ARTICLE

The effect of inter-unit HLA matching in double umbilical cord blood transplantation for acute leukemia

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ABSTRACT

The effects of inter-unit HLA-match on early outcomes with regards to double cord blood transplantation have not been established. Therefore, we studied the effect of inter-unit HLA-mismatching on the outcomes of 449 patients with acute leukemia after double cord blood transplantation. Patients were divided into two groups: one group that included transplantations with inter-unit mismatch at 2 or less HLA-loci (n=381) and the other group with inter-unit mismatch at 3 or 4 HLA-loci (n=68). HLA-match considered low resolution matching at HLA-A and -B loci and allele-level at HLA-DRB1, the accepted standard for selecting units for double cord blood transplants. Patients', disease, and transplant characteristics were similar in the two groups. We observed no effect of the degree of inter-unit HLA-mismatch on neutrophil (Hazard Ratio 1.27, *P*=0.11) or platelet (Hazard Ratio 0.1.13, *P*=0.42) recovery, acute graft-*versus*-host disease (Hazard Ratio 1.17, *P*=0.36), treatment-related mortality (Hazard Ratio 0.92, *P*=0.75), relapse (Hazard Ratio 1.18, *P*=0.49), treatment failure (Hazard Ratio 0.99, *P*=0.98), or overall survival (Hazard Ratio 0.98, *P*=0.91). There were no differences in the proportion of transplants with engraftment of both units by three months (5% after transplantation of units with inter-unit mismatch at ≤2 HLA-loci and 4% after transplantation of units with inter-unit mismatch at 3 or 4 HLA-loci). Our observations support the elimination of inter-unit HLA-mismatch criterion when selecting cord blood units in favor of optimizing selection based on individual unit characteristics.

Introduction

Double umbilical cord blood (UCB) has allowed adults and larger adolescents to proceed to allogeneic transplantation with this donor type when a single adequately dosed unit is not available. At its inception at the University of Minnesota, the UCB units were required to be at least 4/6 HLA-matched to the patient and to each other, not necessarily at the same loci.[1,2] The inter-unit HLA-match requirement was based on what was known about the effect of HLA-mismatching on the outcomes of single UCB transplantation in order to minimize the risk of cross-rejection

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between the two donor units and the potential for increased risk of graft failure. However, as double UCB transplantation became more widely used, the degree of inter-unit HLA-mismatching allowed has been relaxed to adjust for institutional practice.[3-5] The rationale for relaxing inter-unit HLA-matching was to allow for optimization of cell dose and donor-recipient HLA-matching of each unit, instead of compromising one or both criteria in order to find a pair of UCB units that match each other. Such an approach would potentially improve the engraftment potential of each unit despite the fact that a single UCB unit predominates long term in most patients. The

Table 1. Patients', disease and transplantation characteristics.

Variables	≤ 2 inter-unit HLA-mismatch	≥3 inter-unit HLA-mismatch	*P*
Number	381	68	
Age			0.27
≤ 20 years	72 (19%)	12 (17%)	
21 – 40 years	105 (27%)	13 (19%)	
> 40 years	204 (54%)	43 (64%)	
Sex, male	205 (54%)	37 (54%)	0.93
Performance score			0.70
90 – 100	274 (72%)	47 (69%)	
< 90	101 (27%)	19 (28%)	
Not reported	6 (2%)	2 (3%)	
HCT-CI score			0.17
0	136 (36%)	20 (30%)	
1 – 2	121 (32%)	20 (30%)	
≥3	124 (33%)	28 (40%)	
Cytomegalovirus serostatus			0.19
Positive	248 (65%)	49 (72%)	
Negative	129 (34%)	17 (25%)	
Not reported	4 (1%)	2 (3%)	
Disease			0.25
Acute myeloid leukemia	247 (65%)	49 (72%)	
Acute lymphoblastic leukemia	134 (35%)	19 (28%)	
Disease status			0.07
First complete remission		224 (59%)	31 (46%)
Second complete remission	125 (33%)	32 (47%)	
Third complete remission	32 (8%)	5 (7%)	
Cytogenetic risk			0.25
Favorable/intermediate	259 (68%)	42 (62%)	
Poor	102 (27%)	19 (28%)	
Not reported	20 (5%)	7 (10%)	
Conditioning regimen			0.43
TBI 200 + cyclophosphamide + fludarabine	142 (37%)	21 (31%)	
TBI 200 + treosulfan	14 (4%)	5 (7%)	
TBI 400 + cyclophosphamide + fludarabine + thiotepa	27 (7%)	4 (6%)	
Melphalan (< 150 mg/m^2) + other agents	12 (3%)	4 (6%)	
TBI≥1000 + cyclophosphamide + fludarabine	186 (49%)	34 (50%)	
Graft-*versus*-host disease prophylaxis			0.15
Tacrolimus + mycophenolate	87 (23%)	21 (31%)	
Cyclosporine + mycophenolate	294 (77%)	47 (69%)	
Donor-recipient HLA-match			N/A
4/6 + 4/6	116 (30%)	48 (71%)	
4/6 + 5/6	87 (23%)	20 (29%)	
4/6 + 6/6	2 (1%)	—	
5/6 + 5/6	120 (31%)	—	
5/6 + 6/6	26 (7%)	—	
6/6 + 6/6	30 (8%)	—	
Transplant period			0.86
2008 – 2010	175 (46%)	32(47%)	
2011 – 2014	276 (54%)	36 (53%)	
Median follow up (range) months	36 (3-77)	36 (4-74)	N/A

