Unrelated donor α/β T cell– and B cell–depleted HSCT for the treatment of pediatric acute leukemia

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Key Points

- URD HSCT with TCRαβ/CD19 depletion is a safe and effective approach to alternative donor transplantation for hematologic malignancies.

- Nonpermissive mismatch at DP was associated with a 16.5-fold risk of acute GVHD and may represent a modifiable target to mitigate GVHD risk.

Unrelated donor (URD) hematopoietic stem cell transplant (HSCT) is associated with an increased risk of severe graft-versus-host disease (GVHD). TCRαβ/CD19 depletion may reduce this risk, whereas maintaining graft-versus-leukemia. Outcome data with TCRαβ/CD19 depletion generally describe haploidentical donors, with relatively few URDs. We hypothesized that TCRαβ/CD19-depletion would attenuate the risks of GVHD and relapse for URD HSCT. Sixty pediatric and young adult (YA) patients with hematologic malignancies who lacked a matched-related donor were enrolled at 2 large pediatric transplantation centers between October 2014 and September 2019. All patients with acute leukemia had minimal residual disease testing, and DP typing was available for 77%. All patients received myeloablative total body irradiation– or busulfan-based conditioning with no posttransplant immune suppression. Engraftment occurred in 98%. Four-year overall survival was 69% (95% confidence interval [CI], 52%-81%), and leukemia-free survival was 64% (95% CI, 48%-76%), with no difference between lymphoid and myeloid malignancies ($P = .6297$ and $P = .5441$, respectively). One patient (1.7%) experienced primary graft failure. Relapse occurred in 11 patients (3-year cumulative incidence, 21%; 95% CI, 11-34), and 8 patients (cumulative incidence, 15%; 95% CI, 6.7-26) experienced nonrelapse mortality. Grade III to IV acute GVHD was seen in 8 patients (13%), and 14 patients (26%) developed chronic GVHD, of which 6 (11%) had extensive disease. Nonpermissive DP mismatch was associated with higher likelihood of acute GVHD (odds ratio, 16.50; 95% CI, 1.67-163.42; $P = .0166$) but not with the development of chronic GVHD. URD TCRαβ/CD19-depleted peripheral HSCT is a safe and effective approach to transplantation for children/YAs with leukemia. This trial was registered at www. clinicaltrials.gov as #NCT02323867.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) can be curative for children and young adults (YAs) with high-risk or relapsed hematologic malignancies for whom chemotherapy alone has a very poor chance for cure. However, only approximately 20% of patients will have an HLA-matched sibling donor.[1] Alternative donor options include unrelated donors (URDs), haploidentical donors, and unrelated cord blood. All have potential benefits but also significant risks, including poor engraftment, delayed immune reconstitution, and graft-versus-host disease (GVHD). The choice of alternative donor is often dictated by the transplant center's preference, expertise, and availability of processing laboratory.

Both ex vivo and in vivo methods of T-lymphocyte depletion have been used to decrease the risk of GVHD for URDs. In vivo methods include antithymocyte globulin,[2-6] alemtuzumab,[7] and posttransplant cyclophosphamide.[8-11] Ex vivo methods range from CD34 selection, which results in a product depleted of nearly all mature immune cells, to a variety of more selective T cell–targeted depletion strategies. Although more complete T-cell depletion techniques may reduce the risk of GVHD, there are concerns regarding the loss of the graft-versus-leukemia (GVL) effect, increasing the risk of disease relapse, and delayed immune reconstitution leading to severe infection. The recent implementation of selective TCRαβ depletion has demonstrated success with haploidentical grafts.[12] Retention of mature natural killer and γδT cells maintain both GVL effect[13] and protection against infection.[12,14,15] Concomitant CD19 depletion may reduce the risk of Epstein-Barr virus (EBV) transmission as well.

Outcomes using TCRαβ/CD19-depletion HSCT for patients with hematologic malignances with haploidentical donors has demonstrated acceptable rates of GVHD and relapse. However, there are limited data using this approach with unrelated donors.[16] Following our use of CD3$^+$ depletion with CD3$^+$ addback,[17] we developed protocols using TCRαβ/CD19 depletion with the goal of improving relapse and survival outcomes and decreasing incidence of severe acute and chronic GVHD.

Here, we report the outcomes of a study performed at 2 large pediatric HSCT centers, Children's Hospital of Philadelphia (CHOP) and Children's Hospital of Wisconsin (CHW), for pediatric patients with hematologic malignancies who lacked an HLA-matched related donor. This study represents 1 of the largest studies of TCRαβ/CD19 depletion for URD HSCT for pediatric and YA patients with hematologic malignancies.

Patients and methods

Patients

Children and YAs, age ≤23 years, with myelodysplastic syndrome (MDS), acute lymphoblastic (ALL), or acute myeloid leukemia (AML), were enrolled between October 2014 and September 2019 in this prospective clinical trial. The trial was approved by the Institutional Review Boards at CHOP and CHW and the US Food and Drug Administration and registered at ClinicalTrial.gov (#NCT02323867). Before participation, written informed consent was obtained from either patients or their legal guardians. This was the first allogeneic HSCT for these patients.

No patient had an HLA 10/10 or 9/10 matched related donor available. Patients who did not have a 10/10 or 9/10 matched unrelated donor readily available were offered haploidentical transplants. Minimal residual disease (MRD) testing by multiparameter flow cytometry was performed on bone marrow aspirates within 2 weeks of starting conditioning. Patients with ALL were required to be in hematologic remission with an MRD < 0.1%; patients with AML were required to have ≤10% bone marrow blasts.

Donor selection and stem cell collection

URD searches were performed through the National Marrow Donor Program). A 10 allele-matched URD was first preference, followed by a 1 antigen- or allele-mismatched URD. Donor peripheral blood stem cells were mobilized with granulocyte colony-stimulating factors according to institutional or National Marrow Donor Program guidelines.

