Better allele-level matching improves transplant-related mortality after double cord blood transplantation

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ABSTRACT

Cord blood transplant requires less stringent human leukocyte antigen matching than unrelated donors. In 133 patients with hematologic malignancies who engrafted after double cord blood transplantation with a dominant unit, we studied the effect of high resolution testing at 4 loci (-A, -B, -C, -DRB1) for its impact on 2-year transplant-related mortality. Ten percent of the dominant cord blood units were matched at 7-8/8 alleles using HLA-A, -B, -C, and -DRB1; 25% were matched at 6/8, 40% at 5/8, and 25% at 4/8 or less allele. High resolution typing at 4 loci showed that there was no 2-year transplant-related mortality in 7-8/8 matched patients. Patients with 5-6/8 matched dominant cord blood units had 2-year transplant-related mortality of 39% while patients with 4/8 or less matched units had 60%. Multivariate regression analyses confirmed the independent effect of high resolution typing on the outcome when adjusted for age, diagnosis, $CD34^+$ cell dose infused, graft manipulation and cord to cord matching. The worst prognostic group included patients aged over 32 years with 4/8 or less matched cord blood units compared with patients who were either younger than 32 years old independent of allele-level matching, or aged over 32 years but with 5-6/8 matched cord blood units (Hazard Ratio 2.2; 95% confidence interval: 1.3-3.7; $P<0.001$). Patients with 7-8/8 matched units remained the group with the best prognosis. Our data suggest that high resolution typing at 4 loci and selecting cord blood units matched at at least 5/8 alleles may reduce transplant-related mortality after double cord blood transplantation.

Introduction

The use of cord blood (CB) for hematopoietic stem cell transplantation (HSCT) provides potential advantages compared with the use of bone marrow (BM) or mobilized peripheral blood progenitor cells (PBPCs), including ease of collection, prompt availability and decreased stringency of human leukocyte antigen (HLA)–matching requirements compared to that of unrelated BM or PBPCs.[1] The greater degree of donor-recipient HLA mismatch tolerated when CB grafts are used without an increased incidence of graft-*versus*-host disease (GvHD) is likely due to the lower numbers of T cells and the relatively immunologically naive status of the lymphocytes in CB compared with BM or PBPCs.[2] Therefore, while unrelated adult donors are selected to be closely matched to recipients at HLA-A, -B, -C, and -DRB1 by high resolution testing,[3] CB units are currently most commonly selected using lower resolution HLA typing (antigen-level) for HLA-A and -B and allele level for HLA-DRB1.[4]

A recent study by the Center for International Blood and Marrow Transplantation Research (CIBMTR) and Eurocord reported better outcomes in single CB transplants (CBT) with improved allele-level matching for 4 HLA loci (-A, -B, -C, and -DRB1).[5] Their results suggest the avoidance of CB transplantation with 3 or more allele level mismatches due to unacceptable non-relapse mortality and inferior survival.

Here, we report our experience of 133 patients with high-risk hematologic malignancies who underwent dCBT. Our aim was to investigate the impact of HLA matching by high resolution testing between the dominant CB unit and the recipient on transplant outcomes.

Methods

Patients with hematologic malignancies who received their first allogeneic HSCT using double CB units between January 2003 and April 2014 and engrafted within 42 days were included in this analysis (n=133). Patients eligible for this study had donor and recipient HLA typing performed at the HLA-A, HLA-B, HLA-C and HLA-DRB1 loci using molecular techniques (probe-based and sequenced-based typing) at intermediate and high resolution levels. This study was performed in accordance with the Declaration of Helsinki and was approved by the local institutional review board (IRB). Written informed consent was obtained from all patients.

Cord blood unit selection prior to cord blood transplant

As per institutional guidelines, the largest available CB unit with

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Table 1. Patients', disease and graft characteristics.

Characteristics	N (%)
Diagnosis to transplant, median (range), months	15 (3-162)
Age, median (range), years	44 (1-73)
Patient weight (kg), median (IQR)	79.1 (61.1-90.8)
Age	
≤17	6 (4)
18-30	23 (17)
31-40	25 (19)
41-50	25 (19)
51-60	33 (25)
>60	21 (16)
Disease	
AML/MDS	78 (59)
ALL	27 (20)
NHL/HD/CLL	23 (17)
CML/MPD	5 (4)
Cytogenetics for AML/MDS patients	
Good	5 (5)
Intermediate	51 (53)
Bad	41 (42)
Disease status at HSCT	
CR1/CP1	29 (22)
CR2/CP2	45 (34)
Other	59 (44)
CMV seropositivity of the recipient	
Non-reactive	12 (9%)
Reactive	122 (91%)
Donor-recipient sex mismatch (dominant unit)	
Female-male	34 (26)
Female-female	32 (24)
Male-female	28 (21)
Male-male	36 (27)
Unknown	3 (2)
Preparative regimen	
Myeloablative	79(59)
Reduced intensity	54 (41)
CB unit manipulation (any of the units)	
None	45 (34)
Mesenchymal stem cell expansion	85 (64)
Fucosylation	3 (2)
Total nucleated cell dose infused, median (range) ($x10^8$/kg)	0.52 (0.15-43)
Total nucleated cell dose of the dominant unit, median (range) ($x10^8$/kg)	0.22 (0.05-29)
CD34 cell dose infused, median (IQR) ($x10^6$/kg)	0.35 (0.14,1.7)
CD34 cell dose of the dominant unit, median (IQR) ($x10^6$/kg)	0.08 (0.06, 0.15)
Dominant CB unit-recipient HLA match	
≤4/8	34 (25)
5/8	52 (40)
6/8	34 (25)
7-8/8	13 (10)
Non-dominant CB unit-recipient HLA match	
≤4/8	34 (26)
5/8	51 (39)
6/8	38 (29)
7-8/8	9 (7)
CB unit-CB unit match	
≤4/8	61 (46)
5/8	37 (28)
6/8	21 (16)
7-8/8	13 (10)
ABO compatibility of the dominant unit and the recipient	
Matched	50 (38)
Minor mismatch	34 (26)
Major mismatch	47 (36)
Time of transplant from diagnosis, median (range)	15 months
Transplant period	
2000-2009	63 (47)
2010 and beyond	70 (53)

