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Factors Associated with Long-Term Risk of Relapse after Unrelated Cord Blood Transplantation in Children with Acute Lymphoblastic Leukemia in Remission

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A B S T R A C T

For pediatric patients with acute lymphoblastic leukemia (ALL), relapse is an important cause of treatment failure after unrelated cord blood transplant (UCBT). Compared with other donor sources, relapse is similar or even reduced after UCBT despite less graft-versus-host disease (GVHD). We performed a retrospective analysis to identify risk factors associated with the 5-year cumulative incidence of relapse after UCBT. In this retrospective, registry-based study, we examined the outcomes of 640 children (<18 years) with ALL in first complete remission (CR1; n = 257, 40%) or second complete remission (CR2; n = 383, 60%) who received myeloablative conditioning followed by a single-unit UCBT from 2000 to 2012. Most received antithymocyte globulin (88%) or total body irradiation (TBI; 69%), and cord blood grafts were primarily mismatched at 1 (50%) or 2+ (34%) HLA loci. Considering patients in CR1, the rates of 5-year overall survival (OS), leukemia-free survival (LFS), and relapse were 59%, 52%, and 23%, respectively. In multivariate analysis (MVA), acute GVHD (grades II to IV) and TBI protected against relapse. In patients in CR2, rates of 5-year OS, LFS, and the cumulative incidence of relapse were 46%, 44%, and 28%, respectively. In MVA, longer duration from diagnosis to UCBT (≥30 months) and TBI were associated with decreased relapse risk. Importantly, receiving a fully HLA matched graft was a strong risk factor for increased relapse in MVA. An exploratory analysis of all 640 patients supported the important association between the presence of acute GVHD and less relapse but also demonstrated an increased risk of nonrelapse mortality. In conclusion, the impact of GVHD as a graft-versus-leukemia marker

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is evident in pediatric ALL after UCBT. Strategies that promote graft-versus-leukemia while harnessing GVHD should be further investigated.

INTRODUCTION

Modern risk-based chemotherapy regimens are curative for many children and adolescents with de novo acute lymphoblastic leukemia (ALL) [1]. An additional subset of patients can achieve long-term remission with allogeneic hematopoietic stem cell transplantation (HSCT), but outcomes are poor for those who relapse after HSCT. Current practice is to offer HSCT to patients with high-risk disease in first complete remission (CR1) or relapsed disease once a second complete remission (CR2) is achieved. The curative effect of HSCT is due to the use of high-dose chemotherapy with or without total body irradiation (TBI) along with the potential for the graft-versus-leukemia (GVL) effect. It is well established that the GVL effect, likely due to immune surveillance provided by donor natural killer and T cells, is important in eradicating leukemia after allogeneic HSCT [2-5], although the GVL effect in ALL may be weaker than in other malignancies [6-8]. Select clinical reports have demonstrated lower rates of relapse in pediatric patients with ALL who experience graft-versus-host disease (GVHD) after HSCT, providing indirect evidence of GVL actions in this disease [9-11].

For children and adolescents with ALL in need of HSCT, the use of unrelated donor cord blood (UCB) offers several benefits, including rapid procurement, more lenient HLA matching, and lower rates of GVHD [12]. Clinical reports have also demonstrated lower rates of relapse after UCB transplantation (UCBT) as compared with other donor sources [13-19]. The benefits of UCBT have historically been offset by higher nonrelapse mortality (NRM). Recent outcomes are similar to those seen with other donor sources [13,20,21]. Outcomes after UCBT for pediatric ALL have been described as part of larger prospective [9,16,22,23] and retrospective [18,24-26] studies, but a focused examination of factors influencing relapse in pediatric ALL recipients after UCBT is needed. In this report we describe the long-term outcomes of a collaborative effort between Eurocord, European Society of Blood and Marrow Transplantation (EBMT), and Duke University Pediatric Blood and Marrow Transplant Program to define risk factors associated with relapse and other outcomes after UCBT for pediatric ALL.

METHODS

Study Design

Eligible patients were <18 years old with a diagnosis of de novo ALL in morphologic CR1 or CR2. All patients received myeloablative conditioning followed by transplantation with a single, nonmanipulated UCB unit as their first graft. All UCBTs were performed from 2000 through 2012 to allow for long-term outcomes to be considered. Patients with diagnoses of biphenotypic leukemia, secondary or treatment-related leukemia, or with history of prior HSCT were excluded. Data on patient and graft characteristics and on outcomes were collected from the Eurocord or Duke University Pediatric Blood and Marrow Transplant Program clinical databases. All participating EBMT transplant centers received the synopsis of the study and gave their approval. The Institutional Review Boards of the Eurocord-Netcord scientific committee and Duke University approved this study. All patients gave informed consent for treatment and for data entry and use for analysis in accordance with the Declaration of Helsinki.

Definitions and Endpoints

The primary endpoint for this analysis was the cumulative incidence of relapse, defined as morphologic recurrence of leukemia at any site. Secondary endpoints included leukemia-free survival (LFS) defined as survival while in continuous CR, overall survival (OS), and NRM defined as death occurring while in remission.

