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Transplantation-Related Mortality, Graft Failure, and Survival after Reduced-Toxicity Conditioning and Allogeneic Hematopoietic Stem Cell Transplantation in 100 Consecutive Pediatric Recipients

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A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) with myeloablative conditioning is associated with a 10%-40% risk of day +100 transplantation-related mortality (TRM). We evaluated the feasibility and safety of reduced-toxicity conditioning and allo-HSCT in 100 consecutive children and adolescent recipients (mean age, 9.2 ± 6.8 years). The mean duration of follow-up was 1278 ± 1042 days. Fifty patients had malignant disease. The median time to neutrophil recovery was 18 days, and the median time to platelet recovery was 43 days. Median donor chimerism in engrafted patients was 98% on day +100 and 98% on day +365. The cumulative incidence of acute graft-versus-host disease (GVHD) was 20% (95% confidence interval [CI], 12.1%-27.9%), and that of chronic GVHD was 13.5% (95% CI, 6.6%-20.4%). TRM was 3% (95% CI, 0%-6.4%) by day +100 and 13.6% (95% CI, 6.7%-20.5%) for the entire study period. The incidence of primary graft failure (PGF) was 16% overall, 31.4% after umbilical cord blood transplantation (UCBT), and 0% after allo-HSCT with matched unrelated or matched sibling donors ($P < .0001$). The incidence of PGF in UCBT recipients was 46.7% (14 of 30) in chemotherapy-naive recipients, versus 9.5% (2 of 21) in non–chemotherapy-naive recipients ($P = .019$). Five-year event-free survival was 59.5% ± 5%, and 5-year overall survival was 72.9% ± 5%. Only PGF and poor-risk disease status were significantly associated with decreased overall survival ($P = .03$). Reduced-toxicity conditioning allo-HSCT in pediatric recipients is associated with low TRM; however, chemotherapy-naive UCBT recipients have a significantly higher incidence of PGF.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) with myeloablative conditioning (MAC) is a well-established curative therapy for children and adults with various malignant and nonmalignant hematologic disorders, primary immunodeficiencies (PID), and metabolic diseases [1]. Reduced-toxicity conditioning (RTC) has emerged as an alternative to traditional MAC. RTC is defined as a regimen associated with various degrees of myeloablation, but with decreased toxicity secondary to conditioning compared with traditional MAC [2,3]. The purpose of RTC is to decrease transplantation-related mortality (TRM) while establishing a platform of host–donor tolerance through immunosuppression before and after transplantation.

Children with malignant and nonmalignant diseases who receive MAC allo-HSCT experience both short-term and long-term late complications [4]. TRM after MAC allo-HSCT depends on various factors, including performance status, disease type, disease status, allogeneic donor source, and quantity of committed stem progenitor cells infused [5-7]. According to recent Center for International Blood and Marrow Transplant Research analyses, the day +100 mortality rate is 5%-20% for patients with malignant and nonmalignant diseases after MAC HLA-matched sibling donor (MSD) allo-HSCT and 10%-40% after unrelated donor allo-HSCT [8]. In another recent study of children with leukemia and myelodysplastic syndrome (MDS), Shaw et al. [9] reported a cumulative 3-year incidence of TRM after MAC allo-HSCT was 10% for MSD graft recipients, compared with 27% for matched unrelated donor (MUD) graft recipients. TRM in children after MAC and umbilical cord blood (UBC) transplantation (UCBT) is significantly higher, ranging from 20% to 52% [6,10-12].

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Socié et al. [4] reported long-term survival and late effects after MAC allo-HSCT in 6691 patients who were free of their original disease for at least 2 years after transplantation. Numerous patients died of other secondary complications, including graft-versus-host disease (GVHD; 31%), infection (6%), secondary malignancies (6%), and organ failure (6%). More recently, Sun et al. [13] reported a 59% 10-year cumulative incidence of a chronic health condition and a 35% 10-year cumulative incidence of a severe life-threatening condition or death from a chronic health condition in MAC allo-HSCT recipients. Surviving MAC allo-HSCT recipients were twice as likely as siblings to develop a chronic condition and 3.5 times more likely to develop a severe/life-threatening condition [13].

These potential short-term and long-term complications sometimes factor strongly in the decision on whether or not to proceed with curative-intent therapy, especially in children with nonmalignant diseases. The feasibility of performing RTC allo-HSCT in medically infirm children has been recently reported by Pulsipher et al. [14] on behalf of the Pediatric Blood and Marrow Transplant Consortium. Jacobsohn et al. [15] also demonstrated the feasibility of RTC allo-HSCT in a small group of children with nonmalignant disorders who were also eligible for conventional MAC allo-HSCT.

Whether a select group of children receiving RTC allo-HSCT will benefit from a reduced risk of disease reoccurrence and at the same time a reduced risk of short-term and long-term complications remains to be determined. In the present single-center study, we examined the risk factors associated with TRM, primary graft failure (PGF), and overall survival (OS) in the first 100 consecutive children and adolescents who underwent RTC allo-HSCT for malignant and nonmalignant diseases at our institution.

PATIENTS AND METHODS

The cohort for this analysis comprised 100 consecutive children and adolescents who underwent RTC allo-HSCT at the New York-Presbyterian Morgan Stanley Children's Hospital between January 2001 and October 2010. Pilot data for a small number of patients receiving RTC with shorter follow-up have been reported [16,17]. The first pilot study [16] consisted of 21 patients, with 14 UCBT recipients, 3 MSC allo-HSCT recipients, and 4 MUD allo-HSCT recipients. In our second report, we described 21 pediatric RTC UCBT recipients.

Indications for transplantation included a variety of malignant and nonmalignant conditions. Allogeneic stem cell sources included bone marrow (BM), peripheral blood stem cells (PBSCs), and UBC. All patients were on a clinical research protocol for allo-HSCT approved by the Institutional Review Board at Columbia University Medical Center, and all research protocols were in compliance with the Declaration of Helsinki. This retrospective study was separately approved by the Institutional Review Board of Columbia University Medical Center.

