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Clinical Research: Alternative Donors

Frequency and Risk Factors Associated with Cord Graft Failure after Transplant with Single-Unit Umbilical Cord Cells Supplemented by Haploidentical Cells with Reduced-Intensity Conditioning

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A B S T R A C T

Delayed engraftment and cord graft failure (CGF) are serious complications after unrelated cord blood (UCB) hematopoietic stem cell transplantation (HSCT), particularly when using low-cell-dose UCB units. The haplo-cord HSCT approach allows the use of a lower dose single UCB unit by co-infusion of a $CD34^+$ selected haploidentical graft, which provides early transient engraftment while awaiting durable UCB engraftment. We describe the frequency, complications, and risk factors of CGF after reduced-intensity conditioning haplo-cord HSCT. Among 107 patients who underwent haplo-cord HSCT, 94 were assessable for CGF, defined as <5% cord blood chimerism at day 60 in the myeloid and CD3 compartments, irrespective of neutrophil and platelet counts. CGF occurred in 14 of 94 assessable patients (15%). Median survival after CGF was 12.7 months with haploidentical or mixed haploidentical–autologous hematopoiesis persisting in the 7 surviving. Median progression-free survival after CGF was 7.7 months and was not statistically different from those without CGF (10.47 months; $P = .18$). In univariate analyses, no UCB factors were associated with CGF, including cell dose, cell viability, recipient major ABO mismatch against the UCB unit, or degree of HLA match. We also found no association of CGF with recipient cytomegalovirus serostatus, haploidentical donor age, or day 30 haploidentical chimerism. However, higher haploidentical total nucleated and $CD34^+$ cell doses and day 30 UCB chimerism < 5% in either the myeloid or CD3 compartments were associated with greater risk of CGF. We conclude that assessing chimerism at day 30 may foretell impending CGF, and avoidance of high haploidentical cell doses may reduce risk of CGF after haplo-cord HSCT. However, long-term survival is possible after CGF because of predominant haploidentical or mixed chimerism and hematopoietic function.

INTRODUCTION

Umbilical cord blood (UCB) is an alternative option to standard graft sources for hematopoietic stem cell transplantation (HSCT) and has been successfully used in both children and adults. Although UCB transplantation appears to achieve similar overall and leukemia-free survival relative to adult donor bone marrow or peripheral blood stem cell transplantation, a major limitation of UCB has been early morbidity and mortality from delayed hematopoietic recovery and a higher incidence of graft failure [1-3]. Delayed engraftment has been attributed to the low-stem-cell and T cell content of UCB units, a higher degree of HLA disparity in the donor–recipient pair, and poor T cell function after

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UCB transplant, leading to higher rates of infections in the early post-transplant period [2,4-6]. Nonetheless, UCB has much appeal, such as its availability to a wider range of recipients, observed low rates of graft-versus-host-disease in single UCB unit approaches, and potent graft-versus-tumor effect [6-8]. Theories attribute the superior graft-versus-tumor effects to aspects of the fetal immune system and its tolerance of the recipient environment, greater HLA-mismatch than conventional matched donors [9-11], and persistence of maternal microchimerism in the UCB graft [11,12]. Application of reduced-intensity conditioning (RIC) has extended UCB HSCT to older and less-fit patients [13,14]. As a result, UCB has become an increasingly used graft source for patients lacking HLA matched related donors.

Graft failure is one of the most feared complications after allogeneic HSCT, historically associated with high mortality [15]. Variably defined in the literature by failure to achieve neutrophil engraftment and/or donor chimerism, it invariably represents failure of sustained hematopoietic function by the intended graft. Factors associated with increased risk of graft failure after allogeneic HSCT include degree of HLA mismatch [16-18], use of unrelated grafts [4,18], T cell depletion [19], low cell dose [6], use of RIC [18,20] presence of donor-specific antibodies (DSAs) [21,22], and major ABO mismatch [23,24]. Mechanisms of graft failure described include infections in the recipient [25], drug toxicity, and rejection, which is thought to be recipient immune-mediated, involving T lymphocytes [26,27], natural killer cells [28-30], and DSAs [31-33]. Reported rates of graft failure in UCB transplantation range from 10% to 30%, with risk factors of greater HLA mismatch, lower cell dose, and RIC [2,4-6].

At our institutions we have advanced a UCB HSCT approach after RIC that allows the use of a lower dose single UCB unit by co-infusion of a $CD34^+$ selected haploidentical graft, which we refer to as haplo-cord HSCT. Although the ultimate goal is durable UCB hematopoiesis, the haploidentical graft provides early hematopoietic function until transition to UCB engraftment. With this approach we have achieved fast engraftment, comparable rates of relapse, and low rates of graft-versus-host-disease [34]. Given that haplo-cord HSCT is performed with the goal of durable umbilical cord engraftment, we sought to define the frequency of cord graft failure (CGF) and to describe subsequent outcomes.