Neutrophil recovery was defined as achieving an absolute neutrophil count $\geq .5 \times 10^9$/L for 3 consecutive days. Platelet recovery was defined as achieving a platelet count $\geq 20 \times 10^9$/L without transfusion support. Full donor chimerism was defined as ≥95% cells of donor origin and mixed chimera was between ≥5% and <95% donor cells, as measured using techniques according to the individual transplant center. The diagnosis and grading of acute (aGVHD) and chronic GVHD (cGVHD) was assigned by the transplant center using standard criteria [27,28]. Myeloablative conditioning was defined as containing either TBI with a dose > 8 Gy, a total dose of busulfan > 8 mg/kg orally, or intravenous equivalent.

HLA matching was assigned at the antigen level for class I –A and –B loci and at the allelic level for class II –DRB1. Cytogenetic findings were considered to be *high risk* if any of the following was present: BCR/ABL [t(9.22)], t(1,19), MLL rearrangements [t(4,11)], hypodiploid, or complex (>3 abnormalities); *intermediate* if abnormalities not considered high risk were present; *normal* if no abnormalities were detected; or *missing* if not available. Presence or absence of minimal residual disease (MRD) before UCBT was available for a subset of the cohort (n = 215) but was not considered in the analysis.

Statistical Approach

Separate analyses were performed for patients in CR1 and CR2 to account for the fact that disease status is a well-established predictor of outcomes after HSCT [23,29]. An exploratory analysis was also performed that included all patients in the study cohort. The probabilities of relapse, NRM, aGVHD, and cGVHD were estimated using the cumulative incidence function method in a competing risk setting treating death and relapse as competing events [30]. Differences between subgroups were compared using the Gray K-Sample test [31]. Probabilities of OS and LFS were calculated with the use of the Kaplan-Meier estimator, and differences between groups were compared using the log-rank statistics [32]. Cox proportional hazards regression was used to create prognostic multivariate models [33]. Factors known as potential prognostic factors and all factors associated with a $P < .10$ in the univariate analysis were included in the final models. The presence of aGVHD or cGVHD was considered as time-dependent variables. Statistical analyses were performed with SPSS 22 (SPSS Inc./IBM, Armonk, NY) and R 3.2.3 (R Development Core Team, Vienna, Austria) software packages.

RESULTS

Patient, donor, and transplantation characteristics are presented in Table 1. From 2000 to 2012, 640 children or adolescents (median age, 6.3 years) with ALL in either morphologic CR1 (n = 257; 40.2%) or CR2 (n = 383; 59.8%) underwent UCBT at an EBMT and Eurocord participating center (99 centers) or Duke University. Most patients were transplanted for B cell lineage ALL (79.2%). Patients and grafts were HLA matched (15.6%) or mismatched at 1 (50.1%) or ≥2 (34.3%) loci.

Most patients (68.8%) received TBI-containing regimens, most commonly TBI + cyclophosphamide (23.9%). Most TBI-containing regimens delivered total fractionated doses of 1200 to 1350 cGy (86%; range, 800 to 1500 cGy). The use of TBI-containing regimens was generally limited to children aged ≥ 3 years (94.5%), whereas 51.3% of patients who received chemotherapy-only regimens were younger than 3 years old. No infants (<12 months of age) received TBI. The most common chemotherapy-based regimen was busulfan + cyclophosphamide (15.8%), with most patients receiving a total busulfan dose of 16 mg/kg (data regarding busulfan pharmacokinetics not available). Most patients (88.0%) received antithymocyte globulin before UCBT. The median total nucleated cell dose was 5.0×10^7/kg (range, .3 to 35.4). GVHD prophylaxis was primarily cyclosporine-based with corticosteroids (72.3%) or

Table 1
Patient and Transplant Characteristics Based on Disease Status at Time of Transplant

Characteristics	All (N = 640)	CR1 (n = 257)	CR2 (n = 383)
	% (n)		
Patient			
Median age at UCBT, yr (range)	6.4 (.5-17.9)	5.3 (.5-17.9)	6.9 (.7-17.9)
Gender, male	58.1 (370)	54.5 (139)	60.5 (231)
Cytomegalovirus serology, positive	44.8 (287)	44.4 (114)	45.2 (173)
Disease			
Immunophenotype			
B cell lineage	79.2 (507)	75.1 (193)	82.0 (314)
T cell lineage	17.5 (112)	21.4 (55)	14.9 (57)
Cytogenetic risk			
High	36.3 (149)	48.5 (83)	27.5 (66)
Intermediate	36.7 (151)	32.7 (56)	39.6 (95)
Normal	27.0 (111)	18.8 (32)	32.9 (79)
Donor			
Number of HLA mismatches			
0	15.6 (95)	18.1 (45)	13.9 (50)
1	50.1 (305)	51.6 (128)	49.0 (177)
≥2	34.3 (209)	30.2 (75)	37.1 (134)
Median TNC, $\times 10^7$/kg infused (range)	5.0 (.3-35.4)	5.5 (.3-29.9)	4.7 (.5-35.4)
Transplantation			
Year of transplantation			
<2007	51.2 (328)	47.9 (123)	53.5 (205)
≥2007	48.8 (312)	52.1 (134)	46.5 (178)
Median time from diagnosis to UCBT, mo (range)	16.4 (1.8-154.7)	6.7 (1.8-32.7)	30.7 (2.3-154.5)
TBI-containing regimen			
Yes	68.8 (439)	55.1 (141)	78.0 (298)
No	31.2 (201)	44.9 (116)	22.0 (85)
TBI-containing regimens			
TBI + cyclophosphamide + fludarabine	9.9 (63)	8.2 (21)	11.0 (42)
TBI + other chemotherapy	58.8 (376)	46.7 (120)	66.8 (256)
Chemotherapy-based regimens			
Busulfan + cyclophosphamide	16.1 (103)	23.7 (61)	11.0 (42)
Thiotepa + busulfan + fludarabine	8.3 (53)	11.3 (29)	6.3 (24)
Other chemotherapy	7.1 (45)	10.1 (26)	5.0 (19)
Received serotherapy	88.0 (500)	86.2 (194)	89.2 (306)
Median follow-up, mo (range)	50.1 (1.7-172.4)	46.5 (2.4-172.4)	54.5 (1.7-172.2)
GVHD prophylaxis			
Cyclosporine + steroids	72.3 (438)	70.6 (168)	73.4 (270)
Cyclosporine + mycophenolate mofietil	21.3 (129)	21.0 (50)	21.5 (79)
Other	6.4 (39)	8.4 (20)	5.2 (19)