Eligibility

Patients age 22 years with both malignant and nonmalignant disorders with or without previous comorbidities were eligible for RTC allo-HSCT. There were no open trials for RTC allo-HSCT in children with acute lymphoblastic leukemia. Adequate pretransplantation organ function was defined by organ system. Adequate renal function was defined as serum creatinine 2 times the normal value, creatinine clearance $\geq$40 mL/min/m^2, or a radioisotope glomerular filtration rate (GFR) >60 mL/min/1.73 m^2 or an equivalent GFR as determined by the institution's normal range. Adequate liver function was defined as total bilirubin <2 times normal and a serum glutamic oxaloacetic transaminase (aspartate aminotransferase) or serum glutamic pyruvate transaminase (alanine aminotransferase) value <5 times normal. Adequate cardiac function was defined as a shortening fraction of >27% detected by echocardiography, or an ejection fraction of >47% by radionuclide angiography or echocardiography. Adequate pulmonary function was defined as diffusing capacity of the lung for carbon monoxide >40% by pulmonary function testing or, in children who are uncooperative, no evidence of dyspnea at rest, no exercise intolerance, and a pulse oximetry reading of >94% in room air. A Lansky or Karnofsky performance score >40 was required for eligibility.

Exclusion Criteria

Patients who received CD34-selected cells and T cell–depleted transplants, haploidentical allo-HSCT, double UCBT, or a second RTC allo-HSCT as a rescue for previous graft failure after the first RTC allo-HSCT were excluded from our analyses.

HLA Typing and Stem Cell Source

HLA-A, -B (antigen match by intermediate resolution), -C, -DRB1, and -DQB1 (allele match by high resolution) typing was determined by hybridization of PCR-amplified DNA with sequence-specific oligonucleotide probes, as described previously [16]. Confirmatory typing was performed at Columbia University Medical Center. The criteria for graft matching included at least 4-6/6 loci for UBC and at least 8/10 loci for unrelated donor PBSCs/BM and 5-6/6 for MSD grafts. allo-HSCT was classified as HLA-mismatched with 1 or 2 differences if disparities were detected in HLA-A and -B antigens or in HLA-C, -DRB1, and -DQB1 alleles.

Conditioning Regimens

Specific conditioning regimens were protocol-driven and disease-specific. For the present study, we combined 3 different regimens that delivered lower chemotherapy doses than provided by the standard MAC regimen or with a second alkylating agent replaced by fludarabine. RTC regimens included BFA (busulfan 6.4-8 mg/kg and fludarabine 150 mg/m^2, with or without rabbit antithymocyte globulin [r-ATG] 8 mg/kg; n = 45), BFC (busulfan 12.8-16 mg/kg, fludarabine 150-180 mg/m^2, alemtuzumab 54 mg/m^2; n = 35), and FCA (cyclophosphamide 60 mg/kg and fludarabine 150 mg/m^2 with or without r-ATG 8 mg/kg; n = 20). RTC regimens in children with malignant disease were restricted to those with acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), lymphoma, and neuroblastoma. Only patients who underwent UCBT and MUD allo-HSCT received r-ATG. The majority of children with severe aplastic anemia, PID, and leukodystrophies received cyclophosphamide 60 mg/kg + fludarabine 150 mg/m^2. A regimen comprising busulfan 6.4-8 mg/kg + fludarabine 150-180 mg/m^2 was administered to children with all malignant diseases except CML, and a regimen consisting of busulfan 12.8-16 mg/kg, fludarabine 180 mg/m^2, + alemtuzumab 54 mg/m^2 was used in children at high risk of graft failure, such as those with hemoglobinopathies and CML. Busulfan pharmacokinetic studies were performed in children who received busulfan 12.8-16 mg/kg, as reported previously [3]. The target steady-state busulfan concentration after the first dose and subsequent doses was 600-900 ng/mL.

GVHD Prophylaxis and Grading

Acute GVHD (aGVHD) prophylaxis consisted of tacrolimus starting at 0.03 mg/kg/day as continuous i.v. infusion or 0.12 mg/kg orally twice a day, with dosage adjustments to maintain blood levels between 5 and 20 ng/mL, along with mycophenolate mofetil (MMF) 15-30 mg/kg every 6-12 hours either orally or i.v., as described previously [18,19]. Tacrolimus was started on the first day of conditioning, and MMF was initiated on day +1. Tacrolimus and MMF were tapered if grade II aGVHD developed between day +30 and day +60 in patients with malignant disease and by day +180 in patients with nonmalignant disease [19]. aGVHD and chronic GHVD (cGVHD) were graded according to the Seattle criteria [20]. Adult recipients of MUD allo-HSCT also received methotrexate 15 mg/m^2 administered i.v. on day +1, followed by 10 mg/m^2 via slow i.v. push on days +3, +6, and +11.

Engraftment and Donor Chimerism

Myeloid engraftment was defined as an absolute neutrophil count (ANC) of $\geq$500 $\times$ 10^9/L on the first of 3 consecutive days. Platelet recovery was defined as the first day of the 7 days on which the platelet count was $\geq$20 $\times$ 10^9/L independent of platelet transfusion. Donor myeloid and/or lymphoid chimerism was measured on days +30, +60, +100, +180, and +365 post-transplantation. Percent donor chimerism was determined by quantifying fluorescent-labeled PCR products from donor and recipient alleles at short tandem repeat loci, as described previously [3,16]. Donor chimerism was determined for whole blood and cell subsets as required by individual disease protocols. Cell subsets were isolated using Miltenyi (Miltenyi Biotec, Bisley, UK) magnetic separation.

Infection Prophylaxis and Supportive Care

All patients received sargramostim 250 μg/m^2/day i.v. from day 0 until a WBC count of >300 $\times$ 10^9/L was measured on 2 days, and were then switched to either i.v. or s.c. filgrastim (10 μg/kg/day) until an ANC of 2500 $\times$ 10^9/L was measured on 3 days, as described previously [21]. Herpes simplex virus prophylaxis consisted of acyclovir 250 mg/m^2 i.v. every 8 hours from day −5 until engraftment and development of grade II mucositis. *Pneumocystis carinii* prophylaxis consisted of trimethoprim/sulfamethoxazole up to day −2 and then 3 times weekly after myeloid engraftment. Patients unable to tolerate trimethoprim/sulfamethoxazole received i.v. pentamidine every

