Reduced intensity haplo plus single cord transplant compared to double cord transplant: improved engraftment and graft-*versus*-host disease-free, relapse-free survival

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ABSTRACT

Umbilical cord blood stem cell transplants are commonly used in adults lacking HLA-identical donors. Delays in hematopoietic recovery contribute to mortality and morbidity. To hasten recovery, we used co-infusion of progenitor cells from a partially matched related donor and from an umbilical cord blood graft (haplo-cord transplant). Here we compared the outcomes of haplo-cord and double-cord transplants. A total of 97 adults underwent reduced intensity conditioning followed by haplo-cord transplant and 193 patients received reduced intensity conditioning followed by double umbilical cord blood transplantation. Patients in the haplo-cord group were more often from minority groups and had more advanced malignancy. Haplo-cord recipients received fludarabine-melphalan-anti-thymocyte globulin. Double umbilical cord blood recipients received fludarabine-cyclophosphamide and low-dose total body irradiation. In a multivariate analysis, haplo-cord had faster neutrophil (HR=1.42, P=0.007) and platelet (HR=2.54, P<0.0001) recovery, lower risk of grade II-IV acute graft-*versus*-host disease (HR=0.26, P<0.0001) and chronic graft-*versus*-host disease (HR=0.06, P<0.0001). Haplo-cord was associated with decreased risk of relapse (HR 0.48, P=0.001). Graft-*versus*-host disease-free, relapse-free survival was superior with haplo-cord (HR 0.63, P=0.002) but not overall survival (HR=0.97, P=0.85). Haplo-cord transplantation using fludarabine-melphalan-thymoglobulin conditioning hastens hematopoietic recovery with a lower risk of relapse relative to double umbilical cord blood transplantation using the commonly used fludarabine-cyclophosphamide-low-dose total body irradiation conditioning. Graft-*versus*-host disease-free and relapse-free survival is significantly improved. Haplo-cord is a readily available graft source that improves outcomes and access to transplant for those lacking HLA-matched donors. Trials registered at *clinicaltrials.gov identifiers 00943800 and 01810588.*

Introduction

Allogeneic transplantation with HLA-identical donors is an effective and potentially curative therapy for hematologic malignancies. Limited availability of HLA-identical donors, particularly in patients from under-represented minority groups,

has generated interest in transplantation using mismatched unrelated umbilical cord blood (UCB) stem cells. The promise of cord blood transplantation resides in its ability to provide a source of stem cells that can engraft across HLA barriers with low rates of graft-*versus*-host disease (GvHD) and exert potent graft-*versus*-leukemia (GvL) effects, possibly mediated by contaminating maternal cells.[1-5] But cord blood transplantation is hampered by the low progenitor cell doses in the grafts, and hence often very delayed recovery of neutrophils and platelets, particularly in adult recipients.[6,7] This in turn leads to prolonged hospitalization, expense, morbidity and early mortality. Though smaller studies have shown encouraging results,[8,9] a recent study found that the outcomes of cord blood transplantation in older adults were inferior to those of 8/8 matched unrelated donor transplant recipients, mostly because of increased early treatment-related mortality.[7]

Several recent studies have been conducted to improve hematopoietic recovery after umbilical cord blood transplantation in adults in order to reduce early morbidity and mortality, and possibly health care utilization. Double UCB transplantation is perhaps the most commonly used of these procedures. But in a recently reported randomized study in pediatric patients, it was not associated with improved outcomes relative to single cord transplant.[10]

We and others have investigated an alternative strategy: the use of third-party CD34 selected adult haplo-identical stem cells to supplement a single UCB stem cell graft.[11-15] In an initial report using a reduced intensity conditioning approach, we showed encouraging rates of engraftment and of long-term outcome.[16] We also showed how the initial engraftment of the haplo-identical stem cells was, in the large majority of cases, ultimately superseded by the outgrowth of UCB cells. Since then, more than 150 additional such transplants have been performed at two institutions in the US, where they have become the preferred form of alternative donor transplantation. Here we conducted a formal comparison with patients undergoing reduced intensity conditioning and double UCB transplantation. The comparison group consisted of adult double UCB blood transplant recipients who had received the most widely used reduced intensity conditioning regimen. Trials were registered at *clinicaltrials.gov identifiers 00943800 and 01810588.*

Methods

Patients and controls

In 2007, a prospective study was initiated at the University of Chicago for haplo-cord transplantation following reduced intensity conditioning (*clinicaltrials.gov identifier 00943800*). As of 2012, this was followed by a joint prospective study of reduced intensity conditioning conducted by Weill Cornell Medical College and University of Chicago (*clinicaltrials.gov identifier 01810588*). The primary objective of the latter study was to define the optimal cell dose of the umbilical cord blood graft for haplo-cord transplantation, and, if possible, to match for inherited paternal antigens and non-inherited maternal antigens. Eligibility criteria for both studies were similar.