Values are number of cases with percents in parentheses unless otherwise noted. TNC indicates total nucleated cell dose.

mycophenolate mofetil (21.3%). The median follow-up was 50.1 months (range, 2 to 172.3) for the overall cohort.

The cumulative incidence of aGVHD (grades II to IV) and cGVHD at 5 years post-transplant was 40.4% (95% confidence interval [CI], 36.5 to 44.2) and 17% (95% CI, 14.1 to 20.2), respectively, for the overall cohort. aGVHD grades II to IV was experienced by 248 patients (grade II, 163; grades III to IV, 85) in a median of 18 days (range, 4 to 99) after UCBT. cGVHD was observed in 105 patients (CR1, 41; CR2, 64); 45 presented with extensive disease (CR1, 21; CR2, 24). Similar rates of aGVHD and cGVHD were observed in patients in CR1 and CR2.

Relapse

Considering the patients transplanted while in CR1, the cumulative incidence of relapse at 5 years was 23.4% (95% CI, 18.0 to 29.0; Figure 1A), with a median time from UCBT to identification of relapse of 7.5 months (range, 1 to 65). In multivariate analysis, 2 factors were independently associated with less relapse in patients in CR1: the presence of aGVHD (grades II to IV) as a time-dependent variable (hazard ratio [HR], .32; 95% CI, .14 to .70; $P = .004$) and receiving TBI-containing regimens (HR, .53; 95% CI, .29 to .99; $P = .04$; Table 2, Figure 2A). Although the median age of patients in CR1 who received TBI was older (8.1 versus 4.3 years), age at transplant was not a significant predictor of relapse. The risk of relapse was also not influenced by the degree of HLA matching, the GVHD prophylaxis used, or the presence of cGVHD in these patients.

The cumulative incidence of relapse in patients transplanted while in CR2 was 27.8% (95% CI, 23.2 to 32.6; Figure 1A), with a median time to relapse of 5.9 months (range, 1 to 65) after UCBT. In multivariate analysis 3 factors were associated with a lower risk of relapse (Table 2): HLA-mismatched cord blood grafts (HR, .53; 95% CI, .30 to .94; $P = .03$), the use of TBI-containing regimens (HR, .56; 95% CI, .34 to .93; $P = .03$), and an interval of ≥30 months from original diagnosis to UCBT (HR, .41; 95% CI, .25 to .67; $P < .0001$).

Leukemia-Free Survival

The 5-year probabilities of LFS for children transplanted in CR1 and CR2 were 52.4% (95% CI, 49.0 to 55.8) and 43.9% (95% CI, 41.2 to 46.6), respectively (Figure 1B). For patients in CR1, the benefit of TBI-containing regimens extended to improved LFS (60.2% versus 42.9%, $P = .01$, Figure 2B). Two factors were predictive of LFS in multivariate analysis of patients in CR1: regimens containing TBI (HR, .53; 95% CI, .35 to .82; $P = .004$) and increasing age (in years; HR, 1.05; 95% CI, 1.01 to 1.09; $P = .02$; Table 2).