HCT-CI: hematopoietic cell transplant co-morbidity index; TBI: total body irradiation; HLA: human leukocyte antigen; N/A: not applicable.

engraftment of each potential unit had to be optimized as we cannot predict the predominant unit at time of selection. Moreover, when a limited number of UCB units are available, as in ethnically and racially diverse populations, it may be very difficult to meet a strict inter-unit HLA-matching criterion.

As institutions tend to follow uniform practices when selecting UCB units,[1-6] opportunities to study the effect of inter-unit HLA-mismatch on hematopoietic recovery and survival at individual transplant centers are limited. Reports from a single institution on the characteristics of the dominant unit in the setting of double UCB transplantation and myeloablative conditioning regimens support the fact that cell dose is the only characteristic independently associated with engraftment.[7,8] Unit-unit HLA match did not affect sustained engraftment, but recipients of units closely matched to each other were more likely to demonstrate initial engraftment of both units.[7] Both reports were from a single institution and included modest sample sizes of 129 and 84 double UCB transplants.

Thus, we designed a study using data reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) to determine if the degree of inter-unit HLA-mismatch defined as 2 or less HLA-loci *versus* 3 or 4 HLA-loci between units would affect early outcomes after double UCB transplantation. We hypothesized that any effect of inter-unit HLA-mismatching would be evident within three months after transplantation as one UCB unit typically predominates beyond this period.

Table 2. Effect of inter-unit UCB HLA-match on early outcomes.

Outcomes	Hazard Ratio (95% Confidence Interval)	*P*
Neutrophil recovery		
Inter-unit mismatch ≤2 HLA-loci	1.00	
Inter-unit mismatch ≥3 HLA-loci	0.83 (0.63 – 1.08)	0.17
Infused TNC (sum unit 1 + unit 2) ≥4 *vs.* <4 x 10^7/kg	1.39 (1.09 – 1.79)	0.008
Cytomegalovirus serostatus		
Positive *vs.* negative	0.75 (0.61 – 0.96)	0.006
Transplant-conditioning regimen Reduced intensity *vs.* myeloablative regimen	1.49 (1.22 – 1.82)	<0.001
Platelet recovery		
Inter-unit mismatch ≤2 HLA-loci	1.00	
Inter-unit mismatch ≥3 HLA-loci	1.13 (0.84 – 1.53)	0.42
Transplant-conditioning regimen Reduced intensity *vs.* myeloablative regimen	1.82 (1.45 – 2.27)	<0.001
Grade II-IV acute GvHD		
Inter-unit mismatch ≤2 HLA-loci	1.00	
Inter-unit mismatch ≥3 HLA-loci	1.17 (0.83 – 1.66)	0.36
Transplant-conditioning regimen Reduced intensity *vs.* myeloablative regimen	0.56 (0.43 – 0.73)	<0.001
Overall mortality		
Inter-unit mismatch ≤2 HLA-loci	1.00	
Inter-unit mismatch ≥3 HLA-loci	0.83 (0.46 – 1.46)	0.52
Infused TNC (sum unit 1 + unit 2) ≥4 *vs.* <4 x 10^7/kg	0.95 (0.57 – 1.57)	0.84
Age		
21 – 40 years *vs.* ≤20 years	0.89 (0.37 – 2.14)	0.80
>40 years *vs.* ≤20 years	2.11 (1.02 – 4.38)	0.04
>40 years *vs.* 21 – 40 years	2.36 (1.26 – 4.42)	0.007

UCB: umbilical cord blood; GvHD: graft-*versus*-host disease;TNC: total nucleated cell.