Patients with hematologic malignancies in need of an allogeneic stem cell transplant (SCT) who lacked an HLA-identical related or unrelated donor were eligible. Additional eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status less than or equal to 2, bilirubin less than or equal to 2 mg/dL, creatinine less than 1.5 times the upper limit of normal, preserved heart and lung function, and no evidence of chronic active hepatitis or cirrhosis. HIV negativity was required, and pregnant females were excluded from the study. The studies were approved by the Institutional Review Board of both institutions, and all patients and donors provided written informed consent. The studies were conducted in accordance with the Declaration of Helsinki and were registered on clinicaltrials.gov.

Cases (n=97) include patients consecutively enrolled on these two studies and receiving reduced intensity conditioning between January 2007 and mid-2013. One pediatric patient was excluded, as were 2 patients undergoing transplant for myelofibrosis and the single patient with myeloma.

The control group consisted of adult patients with leukemia, lymphoma or myelodysplastic syndrome reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) who received a double UCB graft following reduced intensity conditioning using fludarabine, cyclophosphamide, and low-dose total body irradiation between 2007 and 2011 at US transplant centers. This is the most widely utilized reduced intensity conditioning regimen for cord blood transplantation in adults with an acceptable treatment-related mortality and is used as the conditioning regimen in several national clinical trials.[6,7,17] Patients with Karnofsky Performance Score (KPS) less than 60, with incomplete background or follow-up information, who received antithymocyte globulin (ATG) or who did not receive a calcineurin inhibitor after transplant, were excluded. A total of 193 CIBMTR patients fulfilled these criteria and were included as a control group. Seven of the 193 control patients had donors that were poorly matched (HLA 3/6). Their exclusion did not affect the results of the analysis.

Donors and stem cell processing

Cord blood

Cord-blood units for haplo-cord were selected based on HLA-typing and cell count. Grafts were matched for at least 4 of 6 HLA loci by the standard cord criteria (i.e. low resolution for HLA-A and HLA- B, and high resolution for HLA-DR)[18] and contained a minimum cell count of $1x10^7$ nucleated cells per kilogram (kg) of the recipient's body weight before freezing. In contrast with common practice, we prioritized matching over cell dose. As of mid-2012, for graft selection we utilized high-resolution HLA typing for HLA A, B, C and DR.[19]

Haploidentical donor

The haploidentical donor was a relative. Donors underwent stem cell mobilization using filgrastim for four consecutive days. Apheresis was started on day 5 and continued daily until at least $5x10^6$ CD 34^+ cells / recipient kg were collected. After collection, and prior to cryopreservation, haplo-identical grafts were T-cell depleted initially using the Isolex 300i CD34 selection device. As of early April 2010, the Isolex 300i CD34 selection device was no longer available, and instead, the Miltenyi CliniMACS device was used under an Investigational New Device (IND) from the United States Food and Drug Agency. In the initial protocol (*clinicaltrials.gov identifiers 00943800*) the cell dose of the haplo-cord donor was based on CD3 cell dose ($<1x10^6$ CD3 per kg_{rec}).[16] In that study, it was noted that the administration of very high doses of haplo CD34 cells correlated with failure of umbilical cord blood engraftment. Subsequently, the cell dose of the haplo graft has been based on CD34 dose with a target dose of 3-5 $x10^6$ CD34 per kg_{rec}.

Donor directed antibodies

As of the tenth patient enrolled on the initial protocol, UCB and haplo-identical donor selection was also based on avoidance of

Table 1. Pre-transplant characteristics of patients included in the UC/WCMC and CIBMTR study cohorts.

Variable	UC/WCMC Haplo+Cord	CIBMTR Double UCB	P
Total n	97	193	
Age, in years, n (%)			0.15
20-29	8 (8)	11 (6)	
30-39	15 (15)	13 (7)	
40-49	18 (19)	31 (16)	
50-59	24 (25)	63 (33)	
60-69	29 (30)	70 (36)	
70+	3 (3)	5 (3)	
Median (range)	54 (20-73)	57 (20-72)	0.03
Sex, n (%)			0.30
Male	60 (62)	107 (55)	
Female	37 (38)	86 (45)	
Weight in kg, n, median (range)	n=61 80 (49, 136)	n=185 79 (46, 146)	0.48
Sorror Comorbidity Index, n (%)			0.29
0	32 (33)	52 (27)	
1-2	29 (30)	52 (27)	
3+	39 (37)	88 (45)	
Missing	0	1 (1)	
N, median (range)	1 (0, 8)	2 (0, 10)	0.13
Race, n (%)			<0.0001
White	59 (61)	152 (79)	
Black	23 (24)	19 (10)	
Others	6 (6)	19 (10)	
Unknown/declined	9 (9)	3 (2)	
Ethnicity, n (%)			0.05*
Hispanic	9 (9)	20 (10)	
Non-Hispanic	64 (66)	162 (84)	
Unknown/declined	24 (25)	11 (6)	
KPS, n (%)			0.04**
90-100%	77 (79)	119 (62)	
60- 80%	20 (21)	57 (30)	
Missing	0	17 (9)	
Disease, n (%)			0.95
AML	54 (56)	108 (56)	
ALL	12 (12)	21 (11)	
CLL	1 (1)	8 (4)	
CML	4 (4)	3 (2)	
Other acute leukemia	1 (1)	3 (2)	
Other leukemia	2 (2)	0	
Myelodysplastic disorders	11 (11)	18 (9)	
Non-Hodgkin lymphoma	8 (8)	21 (11)	
Hodgkin lymphoma	4 (4)	11 (6)	
Disease risk, n (%)			<0.0001
Low	34 (35)	92 (48)	
Moderate	21 (22)	68 (35)	
High	42 (43)	33 (17)	
Conditioning regimen, n (%)			<0.0001
TBI + fludarabine + Cy	0	193 (100)	
Fludarabine + melphalan + ATG	97 (100)	0	
GvHD prophylaxis, n (%)			0.17
CSA alone	0	4 (2)	
CSA + MMF	97 (100)	185 (96)	
CSA + MTX	0	4 (2)	
HLA-match for CB units,[a] n (%)			<0.0001
6/6	10 (10)	7 (4)	
5/6	64 (66)	59 (31)	
4/6	23 (24)	119 (62)	
≤ 3/6	0	7 (4)	
Missing	0	1b (1)	
TNC cell dose at infusion (x10^7/kg), n, median (range)			–
Unit 1	n=97 1.7 (0.5, 9.0)	n=167 2.1 (0.6,5.2)	
Unit 2	–	n=159 2.0 (0.3, 5.1)	
Sum of units	–	n=159 4.1 (1.1, 9.2)	
Year of transplant, n (%)			<0.0001
2007-2009	17 (18)	88 (46)	
2010-2013	80 (82)	105	(54)