HLA typing

HLA genotyping was conducted by the Immunogenetics Laboratory at CHOP or Versti at CHW. Six HLA genes (-A, -B, -C, -DRB1, -DQB1, and -DPB1) were sequenced using targeted amplicon-based next-generation sequencing with Omixon Holotype HLAV2 kits (Budapest, Hungary). The matching status of DPB1-generated T-cell epitopes (TCEs) was determined as permissive or nonpermissive, based on the work of Fleischhauer et al[18] and Crivello et al.[19] The updated TCE group assignment was available through the IMGT-HLA, which is a public resource.

TCRαβ/CD19 cell depletion

Stem cell processing using the CliniMACS Plus device was performed in the cGMP laboratories of the treating institutions, which are accredited by the Foundation for the Accreditation of Cellular Therapy. Briefly, cells were first washed to remove platelets and then labeled with anti-TCRαβ reagent conjugated to biotin. Excess reagent was washed away and then incubated with anti-biotin and anti-CD19 reagents conjugated to paramagnetic beads. The reagents were again removed by washing, and cells were loaded onto the CliniMACS processor in a closed tubing set. The depletion process on the CliniMACS was automated, and the final product was formulated based on flow markers including CD34, TCRαβ, and CD20. Products were released only after passing release criteria including >70% viability, negative gram stain, and a maximum TCRαβ dose of 5.0×10^5/kg.

Transplantation regimen

All patients received a pretransplant myeloablative conditioning regimen with either busulfan or total body irradiation (TBI). TBI (1200 cGy/6 fractions with lung shielding after 800 cGy) was standard for all patients with lymphoid malignancy. Busulfan pharmacokinetic monitoring was performed to achieve steady-state concentrations of 900 to 1200 ng/mL. All patients received thiotepa 5 mg/kg for 2 days, followed by cyclophosphamide 60 mg/kg with mesna for bladder protection for 2 days (supplemental Figure 1). All patients enrolled before December 2015 received antithymocyte globulin (3 mg/kg for 3 days, starting 5 days before transplantation) for GVHD prophylaxis. No patient received pharmacologic GVHD prophylaxis after transplant.

Supportive care

All patients received prophylaxis for *Pneumocystis jirovecii* and fungal infections. To reduce the risk of complications from EBV, EBV serology-positive patients received rituximab (375 mg/m^2) on day +1. Patients that were serology positive for herpes simplex or varicella virus received prophylactic acyclovir. Cytomegalovirus (CMV) serology-positive recipients received CMV-directed prophylaxis with foscarnet (transitioned to valganciclovir when outpatient). Weekly monitoring for CMV, adenovirus, and EBV was performed by polymerase chain reaction. All viral prophylaxis was continued for 100 days after transplant.

Definitions and statistical analysis

Engraftment was defined as the first of 3 consecutive days with peripheral neutrophil count of $\geq 0.5 \times 10^9$/L and platelet recovery as the first day with a platelet count of $\geq 20 \times 10^9$/L without transfusion during the preceding 7 days.[20] Primary graft failure was defined as a failure of initial engraftment within 28 days of HSCT. Only clinically significant viral reactivations were included, defined either by the need for antiviral therapy as dictated by laboratory criteria (presence of BK virus in blood and urine, HHV6 or adenovirus positivity in the setting of clinical symptoms, CMV polymerase chain reaction > 1000 IU or > 3 log), or the presence of associated clinical signs or symptoms. MRD positivity was defined as detectable leukemia ≥ 0.01% by multiparameter flow cytometry at pretransplant assessment. Patients surviving >14 and >100 days after transplant were assessed for acute (aGVHD) and chronic GVHD (cGVHD), respectively. Consensus guidelines for the diagnosis of aGVHD[21] and cGVHD[22] were used.

Patient characteristics and posttransplantation outcomes were summarized by disease type. Time to neutrophil and platelet engraftment (censored at 100 days after transplant) were summarized using cumulative incidence curves and compared using log-rank test. Univariate Cox proportional hazard models were constructed to estimate hazard ratio (HR) of engraftment. Leukemia-free survival (LFS) was defined as time from transplantation to relapse or death, censoring at the last day of follow-up, and overall survival (OS) was defined as time from transplantation to death from any cause, censoring at the last day of follow-up. Kaplan-Meier curves of LFS and OS were plotted by disease type and compared using log-rank tests. To explore baseline (sex, age, HLA match, disease, MRD positivity, CMV status of recipient and donor, DP match, and ATG exposure) and time-varying (aGVHD, cGVHD, and individual viral reactivations) risk factors of LFS and OS, univariate Cox regression models were constructed using the whole cohort, and proportional hazard assumptions were assessed by log-log plots. Competing risk analyses were performed considering time to relapse and time to nonrelapse mortality (NRM) as competing risks for one another. Cumulative incidence curves for relapse and NRM were plotted and compared using Gray's test, and subdistribution hazard regression models were constructed to estimate subdistribution HR (sub-HR). In all survival outcome regression models, postbaseline variables were treated as time-varying covariates. For secondary outcomes, including viral reactivation and occurrence of aGVHD and cGVHD, logistic regression models were used to explore baseline risk factors. All regression models were univariate, and multivariate models were not constructed because of event size of this study. Analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC). Data used in these analyses were current as of 1 April 2020.

Results

Patient and donor clinical characteristics are shown in Table 1. Seven patients (12%) had MRD-level disease at transplant: 4 patients with AML and 3 patients with ALL. Of the 4 patients with AML, 2 had morphologic evidence of disease (2% and 5% blasts on bone marrow biopsy), and 2 had only MRD-level disease. Of the 3 patients with ALL, none had evidence of morphologic disease, but had measurable MRD > 0.01% (0.2%, 0.16%, and 0.04%). Twenty patients with AML were transplanted in CR1, of which 4 (20%) had a diagnosis of secondary AML; 10 patients with relapsed AML were transplanted in CR2. Ten patients with ALL were transplanted in CR1 for induction failure or refractory disease; with the remainder transplanted for relapsed disease, 3 of them (11%) in CR3. Immunotherapy and targeted cytotoxic therapy, including blinatumomab, inotuzumab, and/or CART19, was prior therapy for 17 (63%) patients with ALL. Thirty-seven patients (62%) had 10/10 HLA-matched unrelated donors (MUDs; 12/12 n = 1 [2.7%], 10/10 [97%]), and 23 had HLA-mismatched unrelated donors (MMUDs; 9/10 n = 22 [96%], 8/10 n = 1 [4.3%]). Of MUDs, 9 (24%) were matched at DP, 9 (24%) had a permissive match, and 10 (27%) had a nonpermissive match. For MMUDs, 1 (4.3%) was matched at DP, 10 (43%) had a permissive match, and 7 (30%) had a nonpermissive match. The median number of CD34$^+$ per kilogram infused was 10×10^6 (range, 2.85-20 $\times$ 10^6), and the median number of CD20$^+$ cells per kilogram was 0.75×10^5 (range, 0-6.24 $\times$ 10^5). In all cases, TCRαβ content was lower than 5×10^5/kg.