Methods

Patients

The CIBMTR is a voluntary group of over 350 transplant centers that contribute data prospectively on consecutive transplants performed at each individual center. All patients are followed longitudinally until death or lost to follow up. Seventy-eight centers contributed patients, and transplants were performed between 2008 and 2014 in the United States. Eligible patients were aged 1 to 70 years with acute myeloid or lymphoblastic leukemia (n=449), and were in a first or subsequent complete remission. All received two UCB units, myeloablative or reduced intensity conditioning regimen, and cyclosporine or tacrolimus with mycophenolate for graft-*versus*-host disease (GvHD) prophylaxis. Exclusion criteria were transplants for relapse or primary induction failure (n=226) and anti-thymocyte globulin (ATG)-containing regimens (n=39). The Institutional Review Board of the National Marrow Donor Program approved this study.

End points

The primary end point was overall survival at three months and one year. Death from any cause was considered an event and surviving patients were censored at last follow up. Neutrophil recovery was defined as achieving an absolute neutrophil count of 0.5 x10^9/L or more for three consecutive days; and platelet recovery as platelets 20x10^9/L or more, without transfusion support for seven days. Graft failure was defined as 5% or less donor chimerism or absence of neutrophil recovery. Incidences of grades 2 to 4 acute GvHD were based on reports from each transplant center using standard criteria. Relapse was defined as leukemia recurrence (morphological, cytogenetic or molecular), and non-relapse mortality was defined as death in remission.

Statistical analysis

Differences between groups were compared using the χ^2 test. The probability of overall survival was calculated using the Kaplan-Meier estimator.[9] The probability of neutrophil and platelet recovery, and acute and chronic GvHD were calculated using the cumulative incidence estimator to accommodate competing risks.[10] Cox regression models were built to study the effect of inter-unit HLA mismatch and other factors associated with hematopoietic recovery, acute GvHD, day-100 mortality and 1-year relapse, non-relapse mortality and overall mortality.[11] Variables tested include: inter-unit HLA mismatch, age, sex, performance score, hematopoietic cell transplant co-morbidity (HCT-CI) score, cytomegalovirus (CMV) serostatus, disease, disease status, and transplant conditioning regimen intensity and transplant period. All variables tested met the assumptions for proportionality, and there were no first order interactions between inter-unit HLA mismatch and other variables held in the final multivariate model. All variables that achieved $P \leq 0.05$ were held in the final multivariate model, with the exception of the variable for inter-unit mismatch that was held in all steps of model building and the final model regardless of level of significance. Transplant center effect on survival was tested using the frailty approach.[12] All *P*-values are two-sided. All analyses were carried out using SAS v.9.3 (Cary, NC, USA).

Results

Patients', disease and transplant characteristics

The characteristics of 449 patients with acute leukemia are summarized in Table 1. Donor-recipient and unit-unit HLA-match considered antigen level matching at HLA-A and -B loci and allele-level at HLA-DRB1, the accepted standard for selecting units for double UCB transplants. Clinical practice tolerated multiple HLA-mismatching between units and no more than 2 HLA-loci mismatches between each, and according to this the two recipient groups were created: 1) units were either matched (n=49) or mismatched to each other at 1 (n=113) or 2 (n=219) HLA-loci; and 2) units were mismatched to each other at 3 (n=60) or 4 HLA-loci (n=8). When inter-unit HLA mismatch was 3 or more, most units were mismatched to the recipient at 2 HLA-loci and double mismatch at the same HLA-locus. The median infused total nucleated cell (TNC) dose was 4.26 (range 2.31-5.76) x10^7/kg for transplants with 2 or less inter-unit mismatch and 4.93 (2.99-6.00) x10^7/kg for transplants with 3 or more inter-unit mismatch.