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; ALL: acute lymphoblastic leukemia; NHL: non-Hodgkin lymphoma; HD: Hodgkin disease; CLL: chronic lymphocytic leukemia; CML: chronic myeloid leukemia; MPD: myeloproliferative disorder; HSCT: hematopoietic stem cell transplantation; CR1: first complete remission; CP1: first chronic phase; TBI: total body irradiation; CB: cord blood. CMV: cytomegalovirus; IQR: interquartile range; HLA: human leukocyte antigen.

higher total nucleated cell (TNC) dose that matched at four or more HLA alleles by intermediate-resolution typing for HLA-A and HLA-B and high-resolution typing for HLA DRB1 alleles were selected prior to HSCT. HLA disparity between the two CB units was not considered in the choice of units. The minimal allowed cryopreserved TNC dose for each CB unit was 1.0×10^7/kg or more before and 1.5×10^7/kg or more after 2007.

Treatment plan

Between 2003 and 2014, patients received two CB units that were either both unmanipulated (n=45) or where one of the two was expanded (n=85)[6,7] or fucosylated (n=3) *ex vivo* prior to infusion.[8]

Patients received myeloablative conditioning (MAC) with melphalan (140 mg/m^2 on day -8), thiotepa (10 mg/kg on day -7), and fludarabine (40 mg/m^2 on days -6 through -3) (n=61); or busulfan (average daily area under the curve 5000 μMol-min), fludarabine (10 mg/m^2), and clofarabine (30 mg/m^2) were administered from days -7 through -4 with total body irradiation (TBI) 2 Gy administered on day -3 (n=18).[9] The reduced intensity conditioning regimen (RIC) included cyclophosphamide (50 mg/kg on day -6) and fludarabine (40 mg/m^2 on days -6 through- 3) and TBI 2Gy (on day -1) (n=34) or melphalan(140 mg/m^2 on day -2), and fludarabine (40

Table 2. Transplant-related mortality, overall survival, disease progression and progression-free survival at two years by human leukocyte antigen (HLA) matching between the cord blood units and recipient by univariate analysis.

	N.	2-year transplant-related mortality			2-year overall survival			2-year progression			2-year progression-free survival		
High resolution typing at HLA –A, -B, -C , DRB1 (dominant unit and the recipient)													
3-4/8	34	Ref			Ref			Ref			Ref		
5/8	52	0.5	0.3-1.03	0.06	0.8	0.5-1.4	0.4	0.4	0.8-5.3	0.1	0.99	0.6-1.6	0.9
6/8	34	0.5	0.2-0.97	0.04	0.5	0.3-1.0	0.06	1.2	0.4-3.4	0.8	0.6	0.3-1.1	0.1
7-8/8	13	*NE			0.5	0.2-1.3	0.2	2.6	0.8-7.9	0.1	0.9	0.4-1.9	0.8
3-4/8	34	Ref			Ref			Ref			Ref		
5-6/8	86	0.5	0.3-0.9	0.02	0.7	0.4-1.1	0.1	2	0.8-4.9	0.1	0.8	0.5-1.3	0.5
7-8/8	13	NE			0.5	0.2-1.3	0.1	2.9	1.0-8.8	0.05	0.9	0.4-1.9	0.8
High resolution typing at HLA –A, -B, -C , DRB1 (non-dominant unit and the recipient)													
3-4/8	34	Ref			Ref			Ref			Ref		
5-6/8	89	0.7	0.4-1.3	0.3	0.8	0.5-1.3	0.3	0.9	0.4-1.9	0.8	0.8	0.5-1.2	0.3
7-8/8	9	NE		<0.001	0.6	0.2-1.5	0.2	2	0.7-5.7	0.2	0.7	0.3-1.7	0.4
High resolution typing at HLA –A, -B, -C , DRB1 between 2 CB units													
3-4/8	61	Ref			Ref			Ref			Ref		
5-6/8	58	0.5	0.3-0.9	0.02	0.7	0.4-1.1	0.1	1.99	1.0-3.9	0.05	0.8	0.5-1.2	0.3
7-8/8	13	0.2	0.05-0.97	0.046	0.3	0.1-0.9	0.03	1.4	0.5-4.1	0.5	0.5	0.2-1.1	0.1

**NE: non-evaluable because of small sample size or absence of failure events. HR: hazard ratio; HLA: human leukocyte antigen.*