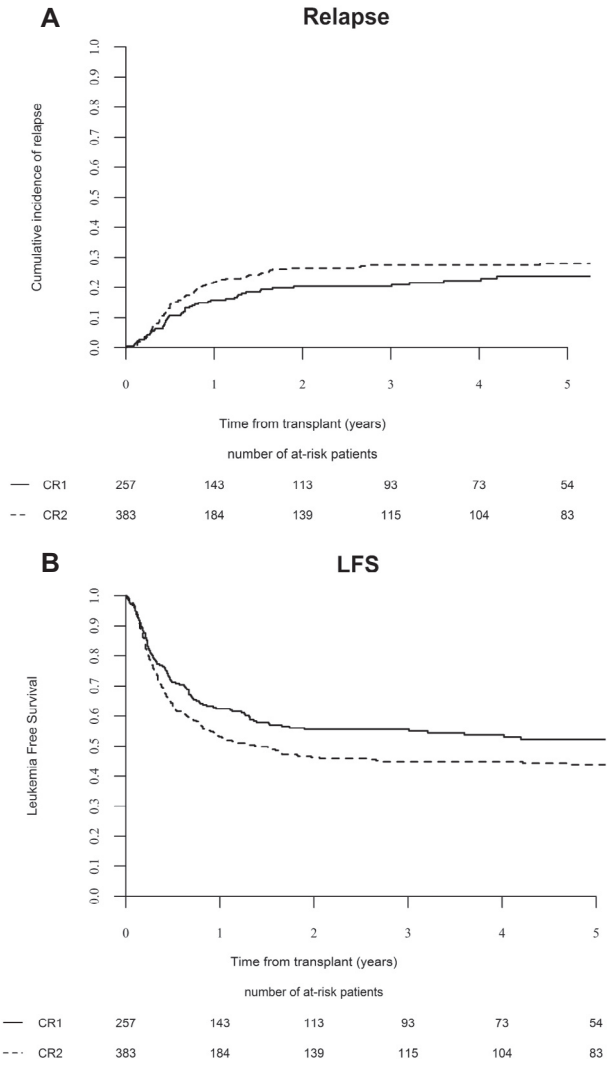

Figure 1. (A) The estimated 5-year cumulative incidence of relapse in children with ALL in CR1 or CR2 at time of UCBT. The cumulative incidence of relapse at 5 years was 23.4% (95% CI, 18.0 to 29.0) and 27.8% (95% CI, 23.2 to 32.6) for children in CR1 and CR2, respectively. (B) The 5-year probability of LFS in children with ALL in CR1 or CR2 at time of UCBT. The LFS was 52.4% (95% CI, 49.0 to 55.8) and 43.9% (95% CI, 41.2 to 46.6) for children in CR1 and CR2, respectively.

Patients in CR2 experienced improved LFS when the interval from initial diagnosis to UCBT was ≥30 months (50.9% versus 36.8%, $P = .001$). Recipients of HLA-mismatched grafts experienced improved LFS, but this was not statistically significant (45.6% versus 32.4%, $P = .09$). In multivariate modeling, longer interval from diagnosis to UCBT (HR, .62; 95% CI, .45 to .88; $P = .006$) and increasing age (in years; HR, 1.05; 95% CI, 1.01 to 1.09; $P = .04$) were both predictive of LFS after adjusting for other variables (Table 2).

OS, NRM, and Causes of Death

The probability of OS at 5 years was 58.8% (95% CI, 55.4 to 62.2) for patients in CR1. Patients who received TBI-conditioning regimens (HR, .51; 95% CI, .32 to .79; $P = .003$) had a lower risk of death at 5 years (Table 2), whereas increasing age (in years) negatively impacted OS (HR, 1.05; 95% CI, 1.01 to 1.09; $P = .03$). Of the 257 patients in CR1, 99 patients died due to relapse (n = 38), NRM (n = 57), or unknown causes (n = 3). Causes of NRM included infection (n = 28), organ toxicity (n = 11), GVHD (n = 9), hemorrhage (n = 6), rejection (n = 1), Epstein-Barr virus–associated lymphoproliferative disease (n = 1), or other (n = 1). Risk factors associated with NRM in multivariate analysis included receiving a mismatched cord blood graft (HR, 3.79; 95% CI, 1.17 to 12.35; $P = .03$) and increasing age (in years; HR, 1.06; 95% CI, 1.01 to 1.12; $P = .03$; Table 2). There was also a trend toward improved NRM in patients who received TBI (HR, .58; 95% CI, .32 to 1.02; $P = .06$).

The 5-year probability of OS was 46.3% (95% CI, 43.5-49.1) in patients in CR2. In multivariate analysis, 3 factors were associated with OS: longer duration from diagnosis to UCBT (>30 months; HR, .61; 95% CI, .43 to .86; $P = .005$; Table 2), increasing age (in years; HR, 1.05; 95% CI, 1.01 to 1.10; $P = .03$; Table 2), and the use of serotherapy (HR, 1.84; 95% CI, 1.01 to 3.36; $P = .04$). Of the 383 total patients in CR2, 191 patients died due to relapse (n = 79), NRM (n = 110), or unknown causes (n = 2). Causes of NRM included infection (n = 46), organ toxicity (n = 21), GVHD (n = 19), rejection (n = 9), hemorrhage (n = 6), Epstein-Barr virus–associated lymphoproliferative disease (n = 4), or other causes (n = 5). The risk of NRM was higher in patients with aGVHD (grades II to IV; HR, 1.67; 95% CI, 1.05 to 2.66; $P = .03$) or with a diagnosis of T cell ALL (HR, 1.78; 95% CI, 1.03 to 3.09; $P = .04$) and increased with age (in years; HR, 1.07; 95% CI, 1.01 to 1.14; $P = .03$; Table 2).

Overall Cohort

Based on the above results and driven by inspection of the data, we then performed an exploratory analysis of the entire cohort. Considering all 640 patients, the 5-year cumulative incidences of relapse, LFS, and OS were 26% (95% CI, 22.4 to 29.7), 47.4% (95% CI, 45.3 to 49.5), and 51.3% (95% CI, 49.2 to 53.4), respectively. The median time to diagnosis of relapse was 6 months (range, 1.1 to 65) after UCBT. Although no differences were noted between patients in CR1 and CR2 with respect to rates of aGVHD, cGVHD, NRM, or relapse in univariate analysis, patients transplanted in CR1 experienced improved LFS (52.4% versus 44%, $P = .03$) and OS (58.8% versus 46.4%, $P = .009$) as compared with those transplanted while in CR2.