Table 1
Demographic Data for Pediatric RTC Allo-HSCT Recipients

Parameter	All (n = 100)	MSD/MUD allo-HSCT (n = 49)	UCBT (n = 51)	*P* Value
Age, years, mean ± SD	9.24 ± 6.79	11.01 ± 6.19	7.54 ± 6.97	.010
Sex, n (%)				.926
Male	71 (71)	35 (71.4)	36 (70.6)	
Female	29 (29)	14 (28.6)	15 (29.4)	
Follow-up, days, mean ± SD	1329.01 ± 1060.9	1404.3 ± 1006	1256.6 ± 1116.3	.489
Diseases, n (%)				.317
Malignant	50 (50)	27 (55.1)	23 (45.1)	
Nonmalignant	50 (50)	22 (44.9)	28 (54.9)	
Disease status, n (%)				.030
Average risk	89 (89)	47 (95.9)	42 (82.4)	
Poor risk	11 (11)	2 (4.1)	9 (17.6)	
Previous autologous SCT, n (%)				.281
No	77 (77)	40 (81.6)	37 (72.6)	
Yes	23 (23)	9 (18.4)	14 (27.4)	
Chemotherapy-naivety, n (%)				.865
Yes	58 (58)	28 (57.1)	30 (58.8)	
No	42 (42)	21 (42.9)	21 (41.2)	
CMV status, n (%)				.864
High	58 (58)	29 (59.2)	29 (56.9)	
Intermediate	12 (12)	5 (10.2)	7 (13.7)	
Low	30 (30)	15 (30.6)	15 (29.4)	
Major ABO incompatibility, n (%)				.390
Yes	22 (22)	9 (18.4)	13 (25.5)	
No	78 (78)	40 (81.6)	38 (74.5)	
Regimen, n (%)				.433
Bu/Flu/alemtuzumab	35 (35)	20 (40.8)	15 (29.4)	
Bu/Flu ± r-ATG	45 (45)	21 (42.9)	24 (47.1)	
Flu/Cy ± r-ATG	20 (20)	8 (16.3)	12 (23.5)	
Performance status, n (%)				.015
Pre-RTC allo-HSCT >70	85 (85)	46 (93.4)	39 (76.5)	
≤70	15 (15)	3 (6.6)	12 (23.5)	

Bu indicates busulfan; Flu, fludarabine; Cy, cyclophosphamide; r-ATG, rabbit antithymocyte globulin.

2 weeks. Fungal prophylaxis consisted of liposomal amphotericin B 3 mg/kg/day i.v. starting on day 0 and continuing through day +100, as described previously [22]. Cytomegalovirus (CMV) prophylaxis was administered as described previously [23]. In brief, allo-HSCT recipients at risk of acquiring CMV infection (CMV-positive donors and/or recipients) after achieving an ANC >750 × 10^9/L received prophylaxis with foscarnet 90 mg/kg/dose every other day, alternating with ganciclovir 5 mg/kg/dose every other day up to day +100.

Definitions

Major ABO incompatibility was defined as donor blood type A, AB, or B and recipient blood type O. CMV risk status was considered high if only the recipient or the donor was CMV-positive, intermediate if both the recipient and donor were CMV-positive, and low if both the recipient and donor were CMV-negative. PGF was defined as failure to achieve a donor-derived ANC >500 × 10^9/L by day +42 and/or ≤50% whole blood donor chimerism by day +60 in all patients except those with immune deficiency. In patients with T cell or combined immune deficiency, PGF was defined as ≤50% T cell (CD3) donor chimerism by day +180. Patients who had received a second stem cell infusion before day +42 for impending graft failure were also considered to have PGF. CMV and adenovirus disease were defined as described previously [24]. TRM was defined as death due to any transplantation-related cause other than disease relapse.

Event-free survival (EFS) for patients with malignant disease was defined as relapse/persistence of the disease and death due to any cause other than disease relapse. EFS for nonmalignant diseases was defined as persistence of disease, graft failure, and death due to any cause. OS was defined as survival with or without the original disease. Disease-free survival (DFS) for patients with malignant disease was defined as survival without the original disease.

Poor-risk patients were defined as patients with malignant disease and either chemotherapy-resistant disease in third or greater complete remission (CR), with induction failure, or progressive disease. All other patients with malignant and nonmalignant diseases were defined as average risk.

Statistical Methods

The continuous variables were summarized as mean ± standard deviation, and categorical variables were summarized as percentages. The Kaplan-Meier product limit estimator was used for estimating OS and PGF. The probabilities of EFS, TRM, aGVHD, cGVHD, neutrophil recovery, and platelet recovery were estimated by the cumulative incidence function estimator. The competing risk for TRM was disease relapse, and the competing risk for relapse was TRM. The cause-specific proportional hazards model was used to analyze TRM. For aGVHD and cGVHD, death without an event, relapse, and PGF were competing risks. The competing risk for neutrophil and platelet recovery was death before recovery. In OS and EFS analysis, PGF was treated as a time-dependent covariate. The estimated probabilities were summarized along with standard error of the mean. The log-rank test was used to assess the difference in these probabilities among different groups. The Cox proportional hazards model was used to adjust risk factors.

In univariate analysis for OS and TRM, risk factors analyzed included age, sex, donor stem cell source, disease status, disease type, chemotherapy-naivety, CMV risk status, ABO incompatibility, regimen received, performance status, infection (viral, bacterial, and fungal), previous autologous HSCT, PGF, aGVHD and cGVHD, percent donor chimerism, infused total nucleated cell (TNC) dose, and infused CD34 cell dose.

In univariate analysis for OS in UCBT recipients who developed PGF, risk factors analyzed included age, sex, disease status, disease type, chemotherapy-naivety, CMV risk status, ABO incompatibility, regimen received, infections (viral, bacterial, and fungal), infused TNC dose (≤5 versus >5 × 10^7/kg), and infused CD34 cell dose (≤1.7 versus >1.7 × 10^5/kg). Any covariates with a *P* value <.20 on univariate analysis were included in the multivariate analysis.

RESULTS

Demographic Data

We prospectively evaluated 100 consecutive patients enrolled in our RTC allo-HSCT protocols (Table 1). There were 71 males and 29 females; with a mean ± standard deviation (SD) age of 9.24 ± 6.79 years. The mean follow-up duration was 1329.01 ± 1060.9 days. Fifty patients had malignant disease, and 50 patients had nonmalignant disease. The distribution of malignant diseases included 14 patients with AML (10 in CR1 and 4 in CR2), 4 with MDS, 6 with chronic phase CML, 7 with relapsed non-Hodgkin lymphoma, 9 with relapsed/refractory Hodgkin lymphoma, and 10 with high-risk neuroblastoma. The distribution of nonmalignant diseases included 23 patients with hemoglobinopathies, 10

with severe aplastic anemia, 9 with PID, 3 with leukodystrophies, and 5 with other diseases. Twenty-three patients with lymphoma and neuroblastoma underwent planned MAC autologous HSCT before RTC allo-HSCT as required by their specific clinical research trial. Eleven patients (11%) had poor-risk disease. Fifty-eight patients were chemotherapy-naive, and 42 patients had received previous chemotherapy. A major blood group mismatch was noted in 22 donor–recipient pairs. Thirty donor–recipient pairs were CMV-negative and 70 patients, either donor or recipient or both, were CMV-positive. Fifteen patients (15%) had a Lansky or Karnofsky score of ≤70, 12 patients (12%) had a score of 80, and 73 patients (73%) had a score of 90-100 (Table 1).