METHODS

Eligibility and Enrollment

Adults with hematologic malignancies who underwent first allogeneic HSCT with haplo-cord HSCT with RIC on 2 consecutive Institutional Review Board–approved protocols at the University of Chicago Medical Center (UCMC) and the Weil Cornell Medical College (WCMC) between 2006 to 2013 were reviewed (clinical trial.gov NCT00943800 and NCT01810588). Inclusion criteria required no available matched related donor or matched unrelated donor. Other protocol inclusion and exclusion criteria were previously reported and similar in both protocols [34]. All patients provided written informed consent.

Treatment Plan

RIC used fludarabine (30 mg/m^2 daily days −7 to −3), melphalan (140 mg/m^2 on day −2 or 70 mg/m^2 daily days −3 and −2), and rabbit antithymocyte globulin (4.5 to 6.0 mg/kg daily divided over 3 to 4 days) and occasionally (n = 5) total body irradiation of 400 cGy (200 cGy twice a day for 1 day) if there was increased risk of central nervous system relapse. Immunosuppression used tacrolimus from days −1 through 180 and mycophenolate mofetil starting on day −2 at either 15 mg/kg every 8 hours or 1000 mg every 8 hours until day 28.

Graft Sources

Preferred haploidentical grafts were related, nonmaternal, and younger donors. Haploidentical donors grafts were harvested after granulocyte colony-stimulating factor mobilization at 5 μg/kg subcutaneously twice a day or 10 μg/kg daily, and $CD34^+$ cells were selected by the Isolex 300i CD34 depletion device (Isolex 300i Magnetic Cell Selection Systems; Nexell Therapeutics, Irvine CA) before April 2010 or the CliniMACS device (Miltenyi Biotec Inc., San Diego, CA) thereafter, aiming for a CD3 cell dose below 1×10^4/kg in the initial phase of the study. As of early 2012 the algorithm was adjusted to also limit the $CD34^+$ cell dose of the haploidentical donor to approximately 3 to 5×10^6/kg of recipient weight.

Preferred UCB units were HLA matched using standard UCB matching criteria: HLA-A and -B, by antigen matching, and at DRB1 by allele matching with at least 4/6 match. The minimum cell dose varied by protocol and protocol stage from $.5 \times 10^7$ total nucleated cells (TNCs)/kg recipient weight to 2.0×10^7 TNCs/kg. Preference was first given to optimal HLA matching once the minimum cell dose requirements were met.

Presence of DSAs against the haploidentical and UCB grafts was evaluated by solid-phase immunoassay (Luminex Corporation, Austin, TX). When DSAs were present, desensitization was performed as previously reported [35]. Haploidentical cells were infused on day 0, and UCB units were infused either later on the same day or on the following day.

Definitions and Study Endpoints

Disease risk was classified based on the American Society for Blood and Marrow Transplantation Request for Information 2006 risk scoring schema (http://www.asbmt.org). Chimerism at UCMC was performed by PCR for variable number of tandem repeats in unfractionated whole blood to assess myeloid chimerism and in the T cell compartment by CD3 sorting. At WCMC, CD33 chimerism was performed instead of unfractionated whole blood chimerism for myeloid chimerism, along with CD3 sorting. Protocol chimerism time points included baseline and post-transplant on days 14, 30, 60, 100, 180, and 360, or more frequently if clinically indicated. Results shown reflect peripheral blood unless only a marrow sample was available. Neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count of at least 500/μL, and platelet recovery was defined as the first of 7 consecutive days with platelet count of at least 20,000/μL free of transfusion.

Umbilical CGF was the primary endpoint, defined as umbilical cord chimerism < 5% at day 60 in the myeloid and CD3 compartments irrespective of hematopoietic function. Death before day 60 excluded patients from the primary CGF analysis because cord engraftment may be either lost or gained at time points before day 60. Primary graft failure referred to a lack of neutrophil recovery by day 28. Postprocessing UCB viability was measured at various cord banks using Trypan Blue staining or, more recently, flow cytometric methodologies as per each cord bank protocol. At UCMC, post-thaw UCB viability was measured using Trypan Blue staining. At WCMC, post-thaw UCB viability was measured using Trypan Blue staining until recently when methodology was changed to flow cytometry with 7-actinomycin D staining. Data were censored as of July 31, 2014.