There were no significant differences in patient age, sex, CMV serostatus, performance score and HCT-CI between the two groups. Although there were no differences between the groups by leukemia type or cytogenetic risk, more transplants with inter-unit mismatch at 2 or less HLA-loci were in first complete remission. Patients were equally likely to receive a myeloablative or reduced intensity conditioning regimen, and all received a calcineurin inhibitor with mycophenolate mofetil for GvHD prophylaxis. The median follow up of surviving patients was 36 months in both groups.

Early outcomes

Results of multivariate analysis for the effect of inter-unit HLA mismatch on outcomes are shown in Table 2. Inter-unit HLA mismatch was not associated with hematopoietic recovery, acute grade II-IV GvHD or overall survival at three months. Independent of inter-unit HLA mismatch, neutrophil recovery was more likely with infused TNC 4 or more x10^7/kg (sum unit1 + unit2). We investigated whether the TNC of a single unit would influence its engraftment in the setting of 2 or less HLA-loci compared to 3 or more HLA-loci inter-unit mismatched transplants and did not see such an effect (P=0.20; paired t-test). Independent of inter-unit HLA-match and total infused TNC, recovery was more likely with reduced intensity transplant conditioning regimen and less likely for CMV seropositive patients. The day-28 incidence of neutrophil recovery after 2 or less HLA-loci inter-unit mismatch transplantation was 67% [95% Confidence Interval (CI): 63-72] and after 3 or more HLA-loci inter-unit mismatch transplantation, 76% (95%CI: 66-