Donor Sources, Hematopoietic Reconstitution, and Donor Chimerism

Forty-one patients received allogeneic hematopoietic stem cells from a 5-6/6 HLA-matched family donor (HLA matching, 33 6/6 sibling donors, 7 5/6 sibling donors, and 1 6/6 maternal donor). Eight patients received an MUD graft (HLA matching, 2 8/10, 5 9/10, and 1 10/10). Twenty-four of the 49 patients who underwent MSD/MUD allo-HSCT had a malignant disease; 18 of these patients received PBSCs, and the other 6 received BM infusions. All 25 MSD/MUD allo-HSCT recipients with nonmalignant disease received BM infusions. Fifty-one patients underwent single-unit UCBT (HLA matching, 25 4/6, 20 5/6, and 6 6/6). Median TNC and CD34 cell doses infused in MRD graft recipients were 10.9×10^7/kg (range, 0.9×10^7-189.7×10^7) and 5×10^6/kg (range, 0.2-16.4 $\times$ 10^6), respectively. Median TNC and CD34 cell doses infused in UCBT recipients were 5.0×10^7/kg (range, 0.9×10^7-42×10^7) and 2.5×10^5/kg (range, 0.3×10^5-9.6×10^5), respectively. Median TNC and CD34 cell doses infused in MUD graft recipients were 76.2×10^7/kg (range, 3.4×10^7-189.7×10^7) and 5×10^6/kg (range, 2.1×10^5-16.4×10^6), respectively. Mean ± SD values are presented in Table 2.

The cumulative incidence of neutrophil engraftment was 98% (95% CI, 92.9%-100%) in MSD/MUD graft recipients and 70.6% (95% CI, 57.8%-83.4%) in UCBT recipients. The cumulative incidence of platelet engraftment was 89.8% (95% CI, 80.8%-98.8%) in MSD/MUD graft recipients and 58.8% (95% CI, 45.0%-72.6%) in UCBT recipients. In the patients who engrafted (n = 84), the median time to neutrophil engraftment was 18 days (Figure 1A), and the median time to platelet engraftment was 43 days (Figure 1B). The median times to neutrophil and platelet engraftment after MSD/MUD allo-HSCT were 15 days (95% CI, 14-17 days) and 18 days (95% CI, 15-27 days), respectively. The median times to neutrophil and platelet engraftment after UCBT were 32 days (95% CI, 27-45 days) and 79 days (95% CI, 56-171 days), respectively. There were no statistically significant differences in donor chimerism values; median (range) donor chimerism for engrafted UCBT recipients was 91% (1%-100%) on day +30, 98% (14%-100%) on day +100, and 98% (55%-100%) on day +365, whereas median donor chimerism for MSD/MUD allo-HSCT recipients was 99% (35%-100%) on day +30, 99% (40%-100%) on day +100, and 100% (75%-100%) on day +365 (Figure 1C).

Table 2
Allogeneic Donor Sources, HLA Disparity, and Cell Dose in Pediatric RTC Allo-HSCT Recipients

Parameter	All	MSD/MUD allo-HSCT	UCBT	*P* Value
HLA match, n (%)				<.0001
4/6	25 (25)	0 (0)	25 (49.0)	
5/6	27 (27)	7 (14.3)	20 (39.2)	
6/6	40 (40)	34 (69.4)	6 (11.8)	
8-9/10	7 (7)	7 (14.3)	0 (0)	
10/10	1 (1)	1 (2.0)	0 (0)	
TNC dose, $\times 10^7$/kg, mean ± SD	40.4 ± 47.4	75.1 ± 46.0	6.5 ± 6.4	<.0001
CD34 cell dose, $\times 10^5$/kg, mean ± SD	26.4 ± 32.3	50.0 ± 31.1	2.8 ± 2.0	<.0001

PGF

PGF occurred in 16 patients (16%) after RTC allo-HSCT, with all cases occurring in UCBT recipients and none in MUD/MSD graft recipients (31.4% versus 0%; $P < .0001$). The incidence of PGF in UCBT recipients who were chemotherapy-naive versus those who were not chemotherapy-naive was 46.7% (14/30) versus 9.5% (2/21) ($P = .019$). Among the 51 UCBT recipients, the incidence of PGF was 54% (6 of 11) with the FCA regimen, 16% (4 of 24) with the BFA regimen, and 37% (6 of 16) with the BFC regimen (Table 3). PGF was noted in 12 of 50 patients (24%) with nonmalignant disease and in 4 of 50 patients (8%) with malignant disease. Of the 29 UCBT recipients with nonmalignant disease, 12 (41%) developed PGF, including 6 of 16 (37%) with the BFC regimen, 6 of 11 (54%) with the FCA regimen, and 0 of 2 with the BFA regimen. The distribution of PGF was 5 hemoglobinopathies, 2 hemophagocytic lymphohistiocytosis, 3 AML/MDS, 1 recessive dystrophic epidermolysis bullosa, 1 scleroderma, 1 CML, 1 mitochondrial neurogastrointestinal encephalomyopathy, 1 PID, and 1 Wiskott-Aldrich syndrome; 8 of 16 patients with PGF are alive.

We performed univariate analysis for various risk factors associated with PGF after UCBT. Only chemotherapy-naivety (hazard ratio [HR], 5.19; 95% CI, 1.18-22.89; $P = .030$) was a significant risk factor for PGF. Nonmalignant disease trended as a risk factor for PGF ($P = .077$). However, disease risk status ($P = .30$), CMV and adenovirus infection ($P = .40$), TNC dose ($P = .60$), and $CD34^+$ cell dose ($P = .70$) were not significant for the risk of developing PGF. However, on multivariate analysis, neither disease type ($P = .90$; HR, 0.9; 95% CI, 0.2-3.6) nor chemotherapy-naivety ($P = .10$; HR, 3.9; 95% CI, 0.6-23.6) was significantly associated with PGF.

aGVHD and cGVHD

The probability of grade II-IV aGVHD in the engrafted cohort was 20% (95% CI, 12.1%-27.9%), and was 24.5% (95% CI, 12.3%-36.7%) in MSD/MUD graft recipients versus 15.7% (95% CI, 5.6%-25.8%) in UCBT recipients ($P = .36$) (Figure 2A). The probability of cGVHD in the engrafted cohort was 13.5% (95% CI, 6.6%-20.4%), and was 21.4% (95% CI, 9.4%-33.4%) in MSD/MUD graft recipients versus 6.1% (95% CI, 0%-12.97%) in UCBT recipients ($P = .024$) (Figure 2B).