*[a]For double UCB blood transplants, degree of HLA-match is defined as the value of the lower HLA-matched unit. HLA-matched data were available for one of two CB units. UC: University of Chicago; WCMC: Weill Cornell Medical College; CIBMTR: Center for International Bone Marrow Transplant Research; KPS: Karnofsky Performance Score. *Calculation excluding category unknown/declined. **Calculation excluding Category Misssing.*

donor-directed HLA antibodies.[20] For this purpose, all donors underwent high-resolution HLA typing including DP typing. A donor targeted by pre-existing recipient HLA-antibodies [i.e. donor specific antibodies or (DSA)] was avoided or, when unavoidable, various strategies were used to limit exposure of the graft to DSA.[21]

Conditioning regimen and post-transplant immunosuppression

Haplo-cord patients received fludarabine 30 mg/m^2/day IV for five consecutive days (days -7,-6,-5,-4,-3), rabbit anti-thymocyte globulin (thymoglobulin, r-ATG) at 1.5 mg/kg every other day for 4 doses (days -7, -5, -3, and -1), and melphalan 70 mg/m^2 /day for 2 doses on day -3 and day -2 (Figure 1). The haploidentical cells were infused on day 0 followed by cord blood later the same day or on day 1. As of mid-2012, ATG was reduced to three doses for patients aged 50 years and older. Double UCB transplant recipients received fludarabine, low-dose total body irradiation (TBI) 200 cGy and cyclophosphamide; these patients did not receive ATG.

All haplo-cord recipients and the majority of double UCB recipients received tacrolimus and mycophenolate mofetil (MMF).

End point definitions and statistical analysis

Engraftment: the time to neutrophil engraftment was defined as the first of three consecutive days with an absolute neutrophil count of 0.5x10^9 per liter or higher, and the time to platelet engraftment as the first of seven consecutive days with a platelet count of 20x10^9 per liter or higher without platelet transfusion. Acute GvHD and chronic GvHD were diagnosed and graded according to consensus criteria.[22] Transplant-related mortality (TRM) was defined as death without evidence of relapse/progression of malignancy. Probabilities of TRM, relapse, acute and chronic GvHD were generated using cumulative incidence estimates to accommodate competing risks. Probability of overall survival (OS) was calculated using the Kaplan-Meier estimator, with the variance estimated by Greenwood's formula. For progression-free survival (PFS), subjects were considered treatment failures at the time of relapse or progression or death from any cause. Patients alive without evidence of disease relapse or progression were censored at last follow up, and the PFS event was summarized by a survival curve. Similarly, the probability of GvHD-free/relapse-free survival (GRFS) was summarized by defining events to include grade 3-4 acute GvHD, extensive cGvHD, relapse, or death.[23]

Cox proportional hazards regression was used to compare outcomes between cases and controls. The following variables were considered in the multivariate models: age (18-59 *vs.* ≥ 60 years), patient gender, Karnofsky Performance Score (90%-100% *vs.* 60%-80%), disease (lymphoma/CLL *vs.* acute leukemia/MDS *vs.* other leukemia), and disease risk (Low *vs.* Medium *vs.* High). Disease risk was defined (low *vs.* medium *vs.* high) using the American Society of Blood and Marrow Transplantation (ASBMT) criteria.[24] The assumption of proportional hazards for each factor in the Cox model was tested using time-dependent covariates. A step-wise model selection approach was used to identify all significant risk factors. Each step of model building contained the main effect for graft source. Factors significant at a 5% level were included in the final model. Potential interaction between main graft source effect and all significant risk factors were tested. Adjusted cumulative incidence functions of neutrophil and platelet engraftment, aGvHD, cGvHD, TRM, relapse and adjusted probabilities of PFS, GRFS and OS were generated from the final regression models stratified on cases *versus* controls.[25,26]

Results

Patients' and graft characteristics

Characteristics of the patients in both groups are shown in Table 1. Median age of haplo-cord recipients was slightly lower (54 *vs.* 57 years; *P*=0.03) while the proportion above 60 years of age was similar between haplo-cord and double UCB recipients (33% *vs.* 39%). There were no significant differences in average weight or comorbity score by the hematopoietic cell transplantation-comorbidity index. The percentage of African Americans (24% *vs.* 10%; *P*=0.0001) was higher among haplo-cord recipients.