Statistical Analysis

Descriptive tables with patient and disease characteristics were tabulated. Univariate analyses were performed on various factors to identify potential risk factors for CGF. The limited number of events precluded multivariable models. Statistical analysis used to determine association between CGF and predictor variables were Fisher's exact test for dichotomous and logistic regression for continuous predictor variables. *P* values reported reflect 2-sided tests with an alpha of .05 considered significant, without adjustment for multiple testing. Overall survival (OS) and progression-free survival (PFS) estimates were generated by the Kaplan-Meier method. Patient death in remission defined transplant- or treatment-related mortality. STATA version 12 (StataCorp LP, College Station, TX) was used for all analyses.

RESULTS

Characteristics of the Study Cohort

A total of 107 adult patients with hematologic malignancies underwent RIC haplo-cord HSCT between January 2007 and December 2013 at UCMC and WCMC. Table 1 depicts baseline characteristics. Diseases indications were acute myelogenous leukemia (AML; 51%), acute lymphoblastic leukemia (12%), myelodysplastic syndrome (11%), and other (25%). Median patient age was 50 years (range, 18 to 73), and many had active disease at time of transplant (47%). The mean UCB cell dose was 2.1×10^7 TNCs/kg (range, .77 to

Table 1
Baseline Characteristics of All Patients and Those Assessable for CGF*

Factor	All Patients (n = 107; 100%)	Assessable for CGF (n = 94; 88%)
Median age, yr (range)	50 (18-73)	51 (18-73)
Sex, male	63 (59)	58 (62)
Disease		
AML/MDS	67 (62)	61 (64)
ALL	13 (12)	11 (11)
CLL	4 (4)	4 (4)
CML	4 (4)	4 (4)
Other	19 (18)	16 (17)
ASBMT risk		
High	41 (38)	39 (42)
Low or intermediate	63 (59)	54 (57)
Missing	3 (3)	1 (1)
Cord HLA match		
4/6	26 (24)	24 (25)
5/6	65 (61)	58 (62)
6/6	14 (13)	12 (13)
Missing	2 (2)	
Haploidentical high resolution		
HLA match		
5/10	40/64 (63)	36/57 (63)
>5/10	24/64 (37)	21/57 (37)
DSAs		
Against cord	3	3
Against haploidentical	7	6
Against both	1	1
Major ABO mismatch (recipient vs. cord)	18/67 (27)	17/60 (28)

MDS indicates myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; ASBMT, American Society for Blood and Marrow Transplantation. Values are number of cases with percents in parentheses, unless otherwise noted.

* Those assessable for CGF include all patients minus those who died by day 60.

8.3), and the cord HLA match was 4/6 in 27% and >4/6 (5/6 or 6/6) in 73%. Five patients had UCB doses below 1×10^7 TNCs/kg.

Primary Engraftment

Median time to neutrophil recovery was 11 days (range, 9 to 62) for the entire group, and median time to platelet recovery was 29 days (range, 10 to 95). Eleven patients (10%) did not recover neutrophils by day 28 (primary graft failure), of which 10 recovered between days 29 and 62. Only 1 of these 11 patients with primary graft failure also had CGF and engrafted neutrophils on day 62 and platelets on day 77. Chimerism studies of this individual showed predominant cord chimerism at day 30 but predominant autologous chimerism at day 60 after early relapse of acute lymphoblastic leukemia on day 21 that was in CR3 at time of transplant. This patient eventually died on day 303 of relapsed disease. Overall, cumulative rate of neutrophil recovery for the whole group at day 28 was 90% at day 48, 99%.

Patients Not Assessable for CGF

Thirteen patients were not assessable for CGF, which required chimerism studies at day 60. Chimerism data were not available on 2 patients, and 11 patients (10.3%) died before day 60, leaving 94 assessable patients for the primary outcome of CGF. Of the 11 patients who died by day 60, disease indications and disease status mirrored the general population, including AML (n = 5), myelodysplastic syndrome (n = 1), acute lymphoblastic leukemia (n = 2), Hodgkin lymphoma (n = 1), and non-Hodgkin lymphoma (n = 2) (Table 2). All 11 patients were high risk according to the American Society for Blood and Marrow Transplantation, and 6 had active disease at time of transplant. Primary causes of death were infections (n = 7), progressive disease (n = 3), or bleeding (n = 1). None of the 11 patients had DSA against the UCB unit. Just 1 of 11 had DSAs against the haploidentical donor.

Patients with CGF

Among 94 assessable patients at day 60, CGF occurred in 14 (15%) (Figure 1). Because haploidentical-derived hematopoiesis serves to protect against delayed UCB engraftment and prolonged cytopenias, most patients who experienced CGF still had timely and adequate hematopoietic function. Median time to neutrophil recovery in those with CGF was 11 days (range, 9 to 62), with only 1 patient meeting criterion for primary graft failure by day 28 who eventually recovered neutrophils on day 62. Median time to platelet engraftment was 21 days, ranging from 14 to 77 days.

