring and treatment strategies worsened the outcome of such donor-recipient pairs [7]. The fact that CMV-seropositive recipients of CMV seronegative grafts had a higher risk of cGVHD may be explained by the well-known GVHD reflaring induced by viral reactivation.

Patients treated with a TBI-based conditioning regimen had a better outcome in this series; nevertheless, chemoconditioning was planned according to the protocol only for children age <2 years and/or carrying a t(4;11) translocation; any other non-TBI conditioning was a protocol deviation, likely adopted for patients with comorbidities, and thus, no conclusions could be drawn in this regard [12,39,40].

All of the countries participating in this study subsequently joined an ongoing prospective randomized trial, running in 110 centers in 28 countries worldwide, aiming at assessing whether a chemotherapy-only conditioning regimen is not inferior to a TBI-based conditioning regimen for transplantation in children, adolescents, and young adults.

Secondary malignancies were relatively low in this series, with a cumulative incidence of <1%, even though a longer follow-up might display further events [41].

In conclusion, children and adolescents with ALL showed similar outcomes after HLA-identical sibling and matched unrelated donor transplantation in terms of EFS, OS, CIR, and NRM. An MSD transplant was better than an MD transplant in patients who underwent HSCT in CR1, and MD graft recipients had significantly slower engraftment. The significantly higher probability and severity of cGVHD in MSD recipients may support the importance of adding MTX in transplantation from HLA-identical sibling donors.

Recipients of PBSCT experienced more aGVHD and cGVHD, without translating into a reduced risk of relapse, which confirms the use of BM as the preferred stem cell source. Adolescents experienced worse cGVHD, higher NRM, and lower survival. Mild aGVHD was associated with better survival, severe aGVHD was detrimental for EFS and survival due to higher NRM, and severe cGVHD was associated with higher NRM

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.07.011.

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