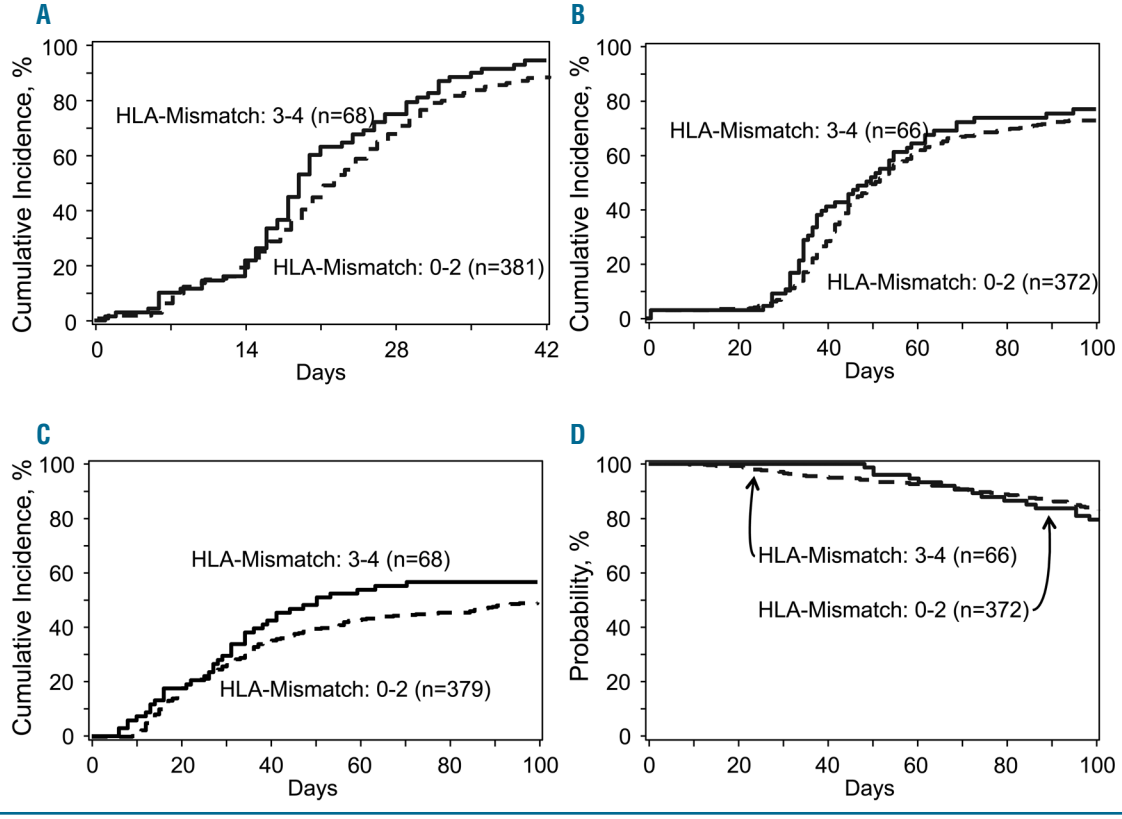

Figure 1. Neutrophil and platelet recovery, grade II-IV acute graft-*versus*-host disease (GvHD) and overall survival. (A) Day 28 neutrophil recovery: the incidence of neutrophil recovery after ≤2 HLA-loci (A) and ≥3 HLA-loci (B) inter-unit mismatched transplants. (B) Day 100 platelet recovery: the adjusted incidence of platelet recovery after ≤2 HLA-loci (A) and ≥3 HLA-loci (B) inter-unit mismatched transplants. (C) Day 100 Grade II-IV acute GvHD: the incidence of acute GvHD after ≤2 HLA-loci (A) and ≥3 HLA-loci (B) inter-unit mismatched transplants. (D) Day 100 survival: the adjusted probability of survival after ≤2 HLA-loci (A) and ≥3 HLA-loci (B) inter-unit mismatched transplants.

Table 3. Effect of inter-unit UCB HLA-match 1-year after transplantation.

Outcomes	Hazard Ratio (95% Confidence Interval)	P
Non-relapse mortality		
Inter-unit mismatch ≤2 HLA-loci	1.00	
Inter-unit mismatch ≥3 HLA-loci	0.92 (0.57 – 1.50)	0.75
Other factors associated with non-relapse mortality		
Age		
21 – 40 years *vs.* ≤20 years	1.50 (0.85 – 2.63)	0.15
>40 years *vs.* ≤20 years	3.06 (1.69 – 5.54)	<0.001
>40 years *vs.* 21 – 40 years	2.03 (1.26 – 3.29)	0.004
Performance score		
<90 *vs.* 90-100	1.78 (1.22 – 2.60)	0.003
Transplant-conditioning regimen		
Reduced intensity *vs.* myeloablative regimen	0.46 (0.29 – 0.73)	<0.001
Relapse		
Inter-unit mismatch ≤2 HLA-loci	1.00	
Inter-unit mismatch ≥3 HLA-loci	1.18 (0.74 – 1.88)	0.49
Other factors associated with relapse		
Transplant-conditioning regimen		
Reduced intensity *vs.* myeloablative regimen	3.14 (2.14 – 4.63)	<0.001
Overall mortality		
Inter-unit mismatch ≤2 HLA-loci	1.00	
Inter-unit mismatch ≥3 HLA-loci	0.98 (0.69 – 1.39)	0.91
Other factors associated with mortality		
Age		
21 – 40 years *vs.* ≤20 years	0.96 (0.62 – 1.48)	0.85
>40 years *vs.* ≤20 years	1.82 (1.26 – 2.64)	0.001
>40 years *vs.* 21 – 40 years	1.90 (1.38 – 2.62)	<0.001
Performance score		
<90 *vs.* 90-100	1.68 (1.28 – 2.22)	<0.001

Table 4. Causes of death.

Causes of death	≤ 2 inter-unit HLA-mismatch	≥ 3 inter-unit HLA-mismatch
Number	205	37
Recurrent leukemia	92 (45%)	17 (46%)
Graft-*versus*-host disease	22 (11%)	5 (17%)
Infection	27 (13%)	6 (16%)
Interstitial pneumonitis	10 (5%)	1 (3%)
Other causes	54 (26%)	8 (22%)

87) (*P*=0.26) (Figure 1A). The corresponding day-42 incidences were 85% (95%CI: 82-89) and 90% (95%CI: 81-96) (*P*=0.28). Independent of inter-unit HLA mismatch, day-28 neutrophil recovery was less likely in patients who were CMV seropositive (64%, 95%CI: 59-70) compared to 74% (95%CI: 67-81) in CMV seronegative patients (*P*=0.03). The incidence of CMV reactivation within 100 days post transplant was higher in CMV seropositive (52%, 95%CI: 46-58) compared to seronegative patients (7%, 95%CI: 4-12) (*P*<0.001). There were no differences in the proportion of patients who died between the two groups: there were 85 deaths (55%; 85 of 156) in the CMV seropositive group and 7 deaths (63%; 7 of 11) in the CMV seronegative group (*P*=0.50).