In the overall cohort the risk of relapse after UCBT was lower in patients who experienced any GVHD (aGVHD grades II to IV or cGVHD; HR, .51; 95% CI, .34 to .76; $P = .0008$). Further analysis revealed that aGVHD grade II (HR, .59; 95% CI, .38 to .94; $P = .025$; Table 3) was associated with less relapse but did not impact NRM, LFS, and OS. Although not associated with relapse, grades III to IV aGVHD increased the risk of NRM (HR, 3.19; 95% CI, 1.93 to 5.27; $P < .0001$), leading to decreased LFS (HR, 1.51; 95% CI, 1.02 to 2.24; $P = .04$) and OS (HR, 1.94; 95% CI, 1.28 to 2.94; $P = .002$). Multivariate models did not demonstrate an association between cGVHD with relapse, NRM, or LFS, but cGVHD was associated with improved OS (HR, .57; 95% CI, .35 to .93; $P = .03$). The impact of disease status on outcomes was also demonstrated in multivariate analysis. Patients transplanted in CR2 were more likely to experience relapse (HR, 1.63; 95% CI, 1.10 to 2.40; $P = .01$) leading to worse LFS (HR, 1.35; 95% CI, 1.02 to 1.79; $P = .04$) and OS (HR, 1.51; 95% CI, 1.11 to 2.06; $P = .008$).

A subgroup analysis performed in the separate cohorts demonstrated improved outcomes in patients who received TBI + fludarabine + cyclophosphamide conditioning. We further explored this finding in the overall cohort. Although the conditioning regimen was not predictive of relapse, the use of TBI + fludarabine + cyclophosphamide was associated with

Table 2
Significant Predictors in Multivariate Analyses of Patients Transplanted in Either CR1 or CR2

Outcome	Variable*	Patients in CR1		Patients in CR2	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Relapse	aGVHD (time-dependent variable)	.32 (.14-.70)	.004		
	TBI-containing regimen	.53 (.29-.99)	.04	.56 (.34-.93)	.03
	HLA-mismatched graft			.53 (.30-.94)	.03
	Longer duration from diagnosis to UCBT (≥30 mo)†			.41 (.25-.67)	<.001
LFS	Age at UCBT, yr	1.05 (1.01-1.09)	.02	1.05 (1.01-1.09)	.04
	TBI-containing regimen	.53 (.35-.82)	.004		
	Longer duration from diagnosis to UCBT (≥30 mo)†			.62 (.45-.88)	.006
OS	Age at UCBT, yr	1.05 (1.01-1.09)	.03	1.05 (1.01-1.10)	.03
	TBI-containing regimen	.51 (.32-.79)	.003		
	Longer duration from diagnosis to UCBT (≥30 mo) †			.61 (.43-.86)	.005
	Use of serotherapy†			1.84 (1.01-3.36)	.04
NRM	aGVHD (time-dependent variable)			1.67 (1.05-2.66)	.03
	Age at UCBT, yr	1.06 (1.01-1.12)	.03	1.07 (1.01-1.14)	.03
	HLA-mismatched graft	3.79 (1.17-12.35)	.03	.81 (.39-1.71)	
	T cell ALL†			1.78 (1.03-3.09)	.04

* Models including patients in CR1 or CR2 adjusted for the following variables: presence of aGVHD (grades II-IV) as a time-dependent variable, presence of cGVHD as a time-dependent variable, age at UCBT (in years), TBI-containing regimen (yes/no), HLA matching (<6 of 6 loci vs. 6 of 6), and year of transplant.
† These additional variables were included in the CR2 multivariate model: longer duration from diagnosis to UCBT (≥30 months), T cell ALL (vs. B cell), and use of serotherapy (yes/no).

a lower risk of NRM, improved OS, and a trend to improved LFS as compared with other TBI-containing regimens (Table 3; NRM: HR, 6.42; 95% CI, 1.33 to 30.94; $P = .02$; OS: HR, 2.84; 95% CI, 1.09 to 7.41; $P = .03$; LFS: HR, 2.32; 95% CI, .99 to 5.46; $P = .05$). OS and LFS was also improved for those who received TBI + fludarabine + cyclophosphamide as compared with chemotherapy-only regimens (OS: HR, 3.08; 95% CI, 1.15 to 8.26; $P = .03$; LFS: HR, 2.89; 95% CI, 1.20 to 6.94; $P = .02$).

DISCUSSION

The aim of this retrospective registry-based study was to identify the impact of clinical factors on long-term outcomes including relapse after UCBT in a cohort of children and adolescents with ALL. These results provide insight into outcomes of pediatric recipients of UCBT beyond the clinical endpoints typically reported in the literature. To our knowledge, this study is the largest analysis to date focused solely on recipients of UCBT for pediatric ALL with a long-term follow-up.

The impact of GVHD and, by extension, GVL effect on relapse after UCBT for pediatric ALL was an important finding of this study. A select number of studies have shown an impact of aGVHD [10,34,35] or cGVHD [11,36] on relapse after related or unrelated bone marrow HSCT for pediatric acute leukemia. Pulsipher et al. [9] observed that aGVHD and pretransplant MRD, independently and in combination, influenced the risk of relapse after HSCT for pediatric ALL after controlling for graft source and other variables. Our results, focused on single cord pediatric recipients with ALL, are consistent with the recent findings of Chen et al. [26] after UCBT (single and double) in patients with ALL or acute myeloid leukemia. They also observed a strong association between antithymocyte globulin use with relapse and NRM, which our results did not support.