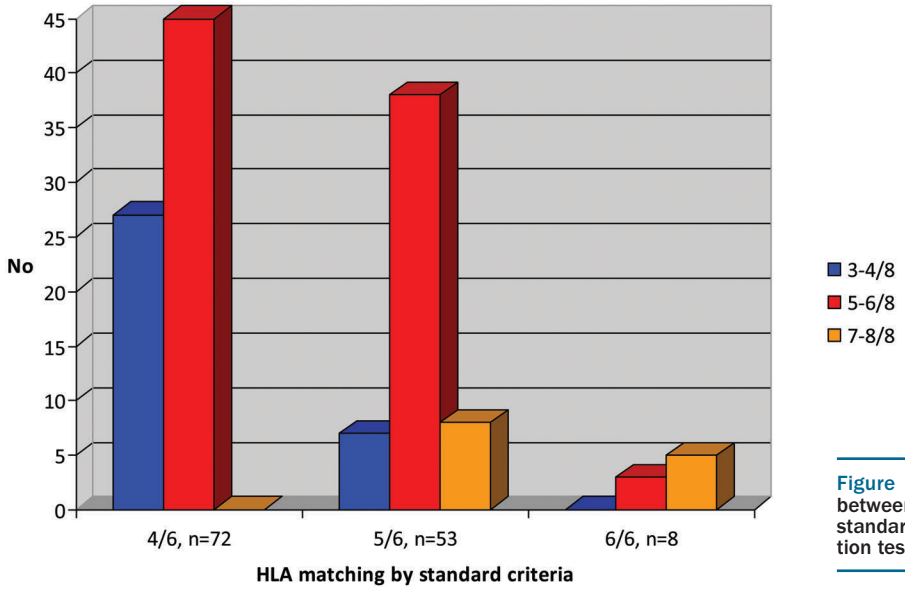

Figure 1. The distribution of HLA matching between the recipient and the dominant unit by standard matching criteria and by high resolution testing at HLA-A, -B and DRB1.

mg/m^2 on days -5 through -2) (n=61) (n=20).[10] All patients received rabbit antithymocyte globulin (thymoglobuline: Genzyme). Prophylaxis against graft-*versus*-host disease (GvHD) consisted of tacrolimus and mycophenolate mofetil.

All patients received subcutaneous granulocyte colony-stimulating factor (Amgen) from day 0 until neutrophil recovery.

Engraftment, donor chimerism and unit predominance

Neutrophil engraftment was defined as the first of three consecutive days of an absolute neutrophil count (ANC) of 0.5×10^9/L or over after the post-transplant nadir. Serial sampling of the BM and/or PB at days 30, 60, 100, 180, and 360 after transplantation determined donor chimerism using eight highly polymorphic microsatellite markers (purchased from Integrated DNA Technologies, Inc., Coralville, IA, USA) in a multiplex polymerase chain reaction (PCR) in recipient and donor units. The dominant unit was defined either as the only unit detected, or, in the case of the presence of 2 units, the unit contributing more than 50% of the total donor chimerism in serial testing. Single-donor dominance was defined as one unit serially contributing more than 70% of the donor chimerism in all cell fractions, or as the sole CB donor present.[11] This was assigned based on either bone marrow or blood chimerism.

End points and definition

The primary study end point was the cumulative incidence of transplant-related mortality (TRM) at two years defined as death due to any cause other than disease persistence or progression. Secondary end points included disease progression, progression-free survival (PFS), overall survival (OS), as well as the rates of acute and chronic GvHD (aGVHD and cGVHD, respectively). PFS was defined as survival without disease progression. Patients who had no engraftment of neutrophils by day 42 were treated as graft failures and were not included in the analyses. Acute GvHD was graded according to the consensus criteria.[12,13] Chronic GvHD was diagnosed when clinical signs were present or developed for the first time after day 100.[14] Donor-recipient HLA match was examined based on the degree of matching between the dominant unit and recipient.

Statistical analysis

The incidence of TRM was estimated using the cumulative incidence method and accounting for disease progression or death attributable to persistence disease as competing risks. Actuarial OS and PFS were estimated using the Kaplan-Meier method. The impact of prognostic factors on OS and PFS were evaluated using Cox's proportional hazards regression on univariate and multivariate analysis. Prognostic factors for TRM and disease progression

Table 3. Univariate analyses for treatment-related mortality and overall survival.

	TRM			OS		
	HR	95%CI	*P*	HR	95%CI	*P*
Age >32	2.2	1.1-4.5	0.03	2.2	1.3-3.9	0.006
Diagnosis						
AML/MDS	Ref			Ref		
ALL	2.1	1.03-4.3	0.004	1.3	0.7-2.2	0.4
NHL/HD/CLL	2.03	1.1-3.9	0.03	0.9	0.5-1.6	0.7
CML/MPD	1.2	0.4-3.8	0.8	0.7	0.2-2.4	0.6
Lymphoid *vs.* myeloid neoplasm	2.04	1.2-3.5	0.01	1.1	0.7-1.7	0.7
Disease status at HSCT						
CR1 *vs.* others	0.5	0.2-1.2	0.1	0.6	0.3-1.1	0.08
Myeloablative *vs.* reduced intensity conditioning	1.4	0.8-2.5	0.2	0.8	0.5-1.3	0.5
Graft manipulation *vs.* no manipulation	1.04	0.6-1.8	0.9	0.9	0.6-1.4	0.6
CD34$^+$ cell dose infused (total) (x10^6/kg)						
≤ 1.75 *vs.* lower	0.8	0.4-1.5	0.4	0.8	0.5-1.4	0.5
CD34+ cell dose of dominant unit (x10^6/kg)						
≤1.75 *vs.* lower	1.03	0.5-1.9	0.9	0.9	0.5-1.6	0.8
TNC infused (total) (x10^8/kg)						
≤ 0.2	Ref			Ref		
> 0.2-0.5	Ref			Ref	0.6-1.6	0.9
> 0.5	0.8	0.5-1.5	0.5	0.96	0.6-1.5	0.9
TNC dose of dominant unit (x10^8/kg)						
≤ 0.2	Ref			Ref		
> 0.2-0.5	1.1	0.6-1.9	0.1	1.03	0.6-1.6	0.9
> 0.5	0.9	0.2-3.1	0.9	0.99	0.4-2.6	0.9
Transplant year						
2003-2009	Ref			Ref		
2010 and beyond	0.8	0.4-1.3	0.4	0.9	0.6-1.4	0.7
Time to dCBT from diagnosis						
>15 months *vs.* ≤ 15 months	1.4	0.8-2.4	0.2	0.9	0.6-1.4	0.7