More specifically, the median neutrophil and platelet counts for CGF patients were 2800/μL and 104 K/μL, respectively, at day 60. Two of 14 patients (14%) experienced neutropenia (ie, absolute neutrophil count < 500/μL or

Table 2
Characteristics of Those Who Died by Day 60 (ie, not assessable for CGF)

Patient No.	Disease	Status at Transplant	Age (yr)	HCT-CI	Days to Death	Day 30 Chimerism,* Myeloid (%)	Day 30 Chimerism,* CD3 (%)	Days to ANC	Cause of Death
1	AML	NR	31	3	32	100/0/0	100/0/0	11	PD, MOF
2	DLBCL	CR2	66	0	40	100/0/0	1/98/1	10	TTP, sepsis
3	HD	Primary refractory	25	4	25	100/0/0	100/0/0	9	Sepsis
4	ALL	CR2	64	2	18	ND	ND	—	Sepsis
5	DLBCL	PR	61	2	48	0/100/0	0/100/0	36	PD
6	AML	CR1	65	1	48	96/4/0	96/4/0	13	Hemorrhage, MOF
7	AML	CR2	69	2	43	64/36/0	61/39/0	21	PNA, sepsis
8	AML	NR	41	NA	7	ND	ND	—	RSV PNA, sepsis
9	AML	NR	62	NA	34	100/0/0	100/0/0	10	Sepsis
10	ALL	CR1	55	NA	33	0/100/0	ND	23	Toxoplasmosis, sepsis
11	MDS	NR	69	NA	37	2/98/0	0/95/5	12	PD

HCT-CI indicates hematopoietic cell transplantation-specific comorbidity index [36]; NR, not in remission; PD, progressive disease; MOF, multiorgan failure; DLBCL, diffuse large B cell lymphoma; CR2, second complete remission; TTP, thrombotic thrombocytopenic purpura; HD, Hodgkin disease; ND, not done; PR, partial response; CR1, first complete remission; PNA, pneumonia; NA, not available; RSV, respiratory syncytial virus.

* Chimerism data: % umbilical cord blood/% haploidentical/% autologous.

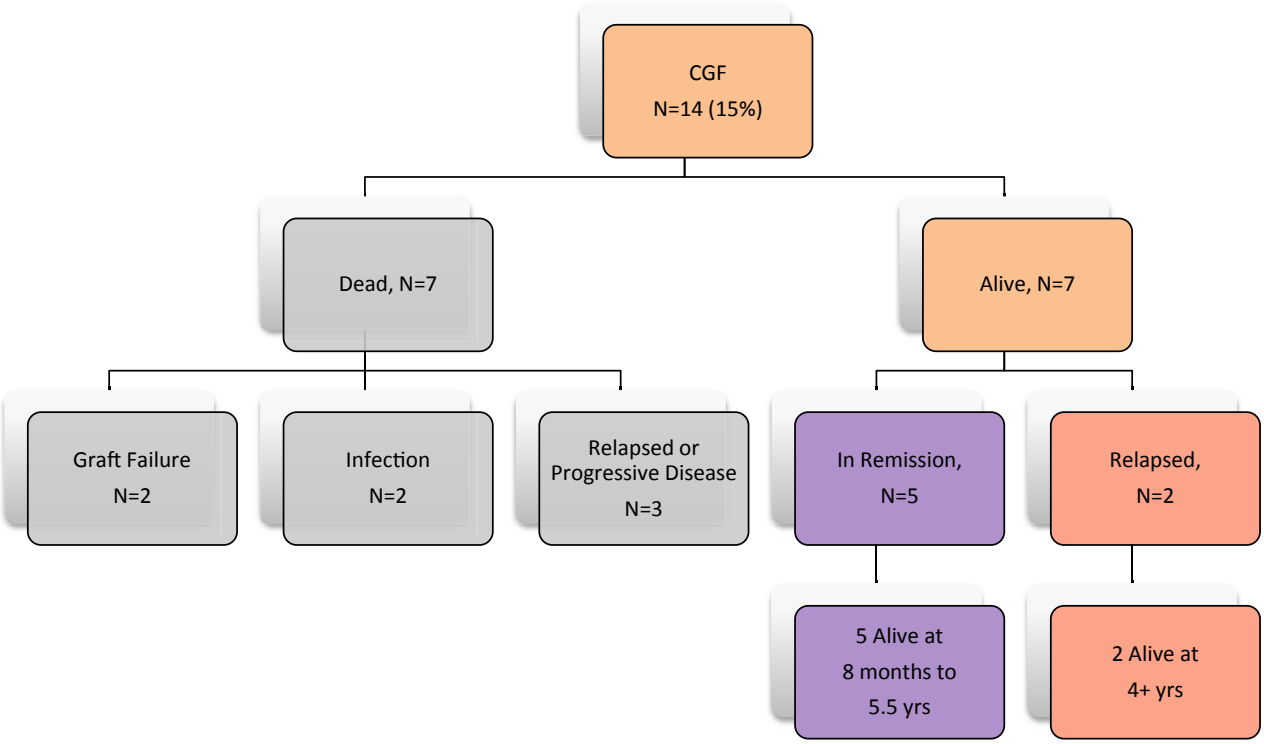

Figure 1. Outcomes of Subjects with Cord Graft Failure after Haplo-cord Transplantation.