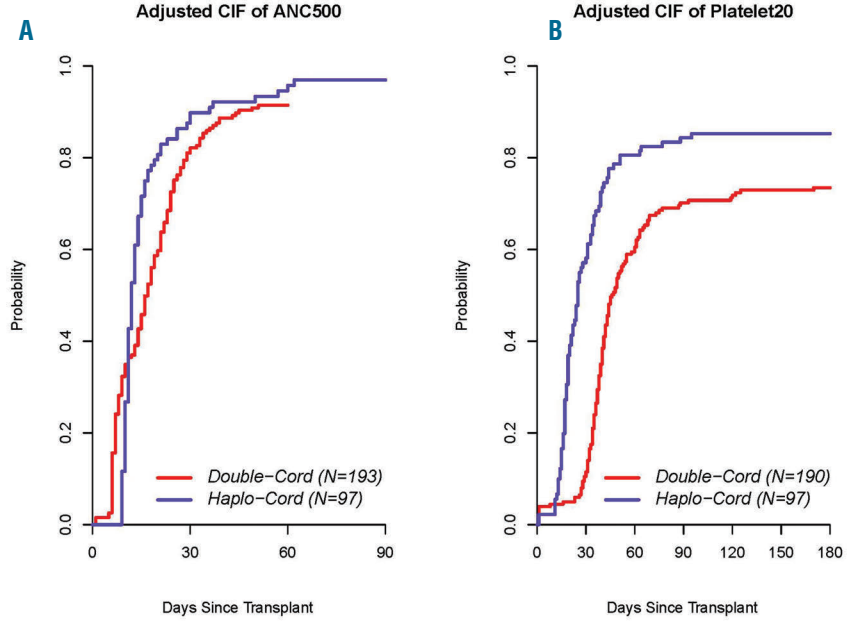

Figure 1. (A) **Adjusted cumulative incidence function for time to neutrophil engraftment.** (B) **Time to platelet engraftment.**

There was a higher percentage of patients with KPS 90-100 among haplo-cord recipients (79% *vs.* 62%; *P*=0.04), but KPS was missing in 9% of controls. Almost two-thirds of patients in both groups had acute myeloid leukemia or MDS but the percentage of patients with high-risk disease was 43% among haplo-cord *vs.* 17% in the double UCB group (*P*<0.0001).

The UCB nucleated cell dose for the haplo-cord recipients was $1.7x10^7$/kg compared to a cumulative dose of $4.1x10^7$/kg for both grafts in the double UCB recipients. Only 24% of haplo-cord recipients received a graft that was 4/6 HLA identical; 66% were 5/6 HLA matched and 10% were 6/6 matched. By contrast, 66% of double UCB recipients received at least one graft that was 4/6 or less well matched (*P*<0.0001). Lastly, the haplo-cord transplant recipients were on average transplanted more recently (82% *vs.* 54% in the period 2010-2013; *P*<0.0001).

Engraftment

By day 30, 90% of the haplo-cord recipients had recovered neutrophil counts *versus* 82% of double UCB recipients. The Hazard Ratio (HR) for neutrophil engraftment was 1.42 (95%CI: 1.10-1.84; *P*=0.007). Similarly 58% of haplo-cord *versus* 12% of double UCB had platelet engraftment by day 30 and the HR for platelet recovery was 2.54 (95%CI: 1.88-3.42; *P*<0.0001) (Figure 1). In multivariable analysis, the only other predictor for platelet recovery was ASBMT high-risk disease score which was associated with slower platelet recovery (Table 2).

Treatment-related mortality, relapse, progression-free, and overall survival

Treatment-related mortality was 30% (95%CI: 21-39) at one year for haplo-cord recipients *versus* 21% (95%CI: 16-27) for double UCB recipients, but this difference was not statistically significant (*P*=0.15) In multivariate analysis, age was the only significant predictor for TRM (HR=2.43, 95%CI: 1.54-3.85, for those ≥ 60 years *vs.* <60 years; *P*=0.0002) (Table 2).

Cumulative Incidence of relapse at one year was 24% (95%CI: 16-33) for haplo-cord recipients *versus* 46% (95%CI: 40-53) for double UCB recipients (HR=0.48; 95%CI: 0.31-0.75; *P*=0.001) (Figure 3). Other risk factors for relapse included ASBMT high-risk score and underlying diagnosis. Patients with lymphoma or CLL had a lower risk of disease recurrence (Table 2).