Engraftment

Primary engraftment was achieved in 98%. One patient did not engraft (infused with 6.6×10^6 CD34$^+$ cells/kg) and subsequently engrafted with a different donor. Of note, donor-specific antibodies were negative. The median time to neutrophil engraftment was 14 days (range 9-30), median time to platelet engraftment was 16 days (range, 9-52; Table 2). Four patients died of NRM before achieving platelet engraftment. In univariate analysis, there was no variable identified that influenced the kinetics of platelet recovery (supplemental Table 1; supplemental Figure 2). Total peripheral blood donor chimerism at day 30 was 100% for 80% of patients with ALL and 42% of patients with AML. At day 100, 100% of patients with ALL and 90% of patients with AML had >95% total donor chimerism.

aGVHD and cGVHD

Thirty-eight patients (63%) did not develop any evidence of aGVHD (Table 2). Fourteen patients (23%) developed grade I to II acute GVHD, and 8 (13%) developed grade III to IV GVHD (Table 2). Low-grade (grade I-II) aGVHD was primarily skin (12 patients), with 3 patients who developed gastrointestinal involvement. Grade 3 to 4 aGVHD included 2 patients with skin-only disease, 1 patient with isolated gastrointestinal disease, and 5 with combination skin and visceral disease (gastrointestinal and/or liver). In univariable analysis, nonpermissive DP match was associated with a higher likelihood of any aGVHD (odds ratio [OR], 16.50; 95% confidence interval [CI], 1.67-163.42; P = .0166; supplemental Table 2), and the use of ATG during conditioning was protective against the development of aGVHD (OR, 0.11; 95% CI, 0.02-0.52; P = .0056). Thirty-five

Table 1. Patient, donor, and transplantation characteristics

	ALL, n = 27 n (%)	AML/MDS, n = 33 n (%)
Patient and disease characteristics		
Female	12 (44%)	16 (48%)
Median age at diagnosis (range), y	11.8 (0.8-20.1)	10.2 (0.7-17.7)
Median age at transplant (range), y	13.9 (1.7-23.2)	11.4 (1.2-18)
Disease phase at HSCT		
CR1	10 (37%)*	20 (61%)
CR2	14 (52%)	10 (30%)
CR3	3 (11%)	0 (0%)
MDS	0 (0%)	3 (9%)
Lymphoblastic leukemia characteristics		
T-ALL	5 (19%)	†
Favorable‡ B-ALL genetics	2 (7%)	†
Intermediate‡ B-ALL genetics	3 (11%)	†
High-risk‡ B-ALL genetics	9 (33%)	†
Myelogenous leukemia characteristics		
Primary AML, reason for transplant	†	11 (33%)
High-risk cytogenetics§	†	5 (15%)
End induction MRD-positive	†	4 (12%)
Secondary AML	†	10 (30%)
Relapsed (HSCT at CR2) AML	†	3 (9%)
MDS		
Prior immunotherapy and targeted therapy		
Blinatumomab	10 (37%)	NA
Inotuzumab	6 (22%)	NA
CD19-directed CAR T-cell	7 (26%)	NA
Disease burden at transplant start		
MRD negative	22 (77%)	26 (87%)
MRD positive	3 (23%)	4 (13%)
Not applicable (MDS)	†	3 (9%)
Transplant characteristics		
HLA compatibility		
MUD	14 (52%)	23 (70%)
MMUD	13 (48%)	10 (30%)
Class I mismatch		
A locus	4	5
B locus	~	4
C locus	4	~
Class II mismatch		
DR locus	2	~
DQ locus	3	~
Class I and II mismatch		
A and DQ	~	1
DP match status		
Match	5 (19%)	5 (15%)
Permissive mismatch	9 (33%)	10 (30%)
Nonpermissive mismatch	7 (26%)	10 (30%)
Unknown	6 (22%)	8 (24%)
Donor sex, female	15 (56%)	14 (42%)
Donor sex mismatch, female donor → male recipient	6 (22%)	6 (18%)
CMV status (recipient/donor)		
Negative/negative	6 (22%)	11 (33%)
Negative/positive	6 (22%)	4 (12%)
Positive/positive	8 (30%)	7 (21%)
Positive/negative	7 (26%)	11 (33%)
Conditioning		
TBI based	27 (100%)	7 (21%)
Busulfan based	0 (0%)	26 (79%)
ATG containing	6 (22%)	15 (45%)
Rituximab	22 (81%)	27 (82%)
Cell dose infused, median (range)		
CD34$^+$ cells $\times$ 10^6/kg	9.6 (3.2-15.3)	10.8 (2.9-20)
TCR$\alpha\beta$ $\times$ 10^5/kg	0.3 (0.0-4.3)	0.3 (0.0-4.5)
TCR$\lambda\delta$ $\times$ 10^6/kg	7.7 (0.3-48.1)	6.8 (1.4-51)

~, Analysis not done.

*Transplant in CR1 for end of induction failure or refractory disease.

†Not applicable.

‡Favorable risk genetics[38]: hyperdiploidy or *ETV6/RUNX1* fusion; intermediate: iAMP21, *IKZF1* deletion, or *TCF3/PBX1*; high risk: *MLL*(*KMT2A*) rearrangements, Philadelphia-chromosome (Ph+), Ph-like, hypodiploidy, and *TCF3/HLF* fusion.