requiring granulocyte colony-stimulating factor) and 1 of 14 (7%) had significant thrombocytopenia (platelet count $< 20/\mu L$ or platelet transfusion dependence). Of the 2 patients with significant neutropenia, 1 had active disease at transplant, had haploidentical predominant chimerism (>50%) at day 30, converting to predominant autologous chimerism at day 60, and died at day 147 of progressive disease. The second had AML in first complete remission at time of transplant, had predominant haploidentical chimerism in the myeloid compartment and predominant autologous chimerism in the CD3 compartment from day 30 and thereafter, relapsed at day 138, and remains alive at 8 months out from transplant. Among those with successful cord engraftment at day 60, CGF was not subsequently observed in the absence of relapse.

Chimerism Among CGF Patients

Day 30 chimerism in 11 of 13 patients with CGF with data available had predominant haploidentical chimerism (>50%) in the myeloid compartment, and 5 of 10 had predominant haploidentical chimerism in the CD3 compartment (Table 3). The rest had predominant autologous chimerism in the respective compartments. Median haploidentical chimerism in the myeloid and CD3 compartments was 95% (range, 0 to 100%) and 20% (range, 0 to 100%), respectively, at day 60.

Of the 14 patients who exhibited CGF at day 60, none subsequently developed any cord blood chimerism. Nevertheless, haploidentical chimerism persisted in many patients. At day 180, 7 of 8 assessable patients retained predominant haploidentical chimerism in the myeloid compartment, although just 1 of 8 retained predominant

Table 3
Characteristics and Outcomes of Patients with CGF

Patient No.	Disease/ Status	Days to Relapse	Days to Death	Cause of Death	Age (yr)	Chimerism (% UCB/% haploidentical/% autologous)					
						Day 30, Myeloid	Day 30, CD3	Day 60, Myeloid	Day 60, CD3	Day 180, Myeloid	Day 180, CD3
1	AML/CR1	894			40	0/100/0	0/31/69	0/100/0	0/100/0	0/100/0	0/100/0
2	AML/CR1		132	Infection	64	0/100/0	0/100/0	0/100/0	0/100/0	Dead	Dead
3	MDS/CR1		234	Graft failure	38	5/95/0	5/95/0	0/85/15	0/34/66	0/62/38	0/4/96
4	CML/blast phase				25	4/96/0	1/99/0	0/72/28	0/5/95	0/84/16	0/27/73
5	t-MDS/NR		93	Graft failure	63	10/90/0	12/66/22	0/15/85	0/0/100	Dead	Dead
6	MDS/NR	714			58	0/86/14	0/9/91	0/71/29	0/5/95	0/13/87	0/4/96
7	ALL/CR1	286	387	Relapse	34	0/100/0	0/100/0	0/88/12	0/9/91	0/90/10	0/29/71
8	CML/CR2		80	Infection	51	0/100/0	0/37/63	0/6/94	ND	Dead	Dead
9	ALL/CR3	21	303	Relapse	42	94/1/5	ND	0/0/100	ND	0/0/100	ND
10	AML/PIF				44	0/0/100	ND	ND	ND	ND	ND
11	HL/CR3				36	0/100/0	ND	0/100/0	0/60/40	0/100/0	0/27/73
12	AML/CR2				65	0/100/0	9/0/91	0/99/1	0/9/91	0/100/0	0/0/100
13	AML/NR		147	PD	69	9/91/0	ND	0/0/100	ND	Dead	Dead
14	AML/CR1				18	1/99/0	84/0/16	0/100/0	0/1/99	0/100/0	0/5/95

t-MDS indicates therapy-related myelodysplastic syndrome; PIF, primary induction failure; CR3, third complete remission.

haploidentical chimerism in the CD3 compartment. By 1 year after transplant, 6 of 7 assessable patients continued with predominant haploidentical chimerism in the myeloid compartment, and 4 of 7 had predominant haploidentical chimerism in the CD3 compartment. Overall, haploidentical chimerism appeared dominant in the myeloid compartment in most who had data available. By contrast, the CD3 compartment was often dominated by autologous chimerism.