Progression-free survival at one year was 45% (95%CI: 33-55) for haplo-cord *versus* 34% (95%CI: 28-41) for double UCB recipients, but this difference was not statistically significant (HR=0.78, 95%CI: 0.56-1.08; *P*=0.13) (Figure 3). Significant predictors of inferior PFS included high ASBMT risk score and age over 60 years (Table 2).

Overall survival at one year was 50% (95%CI: 39-61) for haplo-cord *versus* 52 (95%CI: 45-59) for double cord (HR=0.97, *P*=0.85) recipients (Figure 3). In multivariate analysis, age was the only significant predictor for OS. Patients over 60 years of age had a 50% reduction in the likelihood of OS (HR=2.04, 95%CI: 1.50-2.78; *P*<0.0001) (Table 2).

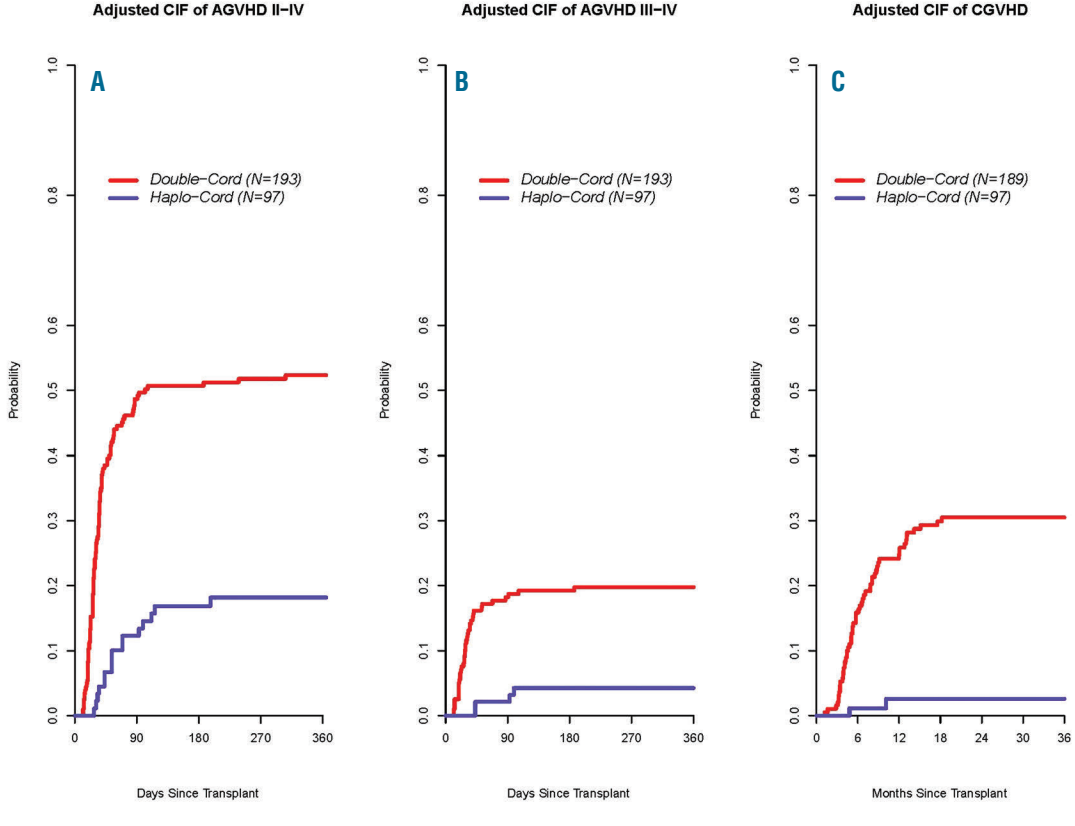

Figure 2. Adjusted cumulative incidence function for (A) **acute graft-*versus*-host disease (GvHD) grade II-IV,** (B) **acute GvHD Grade III-IV and** (C) **chronic GvHD.**

Table 2. Multivariate results.[a]