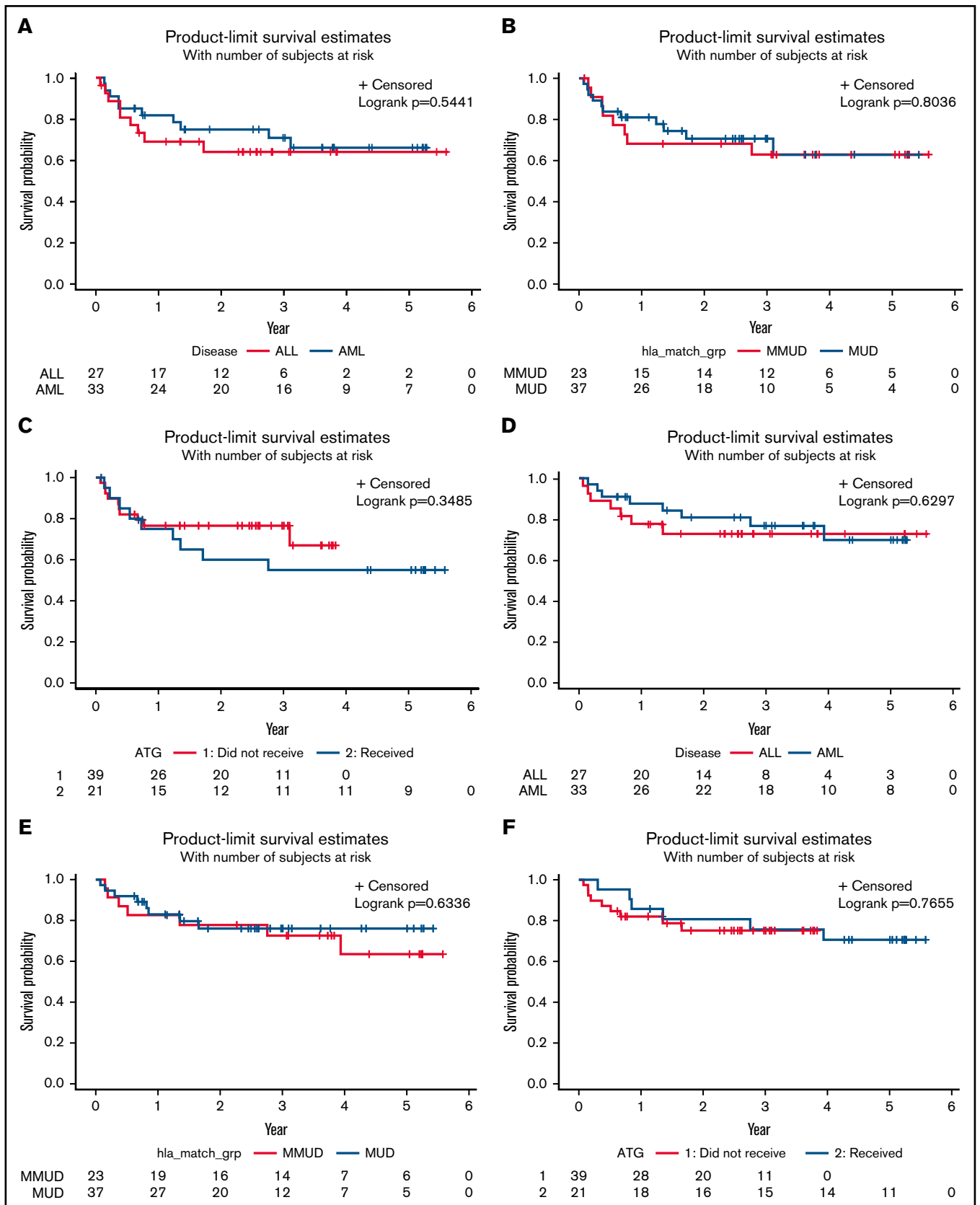§High-risk genetics defined as: −7, −5/5q-, FLT3 high ITD-AR.

Figure 1. LFS and OS by disease type. (A) LFS by type of leukemia, defined as the time from transplantation to relapse or death in the patients who achieved engraftment. (B) LFS by HLA match. (C) LFS by ATG exposure. (D) OS by type of leukemia, defined as the time from transplantation to death from any cause. (E) OS by HLA match. (F) OS by ATG exposure.

Table 2. Post-HSCT outcomes

	ALL, n = 27		AML, n = 33	
Median time to, range (d), n				
Neutrophil recovery*	14 (11-19)	26	14 (9-30)	33
Platelet recovery*	17 (9-19)	23	16 (9-52)	32
Relapse, n (%)	5 (19%)		6 (18%)	
Median time in d, range†	198 (139-625)		199 (49-1132)	
Viral reactivation, n (%)				
Adenovirus	5 (19%)		4 (12%)	
BK	5 (19%)		7 (21%)	
CMV	7 (26%)		5 (15%)	
HHV6	4 (15%)		2 (6%)	
EBV	0 (0%)		0 (0%)	
GVHD				
Acute	12 (46%)		10 (30%)	
Any	6 (23%)		2 (6%)	
Grade 3-4	6 (27%)		8 (26%)	
Chronic‡	3 (14%)		5 (16%)	
Limited	3 (14%)§		3 (9.7%)‖	
Extensive				
Treatment-related mortality				
Before day 100	3 (11%)		1 (3%)	
Overall	4 (15%)		4 (12%)	

*Of those who engraft.
†Of those who relapse.
‡Evaluable patients: ALL n = 22, AML n = 31.
§Sites included: 1 patient with autoimmune hemolytic anemia, skin, and gut involvement, 1 with gut and lung, and 1 with skin and gut involvement that evolved from aGVHD.
‖Sites included: 1 patient with skin, ocular, and presumed lung involvement, 1 with bronchiolitis obliterans, and 1 with isolated skin involvement.

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percent of patients (n = 6) with nonpermissive DP match developed grade III to IV aGVHD. No patient who received ATG (n = 21) developed grade III to IV aGVHD. Those who received a mismatched graft had a threefold increase in odds of developing severe aGVHD, a finding that was not statistically significant (P = .1267; supplemental Table 2). There was no association between TCRαβ cell count and the development of aGVHD or cGVHD (supplemental Table 2).