Clinical Outcomes after CGF

Median OS for the CGF group was 12.7 months from transplant (10.7 months from CGF), whereas median OS for those without CGF was not reached ($P = .51$). Seven of the 14 (50%) with CGF died, all within a year of transplant (Table 3). Causes of death were poor graft function (n = 2), infection (n = 2), and relapsed/progressive disease (n = 3) (Figure 1).

The haploidentical graft generally was maintained in patients with CGF, as discussed in detail above. Seven remain alive (range, 8 months to 5.5 years from transplant), of which 5 remain in remission. No patient required a second allogeneic transplant for graft failure specifically, due to adequate hematopoiesis.

Median PFS was 7.7 months from transplant in those with CGF and was not statistically significantly different from the median PFS of 10.47 months in those without CGF ($P = .18$). Two patients who remain alive developed late AML relapses at days 714 and 894. One achieved a second remission after a second haplo-cord transplant, and the other underwent reinduction for relapsed AML.

Prognostic Factor Analysis for CGF

In univariate analyses, factors not found to be associated with CGF (Table 4) included cord bank–reported post-processing viability, cord bank–reported cell doses, transplant center–measured post-thaw cell doses and viabilities, haploidentical chimerism at day 30 (<5% in either the myeloid or CD3 compartments), age of the haploidentical donor (age > 50 versus <50), recipient major ABO mismatch against the UCB unit, recipient cytomegalovirus serostatus, haploidentical high-resolution HLA match (>5/10 versus 5/10 at HLA-A, -B, -C, -DRB1, and -DQ), and degree of UCB match (HLA match >4/6 versus 4/6). There were limited numbers of patients with DSAs against the grafts, because our institutions generally have avoided them, making this factor not assessable. Of interest, 3 patients did have DSAs against the UCB unit, underwent desensitization, and none experienced CGF. Of the 7 patients with DSAs against the haploidentical graft, 1 died by day 60 and 1 experienced CGF. Of these, just 1 patient had DSAs against both the UCB unit and haploidentical graft; this patient engrafted and is in remission at about 6 months.

Infusion of higher haploidentical cell doses, measured by TNCs or $CD34^+$ as continuous variables, were associated with a greater risk of CGF ($P = .03$ and .055, respectively) (Table 3). Mean of haploidentical cells infused for the entire group were 4.0×10^6 TNCs/kg (standard deviation, 1.7; range, .98 to 11.11) and 4.0×10^6 $CD34^+$/kg (standard deviation, 1.7; range, .95 to 10.95). Additionally, exploratory analysis evaluated the association of CGF with 2 different thresholds of haploidentical $C34^+$ cells, which were not found to be significant: 3.0×10^6 $CD34^+$/kg ($P = .13$) and the median of 3.9×10^6 $CD34^+$/kg ($P = .082$). Day 30 UCB chimerism < 5% in either the myeloid or CD3 compartment was also strongly associated with CGF ($P < .001$ and < .001, respectively).

Table 4
Graft Composition and Traits and Association with CGF at Day 60

	No CGF (n = 80)	CGF (n = 14)	*P*
UCB graft			
Cord bank reported (postprocessing)			
TNCs/kg $\times 10^7$	2.1 ± 1.1	1.9 ± .63	.61
TNCs/kg < 1.5 $\times 10^7$	27/80 (35%)	3/14 (21%)	.54
CD34/kg $\times 10^5$	.88 ± .71	.98 ± .80	.65
Viability (%)	95.6 ± 4.7	96.9 ± 4.6	.38
Viability < 85%	1/80 (1.5%)	1/14 (7%)	.31
Transplant center Reported (post-thaw)			
TNCs/kg $\times 10^7$	1.5 ± .72	1.4 ± .43	.72
TNCs/kg < 1.5 $\times 10^7$	47/80 (60%)	8/14 (57%)	1.0
Viability (%)	91.8 ± 5.3	91.9 ± 5.3	.96
HLA match 4/6 only	19/80 (24%)	5/14 (36%)	.32
Haploidentical graft			
TNCs/kg $\times 10^6$	3.8 ± 1.6	5.0 ± 2.2	.03
CD34/kg $\times 10^6$	3.8 ± 1.6	4.8 ± 2.2	.055
CD34/kg < 3.0 $\times 10^6$	30/80 (38%)	2/14 (14%)	.13
CD34/kg < 3.9 $\times 10^6$	46/80 (58%)	3/14 (27%)	.082
CD3/kg $\times 10^4$	1.2 ± .3	.53 ± .6	.29
HLA match > 5/10*	20/51 (39%)	2/8 (25%)	.67
Haploidentical donor age > 50	9/79 (11%)	2/14 (14%)	1.0
Other characteristics			
Day 30 haploidentical chimerism < 5% (myeloid)	14/77 (18%)	2/15 (14%)	.73
Day 30 haploidentical chimerism < 5% (CD3)	16/66 (24%)	2/10 (20%)	.77
Day 30 UCB chimerism < 5% (myeloid)	14/79 (18%)	10/14 (71%)	<.001
Day 30 UCB chimerism < 5% (CD3)	5/67 (7%)	6/10 (60%)	<.001
Recipient-UCB major ABO mismatch*	41/54 (76%)	6/8 (75%)	.46
Recipient cytomegalovirus seropositive*			1.0

Values are mean ± standard deviation or number of cases/assessable number of cases with percents in parentheses.
* UCMC patients only.