TRM: transplant-related mortality; OS: overall survival, HR: hazard ratio; HSCT: hematopoietic stem cell transplantation; CR1: first complete remission; TNC: total nucleated cell; dCBT: double cord blood transplantation.

were evaluated using Fine and Gray regression model to account for the competing risk. The prognostic factors considered included degree of HLA-matching matching (with the recipient and between the units), recipient's age, disease diagnosis, disease status at HSCT, conditioning regimen, total infused cell dose (TNC and $CD34^+$ separately), and manipulation of one of the CB units. P=0.05 was considered statistically significant. Statistical analyses were performed using STATA 11.0 (StataCorp. 2009: Stata Statistical Software: Release 11: College Station, TX, USA: StataCorp LP).

Results

Demographics

Patients' demographics, disease status and CB graft characteristics for the 133 patients are presented in Table 1. Median age of the study cohort was 44 (range: 1-73) and 21 patients (16%) were over 60 years of age. The majority of diagnoses were acute myeloid leukemia/myelodysplastic syndrome (AML/MDS) in 78 patients (59%) followed by acute lymphoblastic leukemia (ALL) in 27 patients (20%) and non-Hodgkin lymphoma (NHL)/Hodgkin disease (HD)/chronic lymphocytic leukemia (CLL) in 23 patients (17%).

MAC regimens were used in 79 patients (59%) and reduced intensity conditioning (RIC) in 54 (41%) patients. Of 39 patients under 33 years of age, 6 (15%) received RIC compared with 48 of 94 (51%) older patients (P<0.001). Sex-mismatch between the recipient and donor (dominant CB unit) was observed in 62 (47%) patients. As per protocol requirement, 88 patients (66%) had manipulation of one CB unit with either *ex vivo* expansion or fucosylation. The median time to transplant from diagnosis was 15 months.

One unit dominated in all engrafting patients; 110 of 133 patients (82.7%) had complete dominance of a single unit documented as early as 21-28 days after transplantation. The percentage of patients with hematopoiesis entirely derived from a single unit further increased to 72 of 83 assessable patients (86.7%) at day 60 and 71 of 81 assessable patients (87.6%) at day 100.

HLA matching of the dominant cord blood unit

Table 1 presents the distribution of HLA matching between the dominant unit and the recipient by high resolution testing at 4 loci (-A, -B, -C and -DRB1). Of the 133 patients, 34 (25%) had dominant units matched at 4/8 or less, 52 (40%) at 5/8, 34 (25%) at 6/8, and only 13 (10%) at 7-8/8. The distribution of high resolution testing at 4 loci compared with conventional standard criteria by the dominant unit is shown in Figure 1. Among the 75 patients with 4/6 matched dominant CB units using conventional standards, 27 (37.5%) were matched at 3/8 or 4/8 allele-level by high resolution testing. On the contrary, among patients with 5/6 matched dominant CB units by standard criteria, there were only 7 (13%) dominant CB units matched at 3/8 or 4/8 at allele-level. In total, there were only 6 patients (5%) with CB units matched at 3/8 allele-level.

The total cell dose infused was similar between groups by HLA matching. The median dose of TNC infused was ($X10^8$/kg) was 0.6 in 4/8 or less matched, 0.5 in 5-6/8, and 0.4 in 7-8/8 matched patients. Similarly, there was no difference in total infused $CD34^+$ cell dose ($X10^6$/kg) between patients with 4/8 or less and 5-6/8 matched dominant units with medians of 0.475 and 0.43, respectively. The better matched group at 7-8/8 allele-level by high resolution typing had a median CD34 infused dose of 0.21, which was slightly lower compared with worse matched groups.

Median time to transplant from diagnosis was 14 months in 4/8 or less matched, 15.4 months in 5-6/8 matched, and 11.4 months in 7-8/8 matched CB units recipients.

Table 4. Multivariate regression model for 2-year treatment-related mortality and overall survival.

	HR	95%CI	P
TRM at 2 years			
HLA matching between the dominant unit and the recipient and the age			
Either age ≤ 32 or HLA matching >4/8	Ref		
Age >32 and HLA matching ≤ 4/8	2.3	1.1-4.3	0.02
HLA matching 7-8/8	NE		
HLA matching between units			
≤4/8 *vs.* >4/8	1.9	0.9-3.8	0.07
Lymphoid *vs.* myeloid malignancy	2.5	1.2-3.5	0.01
$CD34^+$ cell dose infused (total) ($x10^6$/kg)			
≥ 1.75 *vs.* <1.75	0.8	0.4-1.8	0.7
Graft manipulation *vs.* no manipulation	0.9	0.4-1.6	0.6
OS at 2 years			
HLA matching between the dominant unit and the recipient and the age			
Either age ≤ 32 or HLA matching >5/8	Ref		
Age >32 and HLA matching ≤ 5/8	2.8	1.1-7.2	0.03
HLA matching between units			
≤4/8 *vs.* >4/8	0.3	0.1-1.1	0.06
$CD34^+$ cell dose infused (total) ($x10^6$/kg)			
≥ 1.75 *vs.* <1.75	1.1	0.6-1.9	0.9
Graft manipulation *vs.* no manipulation	1.3	0.8-2.2	0.3

TRM: transplant-related mortality; OS: overall survival, HR: hazard ratio; HSCT: hematopoietic stem cell transplantation; HLA: human leukocyte antigen.