Event	N	Hazard Ratio (95%CI)	P Overall	P Pairwise
ANC > 500x10⁹/L				
Study cohort			0.007	
Double UCB	193	1		
Haplo+Cord	97	1.42 (1.10-1.84)		
Platelet > 20x10⁹/L				
Study cohort			<0.0001	
Double UCB	190	1		
Haplo+Cord	97	2.54 (1.88-3.41)		
Disease risk-ASBMT			0.02	
Medium *vs.* Low	124 (low)	0.97 (0.72-1.31)		0.85
High *vs.* Medium	89 (med)	0.63 (0.43-0.92)		0.02
High *vs.* Low	74 (high)	0.61 (0.43-0.88)		0.007
Sex				0.048
Male	165	1		
Female	122	1.31 (1.00-1.71)		
Grade II – IV acute GvHD				
Study cohort			<0.0001	
Double UCB	193	1		
Haplo+Cord	97	0.26 (0.15-0.45)		
Disease risk-ASBMT			0.04	
Medium *vs.* Low	126 (low)	1.42 (0.97-2.09)		0.07
High *vs.* Medium	89 (med)	0.53 (0.31-0.91)		0.02
High *vs.* Low	75 (high)	0.75 (0.44-1.29)		0.30
Grade III – IV acute GvHD				
Study cohort			0.002	
Double UCB	193	1		
Haplo+Cord	97	0.24 (0.09-0.60)		
Chronic GvHD				
Study cohort			0.0001	
Double UCB	189	1		
Haplo+Cord	97	0.06 (0.01-0.26)		
Treatment-related mortality				
Study cohort			0.23	
Double UCB	193	1.0		
Haplo+Cord	97	1.34 (0.83-2.16)		
Age in years			0.0002	
20-59	183	1		
≥ 60	107	2.43 (1.53-3.85)		
Relapse progression				
Study cohort			0.001	
Double UCB	193	1		
Haplo+Cord	97	0.48 (0.31-0.75)		
Disease risk-ASBMT			<0.0001	
Medium *vs.* low	126 (low)	1.68 (1.06-2.64)		0.02
High *vs.* medium	89 (med)	1.94 (1.22-3.11)		0.005
High *vs.* low	75 (high)	3.26 (2.08-5.12)		<0.0001
Disease group			0.0002	
Lymphoma/CLL *vs.* acute leukemia/MDS	224b	0.36 (0.20-0.64)		0.0005
Other leukemia *vs.* acute leukemia/MDS	13c	0.94 (0.37-2.23)		0.88
Other leukemia *vs.* lymphoma/CLL	53d	2.59 (0.89-7.49)		0.08
Disease-free survival				
Study cohort			0.13	
Double UCB	193	1		
Haplo+Cord	97	0.78 (0.55-1.08)		
Disease risk-ASBMT			0.002	
Medium *vs.* Low	126 (low)	1.19 (0.85-1.66)		0.31
High *vs.* Medium	89 (med)	1.62 (1.10-2.39)		0.01
High *vs.* Low	75 (high)	1.93 (1.34-2.78)		0.0004
Age at HAPLO-CORDT, in years			0.01	
20-59	183	1		
≥ 60	107	1.45 (1.08-1.93)		

Overall survival			
Study cohort			0.85
Double UCB	193	1.0	
Haplo+Cord	97	0.97 (0.68-1.36)	
Age at HAPLO-CORDT, in years			<0.0001
18-59	183	1	
≥ 60	107	2.04 (1.50-2.78)	
GvHD/relapse-free survival GRFS			
Study cohort			0.002
Double UCB	187	1.0	
Haplo+Cord	97	0.63 (0.46-0.85)	
Karnofsky Score			0.02
60-80% *vs.* 90-100%		0.65 (0.48-0.89)	0.005

[a]Risk factors evaluated: age (18-59 years vs. ≥60 years), sex, Karnofsky Performance Score (KPS) (90%-100% vs. 60%-80% vs. missing), disease [(lymphoma/chronic lymphocytic leukemia (CLL) vs. acute leukemia/myelodysplastic syndromes (MDS) vs. other leukemia)], disease risk (low vs. medium vs. high). [b]Acute leukemia/MDS, n=224. [c]Other leukemia, n=13. [d]Lymphoma/CLL, n=53.

Graft-versus-host disease and relapse-free survival (GRFS)

The cumulative incidence of acute GvHD grade 2-4 by day 120 was 17% (95%CI: 10%-25%) in the haplo-cord patients *versus* 51% (95%CI: 44%-57%) in the double UCB group (P<0.0000). Grade 3-4 acute GvHD at day 120 was similarly reduced in haplo-cord recipients *versus* controls 4% *versus* 19% (P<0.0001). Finally, chronic GvHD was much reduced in haplo-cord *versus* controls with a cumulative incidence at one year of 3% *versus* 30% (P<0.0000) (Figure 4).

Combining these important clinical end points, at one year 38% of haplo-cord recipients were alive without disease progression or serious GvHD *versus* 21% of double UCB recipients. There was a 37% improvement in hazard rate for GRFS (HR=0.63, 95%CI: 0.47-0.85; P=0.002) (Figure 4). A higher KPS (≥90%) was also associated with a superior GRFS (Table 2).

All calculations related to TRM, relapse, PFS, survival, GvHD and GRFS were repeated after excluding from the control group those patients with under 4/6 HLA matched grafts or with missing graft HLA information. This had no impact on any of the outcomes (*Online Supplementary Table S1*).