Our results also demonstrated the delicate balance between GVL effect and GVHD. Although the presence of aGVHD grade II decreased relapse, patients who experienced grades III to IV were at higher risk of NRM without the benefit of less relapse. Therefore, efforts to harness GVHD with the goal of enhancing GVL effect are not without risk. Despite this, it is common practice to rapidly withdraw immunosuppression once relapse has occurred. Others have used also this approach to prevent relapse after HSCT in the setting of increasing host chimerism with tolerable rates of GVHD and some clinical effect [37-39]. It remains unclear if this approach could be extended to patients without signs of impending relapse. Our results suggest that high-risk patients, especially those transplanted in CR1 as compared with patients in CR2, could benefit from more rapid tapers of immunosuppression. Given that most patients relapse beyond the first 100 days post-UCBT, it would be very reasonable to consider weaning immunosuppression in patients without active GVHD at that point. However, further studies are needed to define the optimal timing and the efficacy of this approach. The potential risk of such an approach would be increased risk of GVHD-associated NRM, although patients more commonly died of infectious causes and not GVHD in this current study. Improvements in supportive care could help to offset potential increases in NRM, especially in older children and adolescents. Promising early-phase results using ex vivo cord blood graft expansion technologies may allow for more rapid immune recovery also leading to improved NRM [40-43].

One approach that has been suggested is to use cord blood grafts intentionally mismatched to the recipient in an effort to enhance the GVL effect. Our results indicate that the use of HLA-mismatched grafts decreased the risk of relapse by nearly half in patients in CR2, without any associated increase in NRM. Outcomes of patients in CR2 were nearly identical whether a 4/6 or 5/6 HLA-matched graft was used with respect to relapse, LFS, OS, and NRM. Although there was a trend toward higher cGVHD in recipients of 4/6 HLA-matched grafts, there was no increase in aGVHD (data not shown). Our study supports the earlier finding of Eapen et al. [18], who noted lower relapse in pediatric leukemia patients who received 4/6 conventionally HLA-matched grafts. More recently, Eapen et al. [44] demonstrated the importance of allelic high-resolution matching, including HLA-C loci, on NRM after UCBT, but they did not observe any impact on relapse or OS. Interestingly, HLA matching was not a significant predictor of relapse for patients in CR1 or in the overall cohort, whereas aGVHD was associated with less relapse in both settings. It is possible that HLA matching is serving as a surrogate

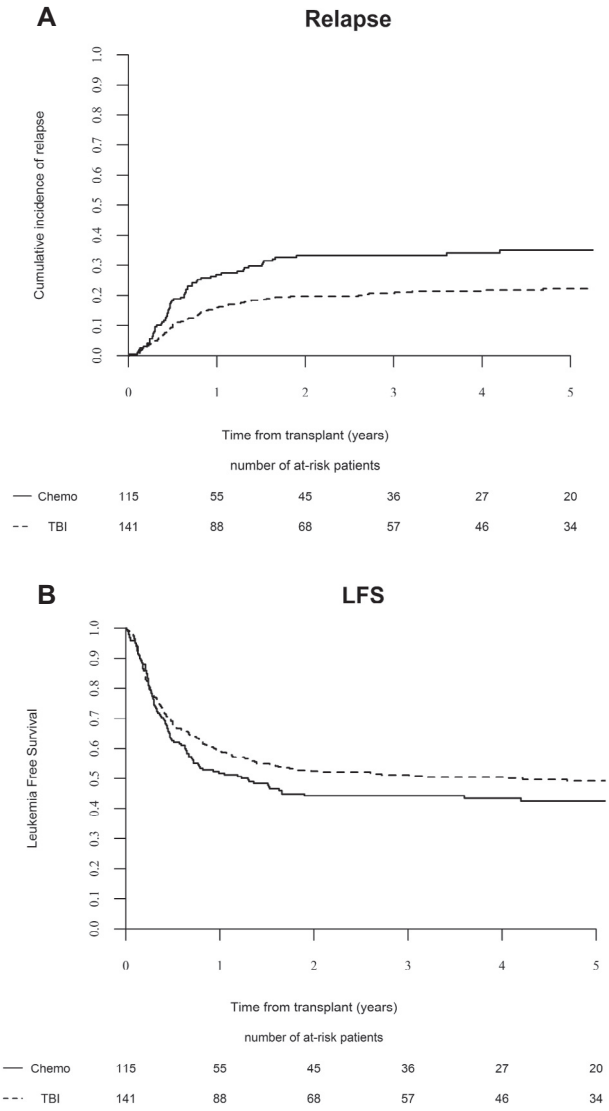

Figure 2. (A) The estimated 5-year cumulative incidence of relapse in children with ALL in CR1 at time of UCBT based on conditioning regimen. The cumulative incidence of relapse was 17.4% and 30.5% for children who received TBI and chemotherapy-based regimens, respectively ($P = .03$). (B) The 5-year probability of LFS in children with ALL in CR1 at time of UCBT based on conditioning regimen. The 5-year probability of LFS for children who received TBI-containing or chemotherapy-containing regimens was 40.9% and 60.2%, respectively ($P = .01$).

for aGVHD in the CR2 cohort, but that is only speculative. Therefore, the use of intentionally mismatched cord blood graft, especially in patients in CR2, is intriguing and warrants further investigation.