HLA matching of the non-dominant cord blood unit

Non-dominant CB units were HLA matched to the recipient at 4/8 or less level in 34 (26%) patients, 5/8 in 51 (39%), 6/8 in 38 (29%), and 7-8/8 in 9 (7%) patients (Table 1). Of 9 patients with 7-8/8 matched non-dominant units, none had dominant CB units matched to the recipient at 4/8 or less allele-level, but 5 had 7-8/8 and the other 4 had 5-6/8 matched dominant CB units. Similarly, of 34 patients with 4/8 or less matched non-dominant units, 17 had 4/8 or less, and 13 had 5/8 matched dominant CB units.

Unit to unit matching analyses revealed that 61 (46%) patients had 4/8 or less HLA matching between two CB units and only 13 (9.8%) had 7-8/8 matching between units.

Transplant-related mortality by HLA matching and other clinical variables

The median follow up among surviving patients was 29 months (range: 3-74). All outcomes were assessed at two years post transplant.

High resolution typing at 4 loci (HLA –A, -B, -C and DRB1) was able to differentiate three different risk groups for 2-year TRM when dominant CB was analyzed (Figure 2A). This was significantly better compared with standard typing (*Online Supplementary Table S1* and *Figure S1A and B*). Patients with 7-8/8 allele-level matched units experienced no TRM during the study period. As expected, TRM was highest in patients transplanted with 3-4/8 allele-level matched CB units (Figure 2A), followed by patients with 5-6/8 allele-level matched CB units [Hazard Ratio (HR) 0.5; 95%CI: 0.3-0.9; *P*=0.02]. Matching between non-dominant unit and the recipient showed that patients with 7-8/8 matched non-dominant units had no TRM over the study period though there was no difference between 5-6/8 and 4/8 or less matched non-dominant units (HR 0.7; 95%CI: 0.4-1.3; *P*=0.7) (Table 2). On the other hand, better matching by high resolution typing between two CB units led to a decrease in TRM on univariate analyses. Patients with CB units matched to each other at 5-6/8 and 7-8/8 level had less TRM compared with 4/8 or less matched unit recipients (HR 0.5; 95%CI: 0.3-0.9; *P*=0.02 and HR 0.2; 95%CI: 0.05-0.97; *P*=0.046, respectively).

We also analyzed the impact of age, diagnosis, disease status at dCBT, conditioning intensity, graft manipulation, $CD34^+$ cell dose infused (post thaw), TNC dose infused, transplant year and time to transplant from diagnosis on 2-year TRM (Table 3). On univariate analyses, age older than 32 years (HR 2.2; 95%CI: 1.1-4.5; *P*=0.03), lymphoid *versus* myeloid malignancy (HR 2.04; 95%CI: 1.2-3.5; *P*=0.01) were the only poor prognostic factors for TRM in addition to HLA matching between the dominant CB unit and the recipient and unit to unit matching. The $CD34^+$ cell dose infused and graft manipulation had no effect on TRM.

Multivariate analyses for treatment-related mortality

We assessed the independent effects of HLA matching by high resolution typing at 4 loci between the dominant unit and the recipient by multivariate analyses adjusting for the factors that were shown to be significant on univariate analysis, including: unit to unit matching, age older than 32 years, and lymphoid malignancies. Graft manipulation and total CD34 cell dose infused were also considered in the multivariate model because of their biological significance, even though these factors were not significant on univariate analysis. The effect of HLA matching between the recipient and dominant unit by high resolution on TRM was not uniform in younger and older patients (Figure 3A). This interaction was taken into consideration in the multivariate regression model by considering the effect of HLA matching according to age groups (>32 and ≤32 years). HLA matching in older patients, and lymphoid malignancies (HR 2.5; 95%CI: 1.2-3.5; *P*=0.01) were the only significant predictors of TRM at two years on multivariate analysis (Table 4). Patients with 7-8/8 dominant units had the best TRM with no event observed. Patients over 32 years of age who had a dominant CB units matched 4/8 or less had the highest TRM at