TRM

TRM was 3% (95% CI, 0%-6.4%) by day +100 and 13.6% (95% CI, 6.7%-20.5%) for the entrire study period (Figure 3). For the entire study period, TRM was 8.4% (95% CI, 0.4%-16.4%) for patients with malignant disease and 18.8% (95% CI, 7.5%-30.0%) for patients with nonmalignant disease. Causes of TRM in the first 100 days included viral infections (n = 2) and veno-occlusive disease (VOD; n = 1). Over the entire study period, 11 patients died due to TRM; these deaths were related to viral infection (n = 4), GVHD (n = 2), complications related to second allo-HSCT in patients with PGF (n = 3),

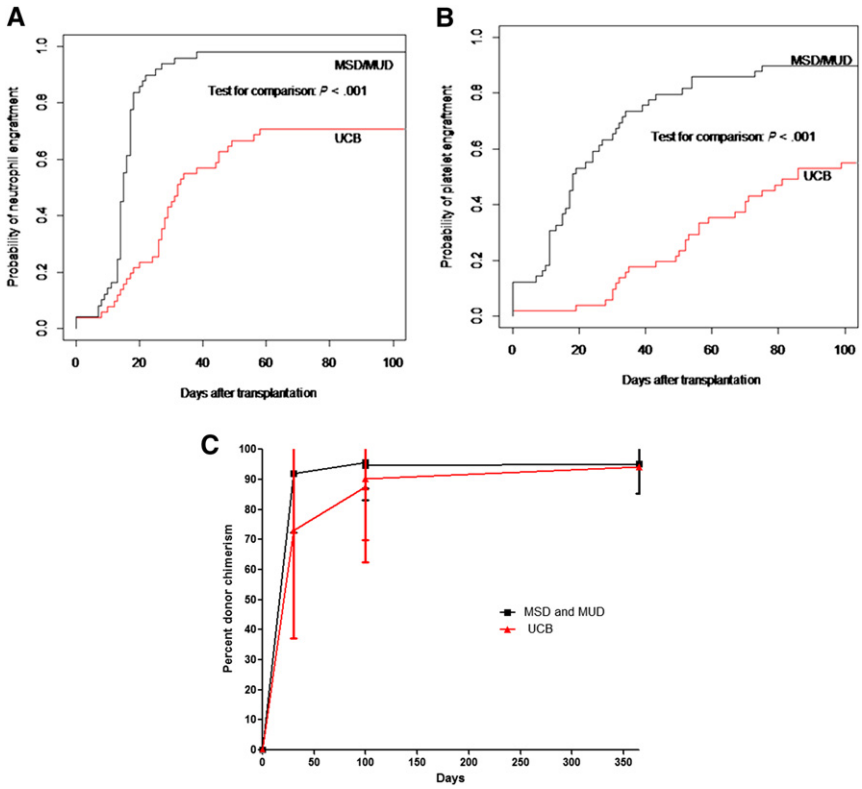

Figure 1. (A and B) Probability of neutrophil engraftment (A) and platelet engraftment (B) after RTC allo-HSCT in children and adolescents with malignant and nonmalignant diseases. (C) Percent donor chimerism (mean ± SD) after RTC allo-HSCT in engrafted children and adolescents with malignant and nonmalignant diseases.

transplantation-associated thrombotic microangiopathy (n = 1), and VOD (n = 1). The vast majority of TRM (10 of 11 cases) occurred within the first year after RTC allo-HSCT. Various risk factors associated with TRM were analyzed with competing-risk regression analysis, with relapse as a competing event. In the univariate analysis, risk factors with a $P \leq .20$ included male sex ($P = .17$), chemotherapy-naivety ($P = .12$), disease type ($P = .11$), high CMV risk status ($P = .18$), fludarabine/cyclophosphamide regimen ($P = .082$), and PGF ($P = .002$). However, in the multivariate analysis of TRM using variables significant at ≤.20 from the univariate analysis, only PGF (relative risk, 3.92; 95% CI, 1.353-11.36; $P = .012$) was significantly associated with TRM (Table 4).

Survival after RTC Allo-HSCT

The 5-year EFS for the entire cohort was 59.5% ± 5% (95% CI, 50.1%-70.6%) (Figure 4A), and was 78.2% (67.1%-91.2%) in

Table 3
Characteristics and Outcomes of Children with PGF after RTC Allo-HSCT with UCB

Patient	Age, Years	Diagnosis	Chemotherapy-Naive	Regimen	TNC, 10^7/kg	CD34, 10^5/kg	Probable Cause of PGF	Autologous Recovery	Outcome
1	1	β-thalassemia	Yes	FCA	9.5	3.69	Unknown	Yes	Alive after MAC UCBT (day +3294)
2	14	CML	Yes	BFA	1.26	0.77	Unknown	Yes	Lost to follow-up
3	15	HLH	No	FCA	1.4	0.34	Unknown	Yes	Alive, NED (day +2929)
4	1	MDS	Yes	BFA	7.99	2.88	Unknown	Yes	Alive after MAC UCBT (day +2864)
5	1	HLH	No	FCA	5.88	2.55	Unknown	Yes	Alive after MAC UCBT (day +2771)
6	3	AML	No	BFA	3.96	2.68	Disease	Yes	Died, progressive disease
7	2	WAS	Yes	FCA	4.97	3.65	Unknown	No	Died after second RTC UCBT
8	21	MNGIE	Yes	FCA	3.02	0.62	Unknown	Yes	Died, progressive disease
9	16	MDS	Yes	BFA	1.7	0.57	Unknown	No	Died due to bacterial infection
10	1	SCD	Yes	BFC	4.3	2.58	Unknown	Yes	Alive with disease (day +1797)
11	10	Scleroderma	Yes	BFC	4.8	4.18	CMV	Yes	Alive with disease (day +1222)
12	2	SCD	Yes	BFC	6.95	2.09	CMV	Yes	Died due to CMV
13	6	SCD	Yes	BFC	3.9	1.9	CMV	No	Died after second MAC-MUD
14	0.6	PID	Yes	FCA	15.2	17	Unknown	Yes	Alive after MAC UCBT (day +766)
15	2	SCD	Yes	BFC	5.34	0.6	Adenovirus	No	Died after second RTC UCBT
16	1	RDEB	Yes	BFC	12.48	3.28	Strenotrophomonas infection	No	Died after second RTC UCBT (VOD)

HLH indicates hemophagocytic lymphohistiocytocis; WAS, Wiskott-Aldrich syndrome; MNGIE, mitochondrial neurogastrointestinal encephalopathy; SCD, sickle cell disease; RDEB, recessive dystrophic epidermolysis bullosa; FCA, fludarabine/cyclophosphamide/antithymocyte globulin; BFA, busulfan/fludarabine/antithymocyte globulin; BFC, busulfan/fludarabine/Campath; NED, no evidence of disease.