Fifty-three patients survived >100 days after HSCT and were evaluated for cGVHD. Fourteen children (26%) developed cGVHD, of which 8 had limited (15% of evaluable patients), and 6 (11% of evaluable patients) had extensive disease (Table 2).Using the NIH Consensus Criteria classification,[22] 7 had mild, 2 had moderate, and 5 had severe disease. Of those with cGVHD, 8 patients (57%) had preceding aGVHD. The use of ATG during conditioning demonstrated a protective effect against cGVHD, but the data did not reach statistical significance (OR, 0.24; 95% CI, 0.05-1.21; P = .0836; supplemental Table 2).

Viral reactivation and infection

Twenty-eight patients (47%) developed viral reactivation (Table 2). No patient developed EBV reactivation. Univariate analysis did not reveal any associated risk factors for viral reactivation, including ATG use (supplemental Table 3). There was also no associated found between TCRλδ content and the development of viral reactivation (supplemental Table 3). Two patients died as a result of respiratory viruses, 1 with rhinovirus and 1 with coinfection of influenza A, human metapneumovirus, and *Pneumocystis jirovecii*, both while receiving immunosuppression for GVHD.

Bacterial blood infections reported in the first 100 days after transplantation included *Staphylococcus epidermidis* (n = 2), *Staphylococcus aureus*, *Weissella confusa, Pantoea* species, and *Leclercia adecarboxylata*. One patient died in association with *Enterobacter cloacae* infection identified at day +125, and 1 patient died in the setting of multiple infections, including disseminated candidiasis, pulmonary aspergillosis, disseminated *Mycobacterium avium*, and *Clostridium difficile* (supplemental Table 4). No other fungal infections were reported.

Relapse

With a median follow-up of 3.1 years (range, 0.6-5.6 years), 11 patients (18%) relapsed at a median of 6.6 months (range, 2-38 months) after HSCT, 8 occurring within the first year. Overall, 4-year LFS was 64% (95% CI, 48%-76%; Figure 1A), and there was no statistically significant difference by HLA-mismatch (Figure 1B) or ATG-exposure (Figure 1C). In the univariate analysis for LFS, adenovirus infection (HR, 4.0; 95% CI, 1.45-11.30; P = .0076) was associated with worse LFS, whereas HLA mismatch was not (HR, 1.12; 95% CI, 0.45-2.80; P = .8039; Table 3). aGVHD was associated with worse LFS (HR, 2.8; 95% CI, 1.12-6.85; P = .0275), but this association was driven by an increase in NRM and not relapse (relapse: sub-HR, 1.02; 95% CI, 0.31-3.32; P = .9744; NRM: sub-HR, 7.54; 95% CI, 1.62-35.18; P = .0101). The association of cGVHD with relapse was in the protective direction but was not statistically significant (sub-HR, 0.43; 95% CI, 0.053-3.499; P = .4309), but cGVHD was significantly associated with higher risk of NRM (sub-HR, 8.79; 95% CI, 1.66-46.65; P = .0107).

The overall cumulative incidence of relapse was 21% (95% CI, 11%-34%), with no difference between ALL and AML (P = .8628; Figure 2A). MRD positivity conferred an almost fourfold increase in hazard of relapse (sub-HR, 3.90; 95% CI, 1.07-14.27; P = .0399), and the use of ATG during conditioning was similarly associated with higher hazard of relapse that approached statistical significance (sub-HR, 3.27; 95% CI,

Table 3. Univariable analysis of risk factors for relapse, NRM, OS, and LFS

	LFS			OS			Relapse			NRM		
	HR	95% CI	P	HR	95% CI	P	Sub-HR	95% CI	P	Sub-HR	95% CI	P
Baseline covariates												
Female	0.584	0.229-1.488	.2595	0.730	0.260-2.052	.5504	0.367	0.099-1.359	.1333	1.147	0.290-4.541	.8453
Age ≥ 12 y	1.101	0.446-2.720	.8343	1.161	0.420-3.212	.7734	1.064	0.329-3.438	.9173	1.247	0.320-4.865	.7508
MMUD (ref: MUD)	1.123	0.450-2.803	.8039	1.281	0.462-3.549	.6345	0.822	0.240-2.823	.7560	1.529	0.399-5.866	.5356
ALL (ref: AML)	1.323	0.534-3.281	.5458	1.285	0.463-3.567	.6305	1.091	0.343-3.466	.8827	1.393	0.371-5.235	.6237
MRD-positive	1.521	0.434-5.331	.5121	0.460	0.060-3.520	.4545	3.899	1.065-14.271	.0399	*	*	*
CMV status (rec/don)												
Neg/neg	REF	REF	REF	REF	REF	REF	REF	REF	REF	REF	REF	REF
Neg/pos	1.708	0.493-5.912	.3985	1.988	0.443-8.926	.3697	1.743	0.478-6.352	.3999	1.625	0.110-24.007	.7237
Pos/neg	0.932	0.270-3.223	.9114	1.170	0.261-5.231	.8376	0.439	0.084-2.297	.3295	2.875	0.298-27.744	.3612
Pos/pos	1.157	0.310-4.321	.8283	1.871	0.416-8.425	.4143	0.301	0.033-2.761	.2886	3.951	0.402-38.855	.2388
DP match												
Match	REF	REF	REF	REF	REF	REF	REF	REF	REF	REF	REF	REF
Nonpermissive	3.211	0.692-14.889	.1362	6.078	0.759-48.682	.0891	0.806	0.140-4.649	.8097	*	*	*
Permissive	1.013	0.185-5.545	.9884	1.519	0.158-14.618	.7176	0.465	0.065-3.339	.4462	*	*	*
Unknown	1.295	0.237-7.086	.7655	2.107	0.219-20.265	.5189	1.308	0.249-6.864	.7511	0.998	0.646-1.541	.9927
ATG-containing (ALL + AML)	1.537	0.621-3.807	.3526	0.846	0.282-2.539	.7657	3.265	0.928-11.489	.0653	0.508	0.121-2.141	.3562
ALL patients only	~	~	~	~	~	~	5.954	1.100-32.222	.0384	~	~	~
AML patients only	~	~	~	~	~	~	2.279	0.403-12.877	.3513	~	~	~
Rituximab-containing	0.752	0.249-2.270	.6130	0.855	0.241-3.033	.8085	0.518	0.138-1.948	.3304	1.550	0.189-12.685	.6827
Time-varying covariates												
aGVHD, any (ref: none)	2.769	1.120-6.845	.0275	4.229	1.467-12.190	.0076	1.020	0.314-3.317	.9733	7.543	1.617-35.179	.0101
aGVHD												
None	REF	REF	REF	REF	REF	REF	REF	REF	REF	REF	REF	REF
Grade 1/2	1.444	0.360-5.787	.6043	1.817	0.445-7.424	.4056	1.238	0.320-4.795	.7572	1.611	0.145-17.891	.6980
Grade 3/4	9.364	2.861-30.650	.0002	15.359	4.404-53.559	<.0001	1.587	0.190-13.274	.6697	32.499	4.804-210.871	.0004
cGVHD	1.524	0.472-4.919	.4814	2.457	0.700-8.629	.1607	0.431	0.053-3.499	.4309	8.791	1.657-46.650	.0107
Adenovirus infection, n = 9	4.049	1.450-11.303	.0076	4.829	1.621-14.383	.0047	1.531	0.331-7.075	.5858	5.258	1.289-21.452	.0207
BK virus infection, n = 12	1.383	0.454-4.210	.5683	2.044	0.643-6.494	.2257	0.429	0.059-3.132	.4041	3.854	0.923-16.085	.0642
CMV infection, n = 12	1.945	0.699-5.408	.2024	3.5573	0.960-8.374	.0593	0.895	0.202-3.956	.8834	3.155	0.732-13.600	.1232
HHV6 infection, n = 6	2.631	0.759-9.114	.1271	3.757	1.046-13.496	.0425	#	#	#	8.485	2.063-34.901	.0030