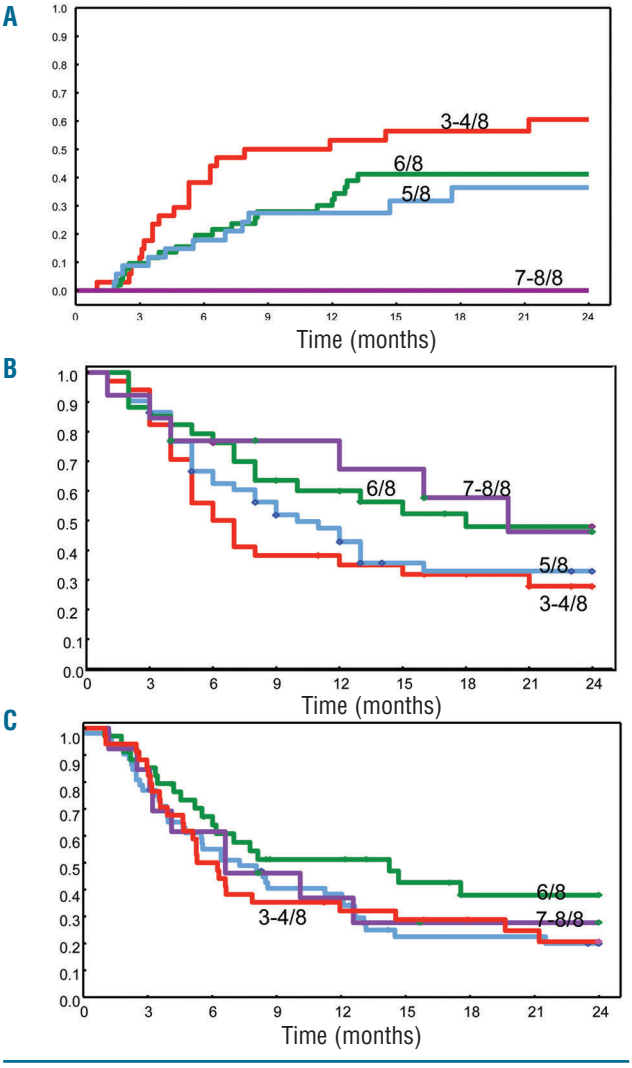

Figure 2. The effect of HLA matching between the dominant unit and the recipient by high resolution testing at HLA-A, -B, -C and –DRB1 stratified by age on (A) treatment-related mortality (TRM) (B) overall survival (OS) and (C) progression-free survival (PFS). High resolution typing at 4 loci revealed that patients with 7-8/8 matched CB units had no 2-year TRM compared with 39% (95%CI: 30%-52%) in patients with 5-6/8 matched and 60% (95%CI: 46%-80%) in ≤4/8 matched patients. For OS, patients matched at >5/8 had better outcomes compared with less matched patients, the 2-year OS estimates were 47% (95%CI: 31%-62%) *versus* 31% (95%CI: 21%-41%). However, PFS was comparable between patients with less matched and better matched CB units with two years of 35% (95%CI: 21%-50%) and 20% (95%CI: 12%-30%), respectively.

two years compared with patients who were either aged 32 years or under or aged over 32 but with 5-6/8 matched dominant units (HR 2.3; 95%CI:1.1-4.3; *P*=0.02). The cumulative incidence of TRM by age and HLA matching adjusted by all other variables is shown in Figure 4.

Overall survival, progression-free survival and disease progression

For OS, each of the 3 HLA matching criteria revealed two different prognostic groups (Table 2 and *Online Supplementary Table S1*). High-resolution testing at 4 loci showed that transplants matched at least more than 5/8 allele-level had improved OS compared with less matched transplants (HR 0.7; 95%CI: 0.4-1.1; *P*=0.09) (Figure 2B) though this did not reach statistical significance. Similarly, HLA matching at 7-8/8 level between two CB units improved OS (HR 0.3; 95%CI: 0.1-0.9; *P*=0.03) compared with 4/8 or less matching between units.

Among other variables tested, age over 32 years was the only prognostic factor for OS (HR 2.2; 95%CI: 1.3-3.9; *P*=0.006).

Similar to TRM, the effect of high resolution matching between the recipient and dominant unit on OS was not uniform in younger and older patients (Figure 3B). Therefore, this interaction was taken care of by stratification of HLA matching and age in multivariate regressions. When adjusted for the factors that were either significant on univariate analysis, including unit to unit HLA matching or clinically important, including CD34$^+$ cell dose and graft manipulation, multivariate regressions showed that patients aged 32 years or over who had CB dominant units matched 5/8 or less had inferior OS compared with patients either aged 32 years or under or had more than 5/8 matched units (HR 2.8; 95%CI: 1.1-7.2; *P*=0.03) (Table 4).

We did not observe any prognostic impact of the HLA matching between the CB units and the recipient on PFS in contrast to TRM and OS (Figure 2C and *Online Supplementary Figure S2C and F*). Patients with dominant units matched at 5-6/8 and 7-8/8 had comparable PFS with 4/8 or less matched patients (HR 0.8; 95%CI: 0.5-1.3; *P*=0.5 and HR 0.9; 95%CI: 0.4-1.9; *P*=0.7, respectively). Similarly, HLA matching between non-dominant unit and the recipient, as well as matching between two CB units, did not affect PFS (Table 2).

Disease progression was higher in patients with 7-8/8 matched dominant units compared with 4 or less matched units (HR 2.9; 95%CI: 1.0-8.8; *P*=0.05) though there was no difference between 5-6/8 and 4/8 or less matched patients for the cumulative incidence of disease progression (HR 2; 95%CI: 0.8-4.9; *P*=0.1). The HLA matching between non-dominant CB unit and the recipient did not have any impact on the incidence of disease progression, but unit to unit matching at 5-6/8 level was associated with higher disease progression compared with 4/8 or less matching between two CB units (HR 1.99; 95%CI: 1.0-3.9; *P*=0.05).

There were 83 deaths observed in our study population.