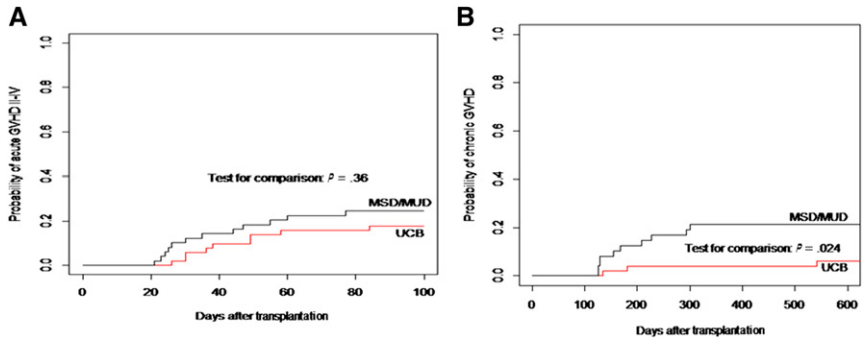

Figure 2. Probability of grade II-IV aGVHD (A) and cGVHD (B) after RTC allo-HSCT in children and adolescents with malignant and nonmalignant diseases.

MSD/MUD RTC allo-HSCT recipients versus 41.6% (95% CI, 29.3%-59.2%) in UCBT recipients ($P < .0001$). The 5-year OS for the entire cohort was 72.9% (95% CI, 64.2%-82.8%), and was 84.3% (95% CI, 74.2%-95.8%) in MSD/MUD RTC allo-HSCT recipients versus 62.0% (95% CI, 49.3%-78.1%) in UCBT recipients ($P < .0001$), respectively (Figure 4B).

All of the surviving patients with malignant diseases had more than 1 year of follow-up. Twenty-five patients received RTC allo-HSCT in CR, 19 remain in CR, 5 relapsed (died), and 1 experienced TRM. Twenty-five patients underwent RTC allo-HSCT without achieving CR, 11 relapsed (8 died, 2 are alive, and 1 was lost to follow-up), 10 achieved CR, and 4 experienced TRM. Our patients who underwent RTC allo-HSCT for malignant disease had a 32% incidence of relapse, a TRM of 10%, an OS of 64%, and a DFS of 58%.

In our multivariate analysis of risk factors associated with OS, only poor-risk disease status (HR, 3.73; 95% CI, 1.2-11.5; $P = .02$), intermediate CMV risk status (HR, 5.6; 95% CI, 1.4-22.33; $P = .01$), and PGF (HR, 4.06; 95% CI, 1.39-11.81; $P = .01$) were associated with significantly worse OS (Table 5). Because RTC UCBT was associated with a significantly poorer OS compared with MUD/MSD allo-HSCT, we further analyzed various risk factors that could be associated with poor OS in children after RTC UCBT. Risk factors in the univariate analysis with $P \leq .20$ included age ($P = .09$), poor-risk disease ($P = .002$), malignant disease ($P = .18$), intermediate CMV risk status ($P = .138$), and PGF ($P = .038$). In the multivariate analysis of OS in children receiving RTC UCBT, only poor-risk disease status (HR, 5.96; 95% CI, 1.57-22.54; $P = .009$), intermediate CMV risk status (HR, 4.92; 95% CI, 1.09-22.26), and PGF (HR, 4.54; 95% CI, 1.49-13.79) were significantly associated with decreased OS (Table 6).

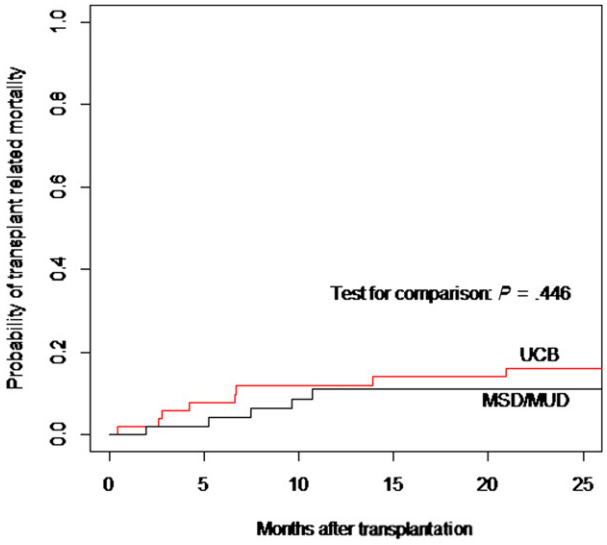

Figure 3. Probability of TRM after RTC allo-HSCT in children and adolescents with malignant and nonmalignant diseases.

DISCUSSION

Here we report the largest study to date of RTC allo-HSCT using both related and unrelated allogeneic stem cell sources in pediatric recipients with both malignant and nonmalignant diseases. In the last several years, the use of RTC allo-HSCT has expanded from adults with high comorbidity indices to adult allo-HSCT candidates without comorbidities [25,26]. The notion is prevalent that because children usually do not have the comorbidities typical of adults (eg, chronic hypertension, diabetes mellitus, chronic coronary ischemic disease, smoking-related illnesses), they can tolerate MAC. However, heavily pretreated children are at risk for early TRM and long-term morbidities. A recently published multicenter retrospective study analyzed the impact of an allo-HSCT–specific comorbidity index (HCT-CI) on TRM in children with malignant and nonmalignant diseases. Children with an HCT-CI score of 3+ had a 1-year TRM of 36% after MAC allo-HSCT and 19% after reduced-intensity/non-

Table 4
Multivariate Competing-Risk Analysis of TRM with Variables Significant at $P \leq .20$ in Univariate Analysis in Pediatric RTC Allo-HSCT Recipients

Parameter	RR	95% CI	*P* Value
Sex			
Male (n = 71)	1		
Female (n = 29)	1.70	0.442-6.53	.440
Diseases			
Nonmalignant (n = 50)	1		
Malignant (n = 50)	0.59	0.155-2.25	.440
Chemotherapy-naive			
No (n = 42)			
Yes (n = 58)	1.96	0.408-9.38	.400
CMV risk status			
Low (n = 32)	1		
High (n = 58)	2.88	0.545-15.28	.210
Intermediate (n = 12)	1.24	0.059-25.97	.890
Regimen			
Bu/Flu/r-ATG (n = 45)	1		
Bu/Flu/alemtuzumab (n = 35)	1.01	0.264-3.89	.980
Flu/Cy/r-ATG (n = 20)	1.44	0.297-7.00	.650
PGF			
No (n = 84)	1		
Yes (n = 16)	3.92	1.353-11.36	.012

Bu indicates busulfan; Flu, fludarabine; r-ATG, rabbit antithymocyte globulin; Cy, cyclophosphamide; RR, relative risk.