TBI has been a mainstay of conditioning regimens used in patients with ALL [45-47]. Concerns regarding the considerable late effects associated with TBI [48], including neurocognitive effects [49-51], have led to the practice of using chemotherapy-based regimens in young children. Older children and adolescents routinely receive TBI as part of conditioning, although TBI-sparing strategies in this population continue to be explored [52-54]. Our results, after adjusting for age and other clinical factors, support the use of TBI as a critical component of treatment. Less relapse was observed in patients in CR1 and CR2 receiving TBI-containing regimens without any increase in NRM. TBI-containing regimens were also associated with improved LFS and OS in patients in CR1. Recently, Eapen et al. [55] showed an advantage after UCBT for pediatric patients who received TBI + fludarabine + cyclophosphamide conditioning. This led us to perform an exploratory analysis in the overall cohort. In multivariate analysis of the larger cohort, the use of the TBI + fludarabine + cyclophosphamide regimen did not improve the risk of relapse but was associated with lower NRM, leading to improved 5-year LFS and OS. Two prospective UCBT trials in pediatric leukemia have reported low overall rates of relapse associated with this regimen [16,22], making this an intriguing conditioning regimen in pediatric ALL.

Our results do not necessarily suggest that the use of TBI should be extended to young children who may be subject to significant neurocognitive deficits after TBI [56-58]. Young children are also at risk for certain late effects related to myeloablative conditioning [59]. Therefore, the decision to use TBI in younger children should balance disease-related factors with these known potential late effects. It is also important to acknowledge that clinicians may have selected non-TBI regimens based on patient-specific comorbidities, including age. Importantly, we also investigated in a subgroup analysis whether very young patients (<2 years old) and those with t(4,11) influenced the outcomes in patients receiving chemotherapy-based regimens. Our results (data not shown) indicated no significant differences in outcomes between recipients of chemotherapy-only regimens (<2 years versus older children). In summary, our results support the use of TBI as currently practiced for children and adolescents, especially in patients in CR1, in reducing relapse without additional NRM.

Limitations to this study include the retrospective nature and the use of registry-based data. Although pretransplant MRD status was available for a portion of the patients, we elected not to include these data in the analysis because of the amount of missing data and acknowledge that it is a limitation of the study. Pretransplant MRD status has been shown in pediatric ALL to be a strong independent predictor of outcomes after HSCT [9,29,60,61], and although techniques have been significantly refined over the years, the optimal timing and clinical application of post-HSCT MRD testing continues to be explored. Continued advances in cellular and immunotherapy may also provide more effective approaches to management of MRD and relapse post-HSCT.

In summary, our results support the presence of a GVL effect in pediatric ALL after UCBT. We observed that patients who experienced aGVHD, especially those transplanted while in CR1, were less likely to relapse. Strategies to enhance the GVL effect after UCBT should be further explored. On the basis of this study, the use of TBI in conditioning regimens should be strongly considered for patients given the lower cumulative incidence of relapse observed in all patients and a survival advantage in patients in CR1. Altogether, optimizing these clinical factors could lead to improved outcomes in pediatric patients receiving a UCBT transplant for ALL.

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Table 3
GVHD and Other Significant Predictors of Outcomes in Multivariate Analyses of All Patients

Outcome	Variable (Reference)	HR (95% CI)	*P*
Relapse	aGVHD grade II as a time-dependent variable	.59 (.39-.94)	.03
	CR2	1.63 (1.10-2.40)	.01
LFS	aGVHD grades III-IV as a time-dependent variable	1.51 (1.02-2.24)	.04
	Conditioning regimen (TBI + fludarabine + cyclophosphamide)		
	TBI + other	2.32 (.99-5.46)	.05
	Chemotherapy only	2.89 (1.20-6.94)	.02
	CR2	1.35 (1.02-1.79)	.04
OS	aGVHD grades III-IV as a time-dependent variable	1.94 (1.28-2.94)	.002
	cGVHD as a time-dependent variable	.57 (.35-.93)	.03
	Conditioning regimen (TBI + fludarabine + cyclophosphamide)		
	TBI + other	2.84 (1.09-7.41)	.03
	Chemotherapy only	3.08 (1.15-8.23)	.03
	CR1	.67 (.49-.90)	.009
NRM	aGVHD grades III-IV as a time-dependent variable	3.19 (1.93-5.27)	<.0001
	Age at transplant in years	1.07 (1.02-1.14)	.01
	Conditioning regimen (TBI + fludarabine + cyclophosphamide)		
	TBI + other	6.42 (1.33-30.94)	.02
	Chemotherapy only	4.88 (.96-24.68)	.05

All models included the following variables: grade II aGVHD as a time-dependent variable, aGVHD grades III-IV as a time-dependent variable, cGVHD as a time-dependent variable, disease state (CR1 or CR2), leukemia phenotype (B cell vs. T cell ALL), history of central nervous system leukemia (yes or no), age at time of transplantation (in years), year of transplantation, total nucleated cell dose adjusted for patient weight, conditioning regimen (TBI + fludarabine + cyclophosphamide vs. other TBI regimens vs. chemotherapy-only regimen), GVHD prophylaxis (cyclosporine + steroids or mycophenolate mofietil or other regimen), and treatment center.