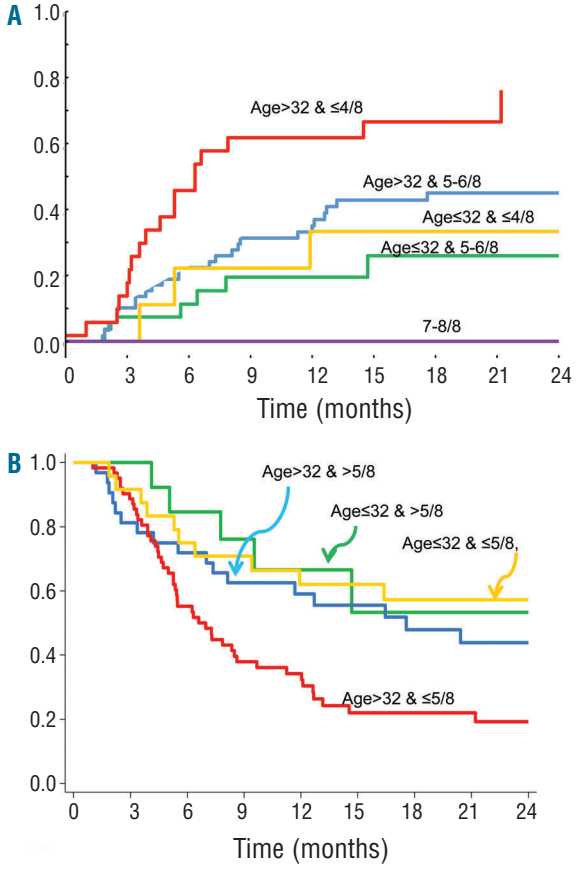

Figure 3. The effect of HLA matching between the dominant unit and the recipient by high resolution testing at HLA-A, -B, -C and –DRB1 stratified by age on (A) treatment-related mortality (TRM) and (B) overall survival (OS). Patients aged over 32 years with CB units matched ≤4/8 had 2-year TRM of 74% compared with patients in the same age group but with 5-6/8 matched units who had 2-year TRM of 45%. Patients aged ≤32 had better TRM independent of their HLA matching with dominant CB unit in the range of 26%-33%. The best OS was observed in patients aged ≤32 and had dominant CB units matched at >5/8 with 2-year estimates of 53% (95%CI: 20-78). On the other hand, patients aged >32 and had ≤5/8 matched units had decreased OS with 2-year estimate of 19% (95%CI: 10-31).

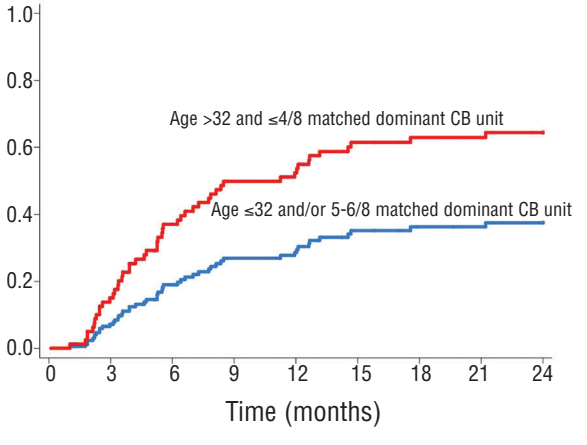

Figure 4. Transplant-related mortality at two years by HLA matching between the dominant cord blood (CB) unit and the recipient and age adjusted by diagnosis, CD34$^+$ cell dose infused, graft manipulation and HLA matching between 2 CB units. The 2-year adjusted incidence of treatment-related mortality (TRM) was significantly higher for patients aged >32 and had ≤4/8 matched dominant CB units compared with patients who are either younger or had better matched units.

The most common causes of death were infection in 9 of 24 deaths (38%) followed by GvHD in 4 (17%) for patients with 4/8 or less matched dominant CB units. This differed in the 5-6/8 matched group, with disease progression the most common cause of death in 20 of 51 deaths (40%) followed by GvHD in 10 (20%) deaths. As expected, there was no TRM in the 7-8/8 matched group in which the sole cause of death was disease progression in 8 deaths.

Graft-versus-host disease

The rate of grades 2-4 acute GvHD increased after transplantation of less matched CB units compared with better matched transplants by any HLA matching criteria but the differences in cumulative incidences did not reach the level of significance except by standard HLA matching criteria (*Online Supplementary Table S2*). There were no significant differences in the risk estimates considering mismatching by high resolution testing despite the less acute GvHD observed in 7-8/8 allele-level matched transplants. The risk of chronic GvHD was not significantly associated with HLA matching, either by standard criteria or by high resolution typing at 4 loci (*Online Supplementary Table S2*).

Discussion

This study demonstrates the importance of high resolution typing of CB units at the HLA–A, -B, -C and –DRB1 loci in the dCBT setting. Better matching of the dominant unit at those 4 loci predicted less 2-year TRM and was an independent and highly significant prognostic factor in patients with different diseases and preparative regimens. With the allele-level typing, three distinct prognostic groups were identified, compared with current standard matching criteria with intermediate resolution testing at HLA–A and –B and high resolution at –DRB1 where only two prognostic groups could be determined. The major impact of HLA matching at 4 loci by high resolution testing was to identify a very poor prognostic group of patients with a 2-year TRM of 74% if CB unit and the recipient were matched at less than 5/8 alleles and the recipient was older than 32 years of age. This high-risk group represented approximately one-third of the patients with 4/6 matched CB units using standard criteria. Our study has limitations inherent in a retrospective design and we believe that our results need to be confirmed by different groups before a paradigm change in CB unit selection in double CB recipients is widely accepted. On the other hand, TRM continues to be the major reason for failure after dCBT, with different series reporting incidence rates ranging from 29% to 52%.[10,15,16] In our study, the magnitude of decrease in 2-year TRM by just selecting 5-6/8 CB units rather than 4/8 or less matched units in patients over 32 years of age was approximately 30%. This supports the notion that high resolution typing at 4 loci and selecting CB units matched at at least 5/8 allele-level in older patients may reduce TRM risk and improve transplant outcomes after dCBT.