DISCUSSION

At our institutions we continue to advance a RIC haplo-cord HSCT approach in those without a matched related or unrelated donor. In this expanded cohort, similar to our initial report, most patients achieved early neutrophil recovery with a median time of 11 days and >90% achieved neutrophil recovery by day 28, irrespective of cord engraftment success [34]. This compares favorably with other UCB approaches where median time to neutrophil recovery falls in the 20 or more day range. Platelet engraftment occurred at a median of 29 days, compared with 40 to 50 day ranges reported in other UCB experiences [3,4,6,8,37,38]. This early hematopoietic recovery is a result of initial haploidentical engraftment, and durable UCB engraftment eventually ensues in most patients, even with the use of smaller UCB units in comparison with that reported in other UCB transplant approaches. Because of less stringent cell dose requirements, better matched UCB units may be chosen.

We sought to describe the frequency and complications of CGF with our haplo-cord platform, because haplo-cord HSCT seeks to achieve durable UCB engraftment. In this report 14 of 94 assessable patients (15%) undergoing RIC haplo-cord transplant experienced CGF, defined as lack of significant (<5%) cord blood chimerism among patients surviving to day 60. Because no patient subsequently developed significant cord engraftment after day 60, lack of cord blood chimerism at this time point reflects failure of the cord graft. In a similar

haplo-cord setting after myeloablative conditioning using similar UCB cell doses (median, 2.4×10^7 TNCs/kg) and more limited haploidentical cell doses (median, 2.5×10^6 CD34/kg with 25 to 75 interquartile range, 2.3 to 3), Kwon et al. [39] reported a CGF rate of 9%. They defined CGF differently as absence of UCB-specific alleles at day 30 (cutoff $<$ 1% for whole bone marrow and peripheral blood samples and $<$5% for leukocyte lineages).

These rates of CGF after haplo-cord HSCT appear on par to the reported rates of 4% to 20% in the myeloablative setting and 6% to 12% in the RIC setting using single and double UCB approaches, with variable graft-versus-host-disease prophylaxis [40-42]. Brunstein et al. [43] reported a 10% rate of CGF in a multi-institutional protocol using a RIC double UCB approach. Outcomes after CGF have universally been very dismal because delayed engraftment after UCB may result in prolonged cytopenias and associated morbidities, prohibiting a second transplantation. In their RIC double UCB approach, Brunstein et al. [43] reported that all 5 with graft failure died between 23 and 193 days after transplant. Another group reported a median survival of 3.8 months after CGF following RIC single UCB transplantation in adults [44].

By contrast, in our series haplo-cord patients who experienced CGF often had persistent predominantly haploidentical chimerism to mixed haploidentical–autologous chimerism with adequate hematopoietic function, and some achieved long-term disease control. As a consequence, median survival after CGF in our series was 12.7 months from transplant (10.7 months from CGF) with 1 alive and in remission more than 5 years out without additional cellular therapy. Of interest, survival was not significantly different between the CGF and non-CGF groups (median OS not reached at date of censor), but this is based on limited follow-up time. PFS also did not differ significantly, with PFS of 7.7 months from transplant in those with CGF compared with 10.47 months in those without CGF ($P = .18$). Thus, although the rate of CGF of 15% appears similar to or slightly higher than rates reported in other UCB alone approaches, the haploidentical graft appears to provide some degree of protection and disease control in the event of CGF after haplo-cord HSCT, diminishing the high morbidity and mortality often associated with graft failure.

In our analyses we found that day 30 UCB chimerism ($<$5%) in either the myeloid or CD3 compartments to be strongly associated with CGF. This suggests that assessing chimerism before day 60 may indicate impending CGF. This is in line with that reported by the Spanish group in the haplo-cord transplant setting [42]. They found that the dynamics of UCB chimerism over time at days 14, 21, and 28 predicts for CGF, which they defined as absence of UCB-specific alleles by short tandem repeat PCR at day 30.