Discussion

Here we conducted a comparison of 97 adults who underwent haplo-cord transplant with a control group of patients reported to the CIBMTR undergoing reduced intensity conditioning and double UCB transplantation. The control group was restricted to patients receiving fludarabine-cyclophosphamide low-dose TBI conditioning. Originally developed at the University of Minnesota, it appears safer than many other conditioning regimens and has been widely adopted.[7,17] In a recent CIBMTR study, it was the regimen utilized in over two-thirds of US adults undergoing non-myeloablative conditioning and UCB transplant, and therefore a logical choice for our control group. The tolerability of this regimen results in part from its minimal myelosuppression,[27] and typically a minimum UCB cell dose of more than $3x10^7$ nucleated cells is considered a requisite.[6,18] For our study patients, we used a regimen that includes thymoglobulin, and that in addition is much more myelosuppressive and may occasionally lead to irreversible myelosuppression.[28] Despite this, we demonstrated more rapid neutrophil recovery and even more notably accelerated platelet recovery after haplo-cord transplantation. This should have considerable impact on duration of hospitalization, transfusion needs, and the expense of alternative donor transplantation in general.[29,30] We were also able to achieve this result despite accepting lower doses of umbilical cord blood cells, a practice that in other studies of cord blood transplantation has been associated with increased failure rates.[18,31,32]

We were unable to show a significant improvement in TRM despite the more rapid engraftment. This is somewhat paradoxical, but the benefits of rapid neutrophil and platelet recovery were possibly offset by the more intensive conditioning regimen used for haplo-cord and potentially by infections related to thymoglobulin-mediated T-cell depletion.

The rate of disease recurrence after haplo-cord transplantation was significantly decreased. Whether the reduction in relapse relates to the difference in conditioning, rather than to a graft-related effect, cannot be ascertained from our data,[32,33] but it occurred despite the use of thymoglobulin in the haplo-cord patients. ATG may be necessary to assure a smooth transition over time between the haplo-graft and umbilical cord blood predominance. In its absence, severe rejection and prolonged second nadirs have been reported.[15,34] The use of ATG has been controversial because of concerns over higher rates of disease recurrence and increased rates of infections, toxicity and post-transplant lymphoproliferative disease.[35] Increasing evidence, supported by our findings, suggests that many side-effects are dose related and that with appropriate dosing and monitoring, rabbit ATG is safe and not associated with increased rates of disease recurrence.[36] Despite the reduction in disease recurrence, progression-free and OS were not significantly improved.

We also observed a very significant decrease in the incidence of acute and chronic GvHD with haplo-cord transplantation. In part, this can be attributed to our use of ATG. The control group did not receive ATG and all patients received double UCB blood transplantation which was recently shown to be associated with more acute GvHD.[10] There may be additional reasons for the decrease in acute and chronic GvHD. For example, since the size of the cord blood unit no longer determines the rate of engraftment, we were able to choose smaller, better matched UCB units; better matching has been shown to be a major determinant of decreased GvHD.[19] Lastly,

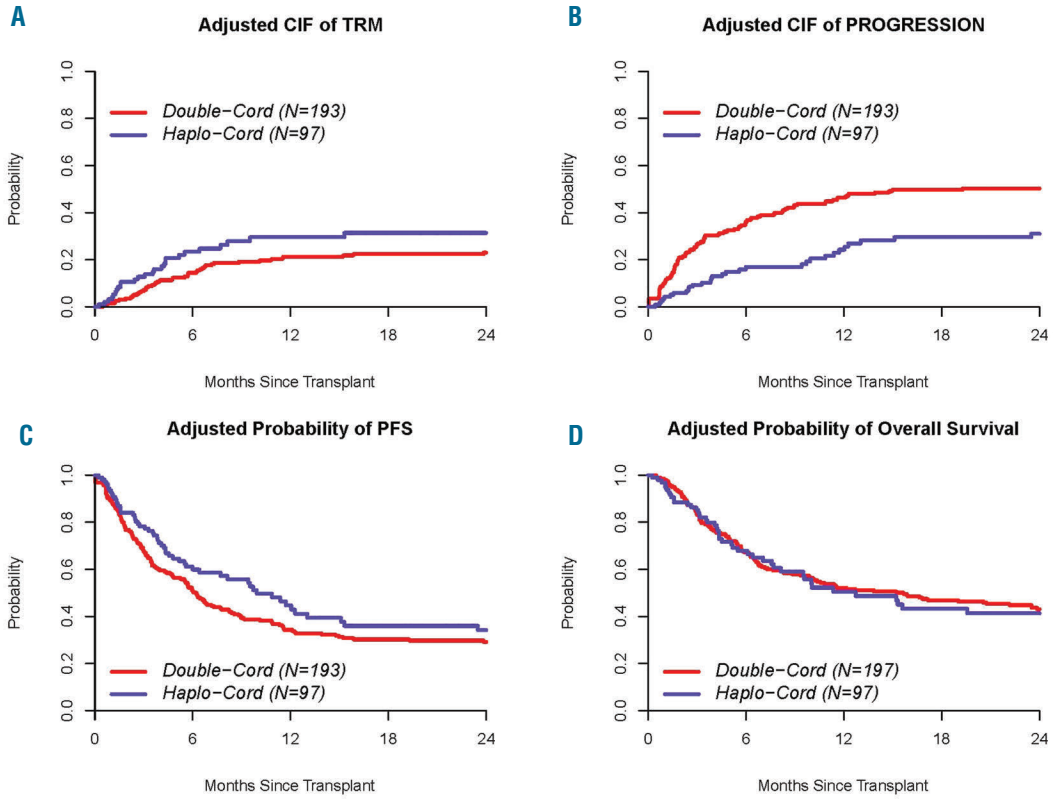

Figure 3. Adjusted cumulative incidence function for (A) **treatment-related mortality (TRM),** (B) **disease progression and adjusted Kaplan-Meier estimate for** (C) **progression-free survival and** (D) **survival.**