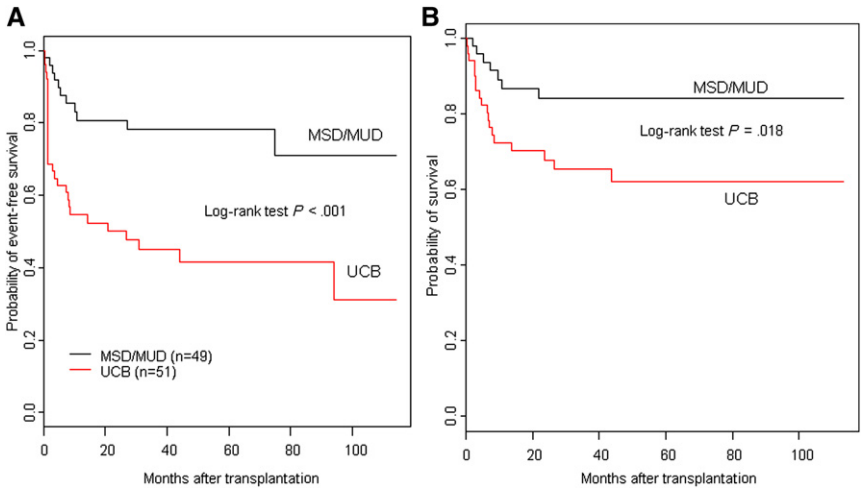

Figure 4. EFS (A) and OS (B) after RTC allo-HSCT in children and adolescents with malignant and nonmalignant diseases.

myeloablative conditioning [27]. Children who may have a 60- to 70-year life expectancy after undergoing allo-HSCT may benefit from the approach of RTC allo-HSCT versus MAC allo-HSCT, especially those with nonmalignant diseases and those with malignant diseases that may have a profound graft-versus-tumor effect [28-30].

TRM is a major concern after allo-HSCT. Several small pediatric studies have reported TRM of 13%-40% after RTC allo-HSCT [14,15,31-33]. However, Jacobsohn et al. [15] reported a 15% day +100 TRM in children undergoing RTC allo-HSCT for nonmalignant disease. One of the most important findings in the present analysis was the extremely low probability of TRM both at day +100 (3%) and in the entire study period (13.6%). Only 3 patients experienced TRM within the first 100 days after RTC allo-HSCT, including 2 patients who died of systemic viral infection (SVI) and 1 patient who died of severe VOD. We previously analyzed the incidence of both viral and fungal infections in pediatric RTC allo-HSCT and MAC allo-HSCT recipients, but were unable to demonstrate a significant reduction in the incidence of SVI in the RTC allo-HSCT recipients [24]. This finding is related in part to our previous report demonstrating no significant differences in T cell immune reconstitution, especially after UCBT, in pediatric RTC allo-HSCT and MAC allo-HSCT recipients [34,35]. More important, the reported TRM in pediatric MAC allo-HSCT recipients is 5%-20% when using HLA-matched related donors and 10%-50% when using unrelated donors, particularly unrelated UBC [8,10,11,36]. Our finding of a TRM of only 3% in the first 100 days after RTC allo-HSCT, even with 50% of the patients receiving UBC grafts, represents a dramatic improvement over MAC allo-HSCT in the pediatric population. Jacobsohn et al. [15] reported a day +100 TRM of 15% in children undergoing RTC allo-HSCT for nonmalignant disease.

In the present analysis, the median time to myeloid and platelet engraftment after RTC allo-HSCT in pediatric recipients was 18 and 43 days, respectively, consistent with reports after MAC allo-HSCT. Most important, in patients who achieved donor engraftment, both early (day 30-100) and late (1 year) donor chimerism were robust, ranging from 95% to 98%. This long-term high-level donor chimerism after RTC allo-HSCT was remarkable and also consistent with reports after MAC allo-HSCT, suggesting that despite the use

Table 5
Multivariate Analysis of OS with Variables Significant at $P \leq .20$ in Univariate Analysis

Parameter	HR	95% CI	*P* Value
Age	1.045	0.976-1.119	.203
Donor			
MSD (n = 41)	1		
MUD (n = 8)	0.593	0.064-5.523	.646
UCB (n = 51)	1.288	0.416-3.984	.661
Disease risk status			
Average (n = 89)	1		
Poor (n = 11)	3.731	1.206-11.542	.022
CMV risk status			
Low (n = 30)	1		
Intermediate (n = 12)	5.614	1.411-22.338	.014
High (n = 58)	2.936	0.858-10.049	.086
Regimen			
Bu/Flu ± r-ATG (n = 45)	1		
Bu/Flu/alemtuzumab (n = 35)	0.926	0.353-2.426	.875
Flu/Cy ± r-ATG (n = 20)	0.675	0.189-2.407	.545
Performance status			
>70 (n = 85)	1		
<70 (n = 15)	1.247	0.318-4.890	.752
Fungal infection			
No (n = 92)	1		
Yes (n = 8)	2.094	0.550-7.968	.278
PGF			
No (n = 84)	1		
Yes (n = 16)	4.058	1.393-11.817	.010

Bu indicates busulfan; Flu, fludarabine; r-ATG, rabbit antithymocyte globulin; Cy, cyclophosphamide.

Table 6
Multivariate Analysis of OS in Children after RTC Allo-HSCT with UCB (n = 51) with Variables Significant at $P \leq .20$ in Univariate Analysis

Parameter	HR	95% CI	*P* Value
Age	1.024	0.941-1.114	.589
Disease risk status			
Average risk (n = 42)	1		
Poor risk (n = 9)	5.957	1.574-22.541	.009
Disease type			
Nonmalignant (n = 30)	1		
Malignant (n = 21)	1.035	0.249-4.298	.963
CMV risk status			
Low (n = 15)	1		
High (n = 28)	2.009	0.604-6.685	.255
Intermediate (n = 8)	4.642	1.019-21.139	.047
PGF			
No	1		
Yes	4.538	1.494-13.786	.008

of a reduced-intensity conditioning regimen, long-term high-level donor chimerism is persistent in pediatric RTC allo-HSCT recipients. These long-term donor chimerism results are consistent with previous smaller studies of pediatric RTC allo-HSCT recipients from our group and other groups [3,14-17,37-39].