Platelet recovery was more likely with reduced intensity transplant conditioning. The day-100 incidence of platelet recovery, adjusted for transplant conditioning regimen intensity, was 72% (95%CI: 67-77) and 72% (95%CI: 60-82) after 2 or less HLA-loci and 3 or more HLA-loci inter-unit mismatch transplantation, respectively (*P*=0.95) (Figure 1B).

Inter-unit HLA mismatch was not associated with graft failure (primary or secondary). Seventy of 381 (18%) patients transplanted with 2 or less HLA-loci inter-unit mismatch and 8 of 68 (13%) patients transplanted with 3 or more HLA-loci inter-unit mismatch developed graft failure (*P*=0.19). We also explored whether engraftment of both UCB units (dual engraftment) varied with inter-unit HLA match and did not find such an effect; 6% of recipients of 2 or less inter-unit HLA-mismatched and 4% of recipients of 3 or more inter-unit HLA-mismatch experienced dual engraftment (*P*=0.65). Dual engraftment was explored on the first reported chimerism assay performed 30 days +10 days after transplantation.

The only factor associated with acute grade 2-4 GvHD was transplant conditioning regimen intensity; risks were lower with reduced intensity conditioning regimens. The day-100 incidence of acute grade 2-4 GvHD, after adjusting for transplant conditioning regimen intensity, was 49% (95%CI: 44-54) and 57% (95%CI: 46-69), after 2 or less HLA-loci and 3 or more HLA-loci inter-unit mismatched transplants, respectively (*P*=0.27) (Figure 1C).

The only risk factor for early mortality was age; risks were higher for patients aged 40 years and older independently of inter-unit HLA-mismatch and TNC. TNC was not associated with early survival. The day-100 probability of survival, adjusted for age was 83% (95%CI: 79-87) and 78% (95%CI: 67-87) after 2 or less HLA-loci and 3 or more HLA-loci inter-unit mismatched transplants, respectively (*P*=0.60) (Figure 1D). Bacterial, viral and fungal infections were common within the first 100 days in both groups; infection rates were 59% and 60% after 2 or less HLA-loci and 3 or more HLA-loci inter-unit mismatched transplants, respectively (*P*=0.82).

We also explored transplant outcomes considering inter-unit HLA-match 0-1 *versus* 2 or more. Compared to transplants with inter-unit HLA-match 0-1, the risks of neutrophil recovery (HR 0.97, 95%CI: 0.77-1.22; *P*=0.82), platelet recovery (HR 0.90, 95%CI: 0.72-1.14; *P*=0.40), grade II-IV acute GvHD (HR 1.14, 95%CI: 0.87-1.50; *P*=0.33) and overall mortality (HR 1.17, 95%CI: 0.83-1.20; *P*=0.35) were not significantly different for transplants with inter-unit HLA-match 2 or more.

One-year overall survival, non-relapse mortality, relapse and chronic GvHD

There were no differences in risks for overall mortality, non-relapse mortality or relapse by inter-unit HLA-mismatch beyond the early post-transplant period (Table 3). We tested for an effect of transplant center; none was found (*P*=0.37). Risks for overall mortality and non-relapse mortality were associated with poor performance scores of 80 or lower, and in patients older than 40 years of age. Transplant-conditioning regimen intensity was associated with non-relapse mortality and relapse. Non-relapse mor-

tality risks were lower and relapse risks higher with reduced intensity compared to myeloablative conditioning regimens. The causes of death are shown in Table 4. Leukemia recurrence was the predominant cause of death in both groups. There was no difference in the 1-year cumulative incidences of chronic GvHD; 28% (95%CI: 24-33) and 35% (95%CI: 24-47) after transplants with inter-unit HLA-mismatch 2 or less and 3 or more, respectively (*P*=0.40).