Cox models were used for OS and LFS and subdistribution hazard models were used for relapse and NRM.
~, Analysis not done.
*Sample size too small.

0.9-11.5; P = .0653; Table 3), with a relapse rate of 33% (n = 7) in patients who received ATG. When relapse risk was stratified by disease type, the use of ATG was associated with a significantly increased hazard of relapse in patients with ALL (sub-HR, 5.95; 95% CI, 1.1-32.2; P = .0384; Table 3), whereas the use of ATG in patients with AML was associated with increased hazard of relapse but was not statistically significant (sub-HR, 2.3; 95% CI, 0.4-12.9; P = .3513; Table 3). HLA mismatch was not associated with an increased hazard of relapse (sub-HR, 0.82; 95% CI, 0.24-2.82; P = .7560).

NRM

Eight patients died of transplantation-related causes, each receiving concurrent or proximate immunosuppression for a diagnosis of GVHD. Of the 4 patients who died within 100 days of transplant, 2 died of multisystem organ failure resulting from shock of unclear etiology and 2 from respiratory failure in the setting of viral infection (rhinovirus, 1 patient) and pulmonary infiltrates of unknown etiology and VOD and severe GVHD (1 patient). The fatal events occurring >100 days after HSCT were similarly split, with 2 patients developing respiratory failure and 2 developing multisystem organ failure all in the setting of documented or presumed infection (identified viral infection, n = 1; fungal infection, n = 1; both leading to respiratory failure). The 3-year cumulative incidence of NRM for the whole cohort of patients was 15% (95% CI, 6.7-26), and there was no difference in cumulative incidence of NRM for ALL vs AML (P = .6218; Figure 2B). In univariate analysis, any aGVHD (sub-HR, 7.54; 95% CI, 1.62-3518; P = .0101), cGVHD (sub-HR, 8.79; 95% CI, 1.66-46.65; P = .0107), adenovirus infection (sub-HR, 5.26; 95% CI, 1.29-21.45; P = .0207), and HHV6 infection (sub-HR, 8.49; 95% CI, 2.06-34.90; P = .0030) were associated with higher hazard of NRM (Table 3). On further categorizing aGVHD grade (grade III/IV, I/II, none), only severe aGVHD (grade III/IV) was significantly associated with NRM (sub-HR, 32.5; 95% CI, 4.8-210.9; P = .0004). Receiving an MMUD graft was not associated with a higher hazard of NRM (Table 3).

OS

Forty-five patients (75%) were alive at the time of last follow-up, with 41 in continuous CR after αβ-depleted HSCT, 3 in remission (26-42 months) after salvage with chimeric antigen receptor (CAR)
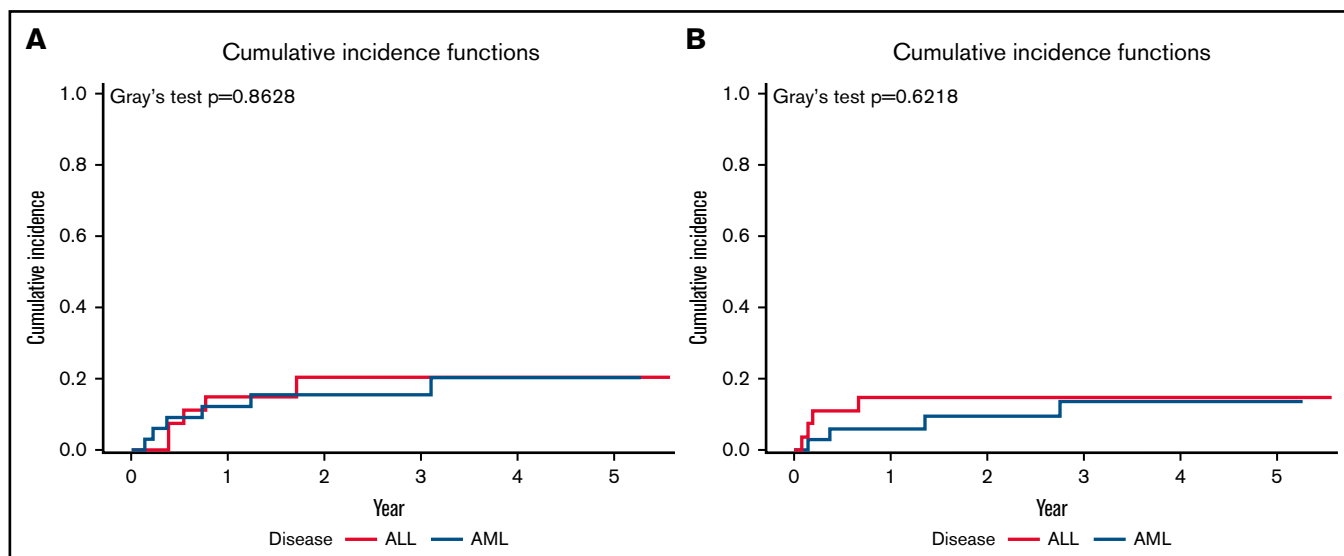
Figure 2. Cumulative incidence (CI) curves for relapse and NRM, considering each other as competing risks, by disease type. (A) CI curve for relapse. (B) CI curve for NRM.