The optimal strategy after cord blood failure has yet to be defined. The Spanish group pursued second transplant for most patients with CGF after myeloablative haplo-cord HSCT [39,42]. We have not standardly pursued a second transplant after CGF in our haplo-cord approach in the absence of inadequate hematopoietic function or relapse. Further study is required to define the importance of UCB engraftment and specifically how achieving full UCB chimerism relative to haploidentical chimerism in myeloid and CD3 compartments affects outcomes after the combined haplo-cord approach. Although it is clear in our data that early haploidentical engraftment is vital in most to produce quick hematopoietic recovery, it remains unclear whether long-term haploidentical or UCB engraftment may be equally effective in long-term disease control. Finally, better understanding the kinetics of achieving or losing donor chimerism may help identify impending graft failure earlier, allowing for potential planning and interventions.

In an attempt to maximize cord engraftment to leverage the favorable advantages of durable UCB engraftment, we explored modifiable pretransplant risk factors associated with CGF, recognizing the relatively limited number of events. Lower umbilical cord cell dose was not associated with CGF, which may be somewhat surprising, given the observed impact of UCB cell dose by other groups on UCB-alone transplant-related outcomes such as engraftment and transplant-related mortality [10,45-47]. However, several groups have discerned a combined effect of HLA match and cell dose [46,48-51]. As most of our subjects received UCB units with $>$4/6 HLA match (73%), which is more than that reported in other UCB transplant experiences due in part to less stringent UCB cell dose requirements with our haplo-cord approach, we were unable to examine combined effects of UCB cell dose and HLA-match.

In addition, we did not find a relationship between UCB viability (measured at the cord banks and at our transplant centers) and CGF. In the haplo-cord HSCT report by Kwon et al. [39], at least 5 of 13 of the CGFs had poor post-thaw colony-forming units, but specific data on pre- and post-processing viability was not provided. Scaradavou et al. [52] described an association between cell viability and UCB engraftment in the double umbilical cord transplant setting. However, all our UCB units exceeded the threshold viability of 75% in that study. Furthermore, techniques to measure UCB viability have evolved from use of Trypan Blue staining, which is limited by the difficulty in distinguishing cell death of different cell subsets, to use of more reproducible methods such as flow cytometry (eg, 7-actinomycin D staining) or fluorescence microscopy (eg, acridine orange), justifying future evaluation as centers continue to optimize measurement of viability.

We did find that cell dose of the co-infused CD34 selected haploidentical graft was associated with CGF, both as measured by TNCs/kg and $CD34^+$ cells/kg, which mirror each other very closely with our CD34 selection method. This supports our initial observation in our first 45 patients [34]. The biology of this observation requires further study. Although umbilical cord stem cells exhibit superior proliferative potential due to a greater number of early and committed progenitor cells [53], higher rate of expansion [54-57], and increased sensitivity to cytokine stimulation [56], risks of limited cell doses in UCB units is well recognized, making it plausible to hypothesize that a larger dose of haploidentical cells may counter UCB stem cells' ability to populate the recipient's marrow and function. Higher haploidentical cell dose also functions as a surrogate for younger donors and/or better mobilization because older unrelated donors may have smaller cell doses collected and greater rates of engraftment failure [58,59]. This raises the intriguing possibility that the optimal donor for a haploidentical alone transplant, a young male donor, may in fact counter the ability to achieve cord blood engraftment in the haplo-cord HSCT setting [60]. However, we did not find a relationship between haploidentical age and CGF in this report.

We did not find other factors previously reported to potentially influence graft failure from different donor sources to be associated with CGF after haplo-cord HSCT, including recipient cytomegalovirus serostatus, recipient

major ABO mismatch against the UCB graft, and degree of UCB graft HLA match (4/6 versus >4/6). These factors may not apply in the haplo-cord setting because of the unique immunobiology involved in the co-infusion of the 2 grafts. We also evaluated haploidentical chimerism at day 30 and did not find a significant relationship with CGF.

The major limitation in this work relates to the limited number of patients experiencing CGF to definitively investigate and establish risk factors. Conversely, the major strength derives from application of prospective trials and use of homogenous treatment regimens, including post-transplant care. However, our results may not apply to different haplo-cord approaches (eg, myeloablative regimens, regimens without ATG, haploidentical plus double UCB, etc.).

In summary, approximately 15% of patients have CGF defined by chimerism data at day 60 after haplo-cord HSCT with RIC. However, some patients with CGF after haplo-cord HSCT experience prolonged survival due to predominantly haploidentical or mixed haploidentical–autologous chimerism and hematopoietic function, with sustained disease control. Higher haploidentical cell doses may impair umbilical cord graft engraftment, and we recommend limiting the haploidentical TNC and/or $CD34^+$ cell dose, which we have incorporated in our current haplo-cord protocols.

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SUPPLEMENTARY DATA

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