For most transplants with inter-unit HLA-mismatch 3 or more, each unit was mismatched to the patient at 2 HLA-loci. Therefore, to ensure the observed results were independent of unit-patient HLA-mismatch, we performed a subset analysis that explored possible differences in survival by inter-unit HLA-mismatch for transplantations mismatched at 2 HLA-loci. There were 203 transplants mismatched at 2 HLA-loci with inter-unit mismatch at 2 HLA-loci and 68 transplants mismatched at 2 HLA-loci with inter-unit mismatch at 3 or 4 HLA-loci. Consistent with the main analysis, we did not observe any differences in survival by inter-unit HLA mismatch (HR 1.09, 95%CI: 0.75-1.59; *P*=0.63), adjusted for patient age. Similarly, there were no differences between the groups with regards to neutrophil recovery (HR 1.26, 95%CI: 0.9-1.75; *P*=0.16), platelet recovery (HR 1.23, 95%CI: 0.88-1.69; *P*=0.22), acute GvHD (1.13, 95%CI: 0.78-1.64; P=0.52), non-relapse mortality (HR 0.99, 95%CI: 0.57-1.72; *P*=0.97) and relapse (HR 1.16, 95%CI: 0.67-2.00; *P*=0.61).

Discussion

We studied the effect of the inter-unit HLA match on outcomes of double UCB transplantation for acute leukemia and did not find an association between inter-unit HLA-mismatch and outcomes. Specifically, there were no differences in hematopoietic recovery, acute GvHD or survival, demonstrating that inter-unit HLA-match is not relevant when selecting UCB units for double UCB transplantation for acute leukemia. The only unit characteristic associated with neutrophil recovery was cell dose. Transplantations of UCB units with a combined infused TNC 4x10^7/kg or more was associated with faster neutrophil recovery, but infused TNC was not associated with survival or non-relapse mortality. There were no differences in engraftment of the dominant and non-dominant units based on inter-unit HLA match or unit TNC within the first month after transplantation. Our observations are not in keeping with a single report of early engraftment of the non-dominant UCB unit when the cell dose of the dominant unit was low ($CD34^+$ <1.20x10^5/kg).[8] The current analyses used TNC dose and the single institution report[8] used $CD34^+$ dose. The heterogeneity of CD34 measurements across laboratories prevents us from studying the effects of CD34 dose in the setting of registry studies. As CD34 is a subset of TNC, a higher infused TNC implies higher infused CD34.

The cryopreserved TNC dose of 2.5x10^7/kg or more, and at least 4/6 HLA-matching to the patient, considering HLA-A and -B at the antigen level and -DRB1 at the allele level, are the cornerstones of initial UCB unit selection for double UCB transplantation. The additional step of matching units to each other has added complexity and has, at times, limited options with respect to selecting the best available UCB unit. Patients with common haplotypes will have several UCB units that meet the above criteria to compose a double UCB graft that includes units that are at least 4/6 HLA-matched to the patient and to each other. In contrast, for racial minorities, identifying multiple UCB units cryopreserved nucleated cell dose 2.5x10^7/kg or more and at least 4/6 HLA-matching to the patient can be challenging, and the added burden of inter-unit matching limited to no more than mismatching at 2 HLA-loci may result in selecting individual units that are less desirable, or at times prohibitive, to the extent that transplantation is denied.[13] Our results support a focus on the selection of each UCB unit with at least the minimum desired dose of 2.5x10^7/kg, and thereafter the best HLA-match to the patient.

In the current analysis, 2-year overall survival for adults with acute leukemia in remission are 47% (95%CI: 42-52) and 45% (95%CI: 33-58) after double UCB transplants with inter-unit HLA-mismatch 2 or less and 3 or more, respectively. The corresponding non-relapse mortality rates were 29% (95%CI: 25-34) and 32% (95%CI: 21-44), and relapse rates 29% (95%CI: 24-34) and 31% (95%CI: 20-43) at two years post transplant. Patients older than 40 years of age and those with performance scores of 80 or lower were at higher risk for overall and non-relapse mortality. Age is not a modifiable factor, but early referral may result in transplantations with better performance scores. The decision to offer an ablative or reduced intensity regimen is based on several factors, including age, fitness and organ function. Consistent with other reports, a potential survival advantage with reduced intensity conditioning regimens was negated by higher relapse.[14,15] These results support the view that UCB transplants are desirable for patients without a fully HLA-matched related or unrelated donor. Although neutrophil recovery was less likely in CMV seropositive patients, and these patients were more likely to experience CMV reactivation, this was not associated with higher mortality when compared to CMV seronegative patients.