The association of better HLA matching and improved transplant outcomes, especially a reduction in TRM, is well established in the unrelated donor setting using BM and PBPCs as the stem cell source.[3,17-21] However, the likelihood of finding an optimal donor who is matched at high resolution at HLA-A, -B, -C, and-DRB1 varies among racial and ethnic groups, with the highest probability among whites of European descent, at 75%, and the lowest probability among blacks of South or Central American descent, at 16%.[22] CB units matched by intermediate resolution at HLA –A, -B and high resolution at DRB1 at 4/6 or higher, which is the current standard for CB selection, are available for almost all patients regardless of racial and ethnic background. A recent analysis on behalf of the CIBMTR and Eurocord investigated whether CB unit selection based on high resolution HLA testing would have improved transplant outcomes in single CBT and, showed that units matched at 4/8 or less were associated with higher TRM and should be avoided.[5]

After dCBT, short- and long-term hematopoietic dominance of a single CB unit is established in the majority of patients. The mechanisms for this observation have been investigated by several groups and are not yet universally conclusive.[23,24] Interestingly, in almost all dCBT transplant recipients, single-unit dominance is frequently detected as early as day 21 after transplantation.[25,26] Potential variables contributing to donor dominance that have previously been evaluated include cryopreserved and infused TNC and $CD34^+$ cell doses, infused $CD3^+$ cell doses, overall viability, degree of HLA or sex matching, ABO typing, and order of unit infusion, all without definitive or reproducible correlation.[27-30] Hence, until further studies establish the definitive predictors of dominant unit more precisely, we believe that selection of both CB units should be based on high resolution testing at 8 allele-level to improve the matching between the patient and CB and decrease TRM. There have been concerns that with better HLA matching, the graft-*versus*-leukemia effect may be diminished leading to increased relapse incidence after CBT. In our cohort, patients with 5-6/8 matched dominant units had similar disease progression and PFS with 4/8 or less matched patients. Patients with 7-8/8 matched dominant units had slightly increased disease progression (which was not statistically significant) but similar PFS compared with 4/8 or less matched patients. These results were similar to the CIBMTR-Eurocord analysis in single CBT that showed decreased TRM with better HLA matching by high resolution testing in CB recipients without deleterious effect on disease progression and PFS. On the other hand, given the potential confounders of patient selection and the relatively small sample size of our study cohort, we do believe that the impact of high resolution HLA matching at 4 loci on disease progression needs to be investigated in larger samples to allow more definite conclusions to be reached.

This new strategy, while improving TRM in the post-transplant setting, may lead to a decrease in the globall CB inventory for patients without a matched unrelated or related donor. In the CIBMTR/Eurocord study with single CBT, approximately half of the patients would not have received a CBT if units matched at 5/8 or less had been avoided. In our study with dCBT, one-fourth of the patients would not have been eligible for transplant if only units matched at 5-8/8 alleles were allowed. It is obvious that, if applied, such selection criterion would have its impact primarily on the 4/6 matched patients using the current standard with intermediate level testing for HLA-A and -B and high-resolution testing for DRB1. On the other hand, a recent analysis by Dahi *et al.*[31] investigated whether the unit selection would change in 100 dCBT patients if both a better HLA match using high resolution

typing and a cryopreserved TNC 2.0×10^7/kg/unit or more were required. Using this model, the graft would have changed in 33 of 100 (33%) transplants, indicating that improved matching was possible in the majority of the patients.

In our cohort, we did not observe decreased TRM or survival advantage using units with TNC and/or $CD34^+$ cell dose in excess of the required minimum dose. The median TNC (total of 2 CB units) delivered post thaw was 0.5×10^8/kg and 75% of transplantations had TNC in excess of 0.15×10^8/kg. Our results are consistent with previous reports that failed to demonstrate improvement in TRM and overall survival with cell dose used in excess of the minimum required, lending support to our observation that TNC in excess of the minimum required dose may not lower mortality risks to overcome the poor prognosis associated with worse HLA.[5,32,33] These data confirm the need for a minimum TNC to ensure engraftment and thereafter prioritize CB unit selection on HLA match considering allele-level HLA typing at 4 loci. Therefore, we need innovative strategies that might help us to overcome the limited availability in the global inventory with stricter criteria for allele-level matching applied. Additional CB units with a greater range of racial/ethnic diversity are needed to improve the chances of finding well-matched units. Graft manipulation might provide a treatment paradigm to use smaller CB units with better HLA allele level matches, and currently there are a number of promising graft manipulation strategies under investigation. We and others have shown that manipulation of one of the CB units with *ex vivo* expansion, fucosylation or treatment with prostaglandin E2 prior to infusion resulted in more rapid and higher levels of neutrophil and platelet engraftment as compared to untreated CB units after dCBT.[7,8,34-37] These graft manipulation strategies, once widely adopted, will allow a paradigm shift for all CB transplantation approaches by allowing us to use smaller, better HLA-matched units in the future.

In spite of the limitations inherent in all retrospective analyses, our study suggests that high-resolution HLA matching at A, -B, -C and –DRB1 could significantly improve the outcome of dCBT patients, and should, when possible, be considered in the CB search algorithm while larger trials are performed to confirm our original findings.

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Authorship and Disclosures

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