The higher-than-expected incidence of PGF (16%) in the present study appeared to be concentrated in the subgroup of RTC UCBT recipients. No PGF was seen in the RTC MSD/MUD allo-HSCT recipients. These findings are in contrast to the results of studies reporting an ~33% incidence of PGF in a subgroup of RTC UCBT recipients, which were 1-1.5 times higher than that in MAC UCBT recipients [38,40]. The risk of PGF was significantly higher in the chemotherapy-naive RTC recipients compared with the non–chemotherapy-naive RTC UCBT recipients. On multivariate analysis, chemotherapy-naivety was not significantly associated with PGF, likely owing to the small number of patients; however, the HR was 3.9. The 10% incidence of PGF in our non–chemotherapy-naive RTC UCBT recipients is similar to the incidence reported in pediatric MAC UCBT recipients. The majority of cases of PGF after RTC UCBT occurred in children with hemoglobinopathies and other nonmalignant conditions, in agreement with previous reports [30,37,38,41]. These patients with PGF and the RTC UCBT recipients were treated on different studies, and owing to the small numbers of graft failures in each study stopping criteria to perform UCBT were not met. However, when we compiled data from all of these studies, we deemed the graft failure rates unacceptable. Thus, we no longer perform RTC UCBT for patients with chemotherapy-naive diseases at our center. Several factors are likely associated with the high incidence of PGF in the chemotherapy-naive RTC UCBT recipients, including 1 log lower TNC and CD34 doses, reduced myeloablation, history of blood cell–sensitizing transfusions, and primary BM disorders, among others; these factors alone or in combination may lead to a increased incidence of PGF after RTC UCBT in pediatric recipients with nonmalignant disease. The optimal RTC regimen before UCBT that will be associated with donor engraftment is unclear. In the present study, the incidence of PGF was identical after the FCA regimen and after the more-intense BFC regimen. Future studies of RTC should explore alternative RTC conditioning regimens to increase the incidence of donor engraftment after UCBT.

We did not notice any PGF in MSD/MUD alloHSCT recipients, and thus did not further analyze differences in the risk of PGF in recipients of BM infusions and recipients of PBSC infusions. However, in a prospective study of 47 children with malignant diseases, PGF was reported in patients who received a BM infusion after RTC [16].

The probability of grade II-IV aGVHD in our pediatric RTC allo-HSCT recipients was only 20%, which was lower than what would have been predicted in MAC allo-HSCT recipients. This lower incidence of grade II-IV aGVHD in pediatric RTC allo-HSCT recipients is similar to what has been described in adults after either reduced-intensity conditioning or RTC allo-HSCT [42-44]. Several factors likely account for this lower incidence of grade II-IV aGVHD after RTC allo-HSCT, including decreased acute tissue damage after RTC allo-HSCT and transient and slower donor chimerism early after allo-HSCT, which may promote host–donor tolerance, thereby reducing early aGVHD [29]. The 13% incidence of cGVHD in the present study is largely consistent with the incidence after MAC UCBT reported previously [7,10,16,35,36,40,45].

The probability of OS in RTC allo-HSCT recipients was 72%, and was significantly higher in MSD/MUD graft recipients compared with UCBT recipients (84% versus 62%). This probability is similar to or, in some instances, improved over previously reported values in pediatric MAC allo-HSCT recipients [8,10,36]. In a multivariate analysis of risk factors associated with poor OS in RTC allo-HSCT recipients, poor-risk disease status and intermediate CMV risk were associated with significantly poorer OS. Why CMV intermediate risk status is associated with poor OS is not clear, given that these patients receive similar CMV prophylaxis as patients with high-risk CMV status. Only 3 of the 11 cases of TRM in the study population can be attributed to PGF, related in part to the RTC regimen. The other 8 deaths (4 due to SVI, 2 due to GVHD, 1 due to transplantation-associated thrombotic microangiopathy, and 1 due to VOD) are all related to known complications after allo-HSCT, and especially after MAC. The excellent 5-year OS after RTC allo-HSCT in our pediatric recipients with malignant and nonmalignant disease is encouraging, but await confirmation in a larger and more uniform cohort.

The presence of measurable disease at the time of RTC allo-HSCT affects outcome. Pulsipher et al. [14] reported DFS of 75% in patients who underwent RTC allo-HSCT in CR, compared with 17% in those who underwent RTC allo-HSCT without achieving CR [14]. In our series, patients undergoing RTC allo-HCST in CR had a DFS of 76%, compared with 40% in those who did not achieve CR. Efforts should focus on achieving CR before RTC allo-HSCT, to decrease the risk of relapse. If this is not possible, then MAC allo-HSCT should be performed if clinically feasible.

This study has several limitations that should be considered when interpreting our results. The patient population was heterogeneous in terms of diseases, disease status, and history of previous chemotherapy. In addition, the allogeneic donor sources were heterogeneous and included HLA MSDs, MUDs, and UBC. The RTC regimens varied somewhat, but were all fludarabine-based; 80% included busulfan, and 20% included cyclophosphamide, and the majority included either r-ATG or alemtuzumab. The analyses were performed retrospectively, and there was no concurrent MAC allo-HSCT cohort. Nonetheless, GVHD prophylaxis and supportive care were uniform in all patients, and the heterogeneity of the patients and donor sources allows for more generalizability across pediatric RTC allo-HSCT recipients. Another limitation of this study is that although theoretically RTC allo-HSCT should be associated with reduced long-term toxicity, we have no long-term data to support or refute this association.

In summary, this study represents the largest series of pediatric RTC allo-HSCT recipients reported to date. The TRM at day +100 and for the entire study period (3% and 13.6%, respectively) is lower than all previously reported series of pediatric MAC and MUD allo-HSCT recipients. The low incidence of grade II-IV aGVHD in our series contributed to this low incidence of TRM, especially in terms of infection- and GVHD-associated deaths. The 84% 5-year OS and absence of PGF in our RTC MSD/MUD allo-HSCT recipients are encouraging. PGF was highly prevalent in the RTC UCBT recipients, especially in the chemotherapy-naive recipients. This high rate of PGF in our subgroup of chemotherapy-naive pediatric patients with malignant (CML, MDS) and nonmalignant diseases after RTC UCBT is unacceptable and should be the subject of future investigations using such strategies as increased immunoablation in RTC regimens, increased UCBT cell doses, double UCBTs, ex vivo expanded UCBTs, and/or

pluripotent or mesenchymal stem cellular adjuvants to promote increased engraftment. Future studies should confirm our preliminary results in larger and more uniformly defined cohorts in prospective trials in pediatric RTC allo-HSCT recipients.

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