T-cell therapy (2 patients) and second HSCT (1 patient), and 1 patient in active posttransplantation leukemia relapse. The 4-year OS probability was 69% (95% CI, 52%-81%), with no difference between ALL and AML (P = .6297; Figure 1D).

Univariate analysis of factors influencing risk of death is displayed in Table 3. aGVHD was associated with a higher hazard of all-cause death (HR, 4.23; 95% CI, 1.47-12.19; P = .0076), whereas cGVHD was not statistically significant (HR, 2.46; 95% CI, 0.70-8.63; P = .1607). Viral infections were also associated with an increased hazard of death, but MMUD grafts were not (HR, 1.28; 95% CI, 0.46-3.55; P = .6345; Table 3). Noteworthy, although not statistically significant, was a sixfold increase in hazard of all-cause death associated with a nonpermissive DP match (HR, 6.08; 95% CI, 0.76-48.68; P = .0891).

Discussion

URD HSCT, while broadening the donor pool, has been limited by GVHD-associated morbidity. TCRαβ/CD19 depletion offers the delivery of a T-cell depletion technique that preserves the γδ T cells, may facilitate engraftment,[23] maintains GVL, and decreases risk of infection.[12] Most studies report its use with haploidentical donor HSCT, with comparatively few URDs. Our data demonstrate that TCRαβ/CD19 depletion for URDs has a high engraftment rate (98%), with excellent OS (69% at 4 years) and LFS (64% at 4 years), unaffected by HLA-mismatch status.

This multi-institutional study is the first to report long-term outcomes in a large population of children and YAs who received TCRαβ/CD19 depletion for unrelated donor HSCT. Further distinguishing this work is that most patients with ALL were pretreated with immunotherapy and that all patients had pretransplant MRD testing. Sixty-three percent of patients with ALL had either relapsed or had B-cell recovery after CAR T-cell therapy or received treatment with blinatumomab or inotuzumab before transplant, 42% of patients with AML were transplanted in CR2 or diagnosed with treatment-associated AML, and 12% of patients had at least MRD-level disease before

conditioning. However, despite many very high-risk patients, few patients with AML in our cohort received TBI-based conditioning regimens in contrast to the haploidentical TCRαβ experience and T-replete marrow studies.[24-27] Thus, the data presented here broaden the donor pool with a strategy that has a low incidence of graft failure and demonstrate encouraging outcomes in a heavily pretreated population.

Previous studies using TCRαβ depletion with haploidentical donors include the large pediatric experience published by Moretta et al[24] that studied 80 patients with a median follow-up of 4 years. Similar to our findings, LFS was 71% at 5 years and OS was 72%. No grade IV aGVHD nor extensive cGVHD was noted, but unlike our study, all patients received ATG as part of conditioning. A small study evaluating TCRαβ T-cell depletion strategies for AML in pediatrics was published by Maschan et al,[28] which included 20 patients who received unrelated donor transplants. Follow-up time was shorter, but 2-year LFS (75%) and OS (65%) were similar to ours at 4 years. No patient in the study of Mashan et al[28] developed grade IV GVHD; however, most participants received ATG in addition to posttransplantation immune suppression, including tacrolimus and methotrexate. In that study, the cumulative incidence of cGVHD for the entire cohort was 25%. A recently published study in adults that included 31 MUDs for hematologic malignancies had markedly higher relapse rates and lower OS than what we present here, with significant NRM, and most patients required DLI,[16] illustrating the importance of pediatric-specific studies.

There is no clear model to dictate the choice of an alternative donor, and the decision typically depends on donor availability, center preference, and expertise. Most studies report similar, or inferior, outcomes in terms of LFS or OS to those presented here but do not consider GVHD-associated morbidity. Comparison with studies using T-replete donor sources is complicated, given the diversity of published donor sources and match characteristics, indications for transplant, and conditioning regimens, as well as the introduction of noncontemporaneous control bias (as supportive care monitoring

and interventions, as well as tissue-typing techniques, have improved over time). The most recent survival probabilities at 3 years for pediatric patients, available from the CIBMTR, after T-replete URD HSCT were approximately 72% and 58% for CR1 (ALL and AML, respectively), 61% and 57% for CR2 (ALL and AML, respectively), and 59% and 26% (ALL and AML, respectively) for patients with advanced disease,[29] but GVHD data are not presented. Other published studies of children with mixed hematologic malignancies have reported modest survival outcomes, clustering around 50% OS, with similar LFS.[30,31] Studies of children with mixed hematologic malignancies receiving T-replete transplants report severe aGVHD rates from 6% to 62%,[25-27,31] and cGVHD rates of 12% to 65%[25-27,31] but uniformly include either pretransplantation ATG or posttransplantation immunologic suppression for GVHD prevention. Unrelated cord blood transplantation also has varying results, with a large retrospective pediatric ALL study finding 5-year LFS probability of 61% and OS of 68%, but severe aGVHD rates of 14% and cGHVD of 22%, despite uniform use of GVHD prophylaxis with either cyclosporine or tacrolimus.[32] Other studies have reported less encouraging relapse-free survival and OS statistics with similar rates of GVHD, despite routine use of GVHD prophylaxis.[33,34] A study of partial CD3+ depletion for alternative donors published by Seif et al[17] reported relapse incidence (cumulative incidence, 26%) and OS at 3 years (62%) that were similar to this work. Despite all patients in that study receiving posttransplantation GVHD prophylaxis with cyclosporine, 21% of patients developed grade III to IV aGVHD, 47% developed cGVHD, and 12% developed extensive cGVHD. In the present study, the rates of high-grade aGVHD and extensive cGVHD were similar, but the rate of overall cGVHD was half (26%), without use of any prophylactic immunosuppression.