there may be an inhibitory effect of the haplo-graft on GvHD. Although the haplo-graft was initially considered merely a "bridge", it contains pluripotent progenitors, which in some cases readily persist in the peripheral blood T-cell compartment. Such persistent "mixed chimerism" may be mitigating the occurrence of GvHD and it is conceivable that the high doses of CD34 cells in the haplo-graft exert a veto-effect, preventing the GvH-like reactions of cord blood lymphocytes, similar to their role in prevention of graft rejection.[37] Regardless of the mechanism, the profound reduction of severe acute and chronic GvHD together with reduced recurrence rates is intriguing.

The long-term detrimental effect of chronic GvHD has been highlighted in numerous recent studies. Chronic GvHD leads to severe chronic morbidity, sequelae of steroid use, increased risk for cardiovascular disease and skin cancer, and dramatically increased risk for late non-relapse mortality.[38-40] GRFS is a novel composite end point that takes into account relapse, non-relapse mortality and severe acute and chronic GvHD.[23] As proposed by the original authors, "GRFS has value as a novel end point for benchmarking new therapies since it measures freedom from ongoing morbidity and represents ideal transplant recovery".[23] GRFS was significantly improved in haplo-cord transplant recipients compared to double UCB recipients.

Lastly, the ability to use smaller UCB units with haplo-cord transplant is of particular interest for transplant in patients of minority descent, and particularly of African descent. They tend to have rare HLA-types, and the genetically better matched UCB units are often quite small.[41,42] Our ability to use these smaller units may at least partially explain the much higher proportion of minority patients (historically underserved[43] and with worse outcomes)[44,45] in the haplo-cord group.

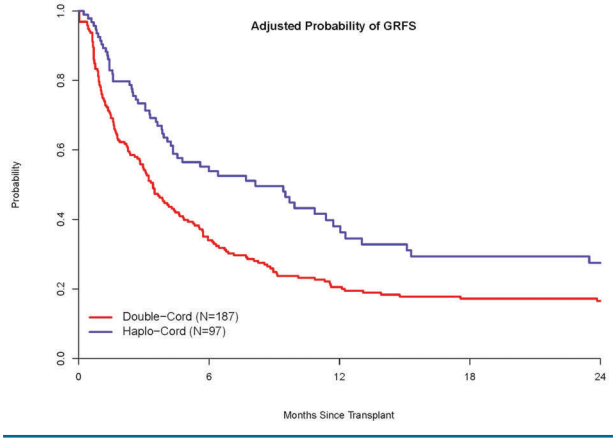

Figure 4. Adjusted Kaplan-Meier estimate for GvHD and progression-free survival (GRFS).

As experience has been gained with haplo-cord transplantation, advances in the field and our own observations have led to modifications, including most importantly: 1) strict monitoring for Epstein-Barr virus reactivation and reduction of the dose of ATG by 25%;[46-49] 2) more stringent selection of CBU units based on viability, bank of origin and high resolution HLA typing;[18,50] 3) limitation of the haplo graft dose to avoid rare instances where the haplo-

graft outcompetes the UCB unit;[16] and 4) avoidance of donors targeted by HLA antibodies, since these were associated with graft failure.[20]

The most noteworthy limitations of this analysis relate to the non-randomized comparison and potential bias of two different data sources (i.e. center-specific data relative to registry data). Adjustment for standard transplant covariates reduces but does not negate the lack of other patient covariates not captured and may influence the results. GvHD outcomes may have been captured differently for the haplo-cord centers (either better or worse) relative to the registry. We believe differences in relapse and PFS are probably accurate, as we would not expect a major difference in relapse detection. Ideally, we would have compared our outcomes to patients receiving a similar conditioning regimen, but this turned out to be impossible. The fludarabine-melphalan-ATG regimen has only been studied in limited numbers (and with different dosing regimens) in double UCB studies.[51,52] In the CIBMTR data-set, fludarabine-alkylator combinations were used in fewer than 10% of older adults with AML receiving reduced intensity conditioning and double UCB transplant. Lastly, there is a remote possibility that the observed advantages in rate of engraftment are simply a result of better HLA-matching, which was achieved because of our CBU unit selection strategy. This is highly unlikely given the well described predominance of the haplo-graft early after transplant.[53]

Several competing technologies are under development involving *in vitro* expansion of UCB cells or other progenitors or methods to enhance homing.[54] Additional trials will be required to determine if any of these procedures will ultimately be superior. CD34 selected haplo-identical cells have the advantage of available technology and rapidity. Haplo-identical transplantation with non-selected cells provides another readily available, affordable and technically less burdensome alternative. In parallel phase II studies it resulted in earlier engraftment than double cord transplant, but had higher rates of disease recurrence.[6] Further studies will be needed to compare outcomes and of these competing technologies, and to further advance the field.

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