Hospital, Sydney; **Belgium** Children's University Hospital BMT Unit, Brussels; Cliniques Universitaires St. Luc, Brussels; Universitair Ziekenhuis, Brussels; University Hospital, Gent; University Hospital Gasthuisberg Dept. of Hematology, Leuven; University of Liege, Liege; **Brazil** University Est. de Campinas, Campinas; Hospital Amaral Carvalho, Jau Sao Paulo; **Canada** Ste-Justine Hospital, Montréal; **Czech Republic** Institute of Hematology and Blood Transfusion, Prague; University Hospital Motol, Prague; **Denmark** Rigshospitalet, Copenhagen; **Finland** Hospital for Children & Adolescents University of Helsinki, Helsinki; Turku University Central Hospital, Turku; **France** Saint Jacques, Besançon; CHU Bordeaux Groupe Hospitalier Pellegrin-Enfants, Bordeaux; Haut-Lévêque, Bordeaux; Hotel Dieu CHU/Jean Perrin, Clermont-Ferrand; Tronche CHU/Albert Michallon, Grenoble; Claude Huriez, Lille; Hôpital Jeane de Flandre, Lille; Debrousse (pédiatrie), Lyon; La Timone (pédiatrie), Marseille; Centre Hospitalier Universitaire La Conception, Marseille Bouches du Rhone; Lapeyronie- adulte, Montpellier; Brabois, Nancy; Hotel Dieu, Nantes; Archet, Nice; Robert Debré, Paris; Saint-Antoine, Paris; Saint-Louis, Paris; Jean Bernard, Poitiers; Pontchaillou, Rennes; Charles Nicolle- pédiatrie, Rouen; Hautepierre (adulte), Strasbourg; **Germany** Universitaetsklinikum Dresden, Dresden; Universitaetsklinikum, Düsseldorf; Martin-Luther-Univ. Halle-Wittenberg, Halle; **Greece** St. Sophia Childrens Hospital, Athens; **Hungary** St. László Hospital, Budapest; Postgraduate Medical School, Miskolc; **Israel** Rambam Medical Center, Haifa; Schneider Childrens Medical Center of Israel, Petach-Tikva; Edmond & Lily Safra Children's Hospital, Chaim Sheba Med Center, Tel-Hashomer; **Italy** Azienda Ospedaliero-Universitaria di Bologna, Bologna; Azienda Ospedaliero Universitaria Meyer—Ospedale di Careggi, Firenze; Institute G. Gaslini, Genova; Monza Ospedale San Gerardo Clinica Pediatrica dell Universita di Milano Bicocca; Clinica di Oncoematologia Pediatrica, Padova; Ospedale dei Bambini, Palermo; Fondazione IRCCS Policlinico San Matteo, Pavia; Università di Perugia, Perugia; Pesaro Hospital, Pesaro; Ospedale Civile Department of Hematology, Pescara; Azienda Ospedaliera Universitaria Pisa, Pisa; Ospedale S. Camillo, Roma; Rome Transplant Network, Roma; Univ.La Sapienza, Roma; Ospedale Infantile Regina Margherita Onco-Ematologia Pediatrica, Torino; Istituto per l'Infanzia Burlo Garofolo, Trieste; Hospital Pausilipon, Napoli; **Jordan** King Hussein Cancer Centre, Amman; **Poland** Wroclaw Medical University, Wroclaw; **Portugal** Inst. Portugues Oncologia, Lisboa; Inst. Portugues de Oncologia do Porto, Porto **Saudi Arabia** King Faisal Specialist Hospital & Research Centre, Riyadh; **Spain** Hospital Santa Creu i Sant Pau, Barcelona; Hospital Universitari Germans Trias i Pujol, Barcelona; Hospital Vall d Hebron, Barcelona, Hosp. Reina Sofia, Córdoba; Hospital Universitario La Paz, Madrid; Niño Jesus Children's Hospital, Madrid; Hospital Universitario Virgen de la Arrixaca, Murcia; University Hospital of Asturias, Oviedo; Hospital Clínico, Salamanca; Hospital U. Marqués de Valdecilla, Santander; Hospital Universitario Virgen del Rocío, Sevilla; Hospital Universitario La Fe, Valencia; Hospital Infantil La Fe, Valencia; **Sweden** Sahlgrenska University Hospital, Goeteborg; University Hospital, Lund; Karolinska University Hospital Children's Hospital, Stockholm; University Hospital, Uppsala; **Switzerland** University Hospital, Basel; Hopitaux Universitaires de Geneve, Geneva; University Children s Hospital, Zürich; **The Netherlands** University Hospital, Leiden; University Medical Centre, Utrecht; **Turkey** Akdeniz University Medical School, Antalya; **United Kingdom** Royal Hospital for Sick Children, Glasgow; Birmingham Childrens Hospital, Birmingham; Royal Hospital for Children, Bristol; Great Ormond Street Hospital, London; Royal Marsden Hospital, London Surrey; Royal Hallamshire Hospital, Sheffield.

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provided data for the study and edited and approved the manuscript.

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