A unique feature of this protocol is the absence of posttransplant immune suppression for GVHD prophylaxis, in addition to elimination of ATG. This is in contrast to prior studies that uniformly included either pre- or posttransplantation GVHD prophylaxis.[17,24-28,32-34] Despite this, severe GVHD incidence was relatively low in comparison with prior studies, with 13% of patients developing grade III to IV aGVHD and 11% developing extensive cGVHD. Our data support prior findings that ATG use during conditioning is protective against the development of aGVHD and was suggestive of a similar protective effect for cGVHD. However, midway through the trial period, ATG was discontinued for URDs because of a concern for increased relapse. This change allowed for a post hoc analysis of ATG exposure and relapse risk in this population that demonstrated that ATG exposure was associated with a 3.3-fold higher incidence of relapse, a finding that approached statistical significance. When stratified by disease type, ATG exposure in patients with ALL was found to be statistically significantly associated with a sixfold increase in risk of relapse, whereas in AML, the association was not as strong. Further work is needed to delineate how best to include ATG in the conditioning regimen, including examination of appropriate dosing and timing, depending on the clinical scenario and the need to minimize GVHD vs enhance a GVL response.

Given the association between grade III and IV aGVHD and NRM in this study, strategies to mitigate severe aGVHD are essential. Another in vivo approach to T-cell depletion and subsequent GVHD prophylaxis is posttransplantation cyclophosphamide, a strategy that has been used extensively for adults with haploidentical donors but has limited experience in URDs. Posttransplantation cyclophosphamide targets alloreactive donor T cells that proliferate early after HSCT, potentially conferring protection against both severe GVHD and graft rejection. Evaluation of this technique in pediatrics, however, has been confined to haploidentical donors,[8,35] so cross-comparison with this modality is currently not possible.

Although immune reconstitution was rapid, with significant T-cell reconstitution noted at 4 months,[36] viral reactivation remains a concern. Viral disease was rare, reflecting meticulous monitoring and prophylaxis. This study did not provide evidence that higher TCRλδ content was more protective against viral reactivation; however, direct comparison with TCRλδ content and viral reactivation rates in other transplant modalities is necessary. Viral infection was the cause of death only in patients who were receiving steroids for severe GVHD (n = 2) and included common respiratory viruses. Notably, there was no evidence of EBV reactivation, most likely related to rituximab in serology-positive patients. This is in contrast to the study by Shelikhova et al,[28] who reported that 50% of patients had EBV reactivation. The fact that patients undergoing TCRαβ/CD19-depletion do not receive posttransplantation immunosuppression provides an ideal platform to introduce immune modulation after HSCT to optimize infectious outcomes. Additional post-HSCT therapy may accelerate immune reconstitution and thus decrease the risk of infection while not endangering the GVL effect or increasing risk of GVHD. Potential therapy may include cytotoxic T lymphocytes, or selected lymphocytes, such as CD45RO+ T cells.

Previous studies of ex vivo T depletion have not evaluated the effect of DP matching. This study demonstrates that, despite ex vivo T depletion, DP may impact outcomes. An array of DP mismatches elicits polyclonal alloreactive T-cell responses and has been associated with a higher risk of aGVHD.[37] In this cohort, 17% of patients were matched at DP, whereas 32% had a permissive mismatch and 28% had a nonpermissive mismatch, with patients who had nonpermissive mismatches at more than a 16-fold risk of aGVHD and a third of them developing severe aGVHD. Larger sample sizes are required, but this may represent a modifiable target to mitigate GVHD risk while not impacting relapse risk, as neither the LFS nor competing risk relapse analysis suggested a protective effect of DP mismatch against relapse. Use of ATG in the setting of DP mismatch is an avenue for future exploration. The use of MRD to define eligibility for transplantation is also important, because MRD positivity, even at low level, conferred an almost fourfold increase in hazard of relapse.

We demonstrated that URD HSCT with TCRαβ/CD19 depletion is a safe and effective approach to alternative donor transplantation for children and YAs with hematologic malignancies. Survival outcomes compare favorably to historical CIBMTR and other published T cell–replete HSCT data and are similar to partially CD3+-depleted PSCT with improved rates of overall aGVHD and cGHVD, with similar rates of severe GVHD despite no routine GVHD prophylaxis. TCRαβ depletion has the advantage of broadening the donor pool with a graft that has good immune reconstitution, preserves the GVL effect, and mitigates the risk of extensive chronic GVHD and graft failure. With further refinements in the approach, including better understanding of the role of DP match status, having a finer delineation of the GVH/GVL tradeoffs involved in the use of ATG, and using additional posttransplantation immune modulation

techniques, TCRαβ T-cell depletion has the potential to minimize morbidity and maximize relapse-free survival for children and YAs requiring unrelated donor transplantation for hematologic malignancy.

Authorship

Contribution: J.-A.T. and N.B. were involved in the conception, design, and planning of the study; A.B.L., J.-A.T., C.W.E., A.E.S., Y.W., B.J., D.S.M., S.K., T.S.O., J.F., L.W., S.A.G., and N.B. collected the data; Y.L. and A.B.L. did the statistical analysis and interpretation; A.B.L. wrote the paper; and all authors reviewed the data analyses, contributed to data interpretation and writing of the report, and approved the final version of the submitted report.

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