Our study has limitations that we have addressed by performing carefully controlled analyses. First, consistent with current clinical practice, only 15% of transplantations chose UCB units with inter-unit mismatch at 3 or 4 HLA-loci, implying that it is more likely to achieve inter-unit HLA matching than not. Among centers that contributed more than 5 patients (n=23 centers), at 6 centers 20%-30% of transplants used UCB units with inter-unit mismatch 3 or more; at the remaining 17 centers, fewer than 5% of transplants used UCB units with inter-unit mismatch 3 or more. Although this is a modest study population, the observed Hazard Ratios are close to 1.00, supporting our recommendation that inter-unit mismatch may be ignored when selecting UCB units for double UCB transplantation for acute leukemia. Second, inter-unit mismatch is confounded by HLA-mismatch. Therefore, a subset analysis limited to 4/6 double UCB transplants was carried out which confirmed the results of the main analysis. Third, our groupings for inter-unit mismatch considered 2 or less HLA-loci mismatch *versus* 3 or more HLA-loci, based on clinical practice. However, we also looked for possible differences, such as 0-1 HLA-locus *versus* 2 or more HLA-loci mismatch, and found none.

Data, mostly from the single UCB transplantation setting, support the use of donor specific anti-HLA antibod-

ies,[16] high resolution HLA-matching,[17] matching at HLA-C,[18] CD34+ cell dose,[7,8,19,20] and cell viability[8] to refine UCB unit selection. However, these data are not always reproducible in the double UCB focused studies.[21,22] Reviewing HLA typing reported to the CIBMTR for the current analyses, the majority of transplantations did not consider allele-level HLA-match or matching at the HLA-C locus for unit selection. It is plausible that matching at the HLA-C locus for units that are matched to the patient at HLA-A, -B and DRB1, or in the presence of a single locus mismatch at A, B or DRB1 may minimize mortality risks.[18] Others have reported that selecting units for double UCB transplants based on high resolution HLA-matching is feasible for most patients without compromising cell dose.[23] Only when UCB unit selection considers matching at the HLA-C locus and high-resolution HLA-match criteria can we design studies to explore the role of better HLA-match for double UCB transplants. In the setting of single UCB transplantation for leukemia, processing and banking practices at the publicly funded US Cord Blood Banks had no effect on early survival,[24] although a single center report concluded that units provided by the non-Netcord Foundation for the Accreditation of Cellular Therapy accredited Cord Blood Banks were associated with low recovery of viable CD34+ cells.[8] Data reported to the CIBMTR suggest approximately 80% of UCB transplants in the US for patients older than 18 years use two cord blood units. Therefore, eliminating inter-unit HLA-mismatch restriction will allow for a larger number of units to be considered when selecting units for double UCB transplants.

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References

1. Barker JN, Weisdorf DJ, DeFor TE, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood. 2005; 105(3):1343-1347.
2. Brunstein CG, Barker JN, Weisdorf DJ, et al. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. Blood. 2007; 110(8):3064-3070.
3. Ponce DM, Zheng J, Gonzales AM, et al. Reduced late mortality risk contributes to similar survival after double-unit cord blood transplantation compared with related and unrelated donor hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2011;17(9):1316-1326.
4. Wagner JE Jr, Eapen M, Carter S, et al. One-unit versus two-unit cord-blood transplantation for hematologic cancers. N Engl J Med. 2014;371(18):1685-1694.
5. Kanda J, Rizzieri DA, Gasparetto C, et al. Adult dual umbilical cord blood transplantation using myeloablative total body irradiation (1350 cGy) and fludarabine conditioning. Biol Blood Marrow Transplant. 2011;17(6):867-874.
6. Ostronoff F, Milano F, Gooley T, et al. Double umbilical cord blood transplantation in patients with hematologic malignancies using a reduced-intensity preparative regimen without antithymocyte globulin. Bone Marrow Transplant. 2013; 48(6):782-786.
7. Avery S, Shi W, Lubin M, et al. Influence of infused cell dose and HLA match on engraftment after double-unit cord blood allografts. Blood. 2011;117(12):3277-3285; quiz 3478.
8. Purtill D, Smith K, Devlin S, et al. Dominant unit CD34+ cell dose predicts engraftment after double-unit cord blood transplantation and is influenced by bank practice. Blood. 2014;124(19):2905-2912.
9. Kaplan EL, Meier P. Nonparametric Estimation from Incomplete Observations. J Am Stat Assoc. 1958;53(282):457-481.
10. Lin DY. Non-parametric inference for cumulative incidence functions in competing risks studies. Stat Med. 1997;16(8):901-910.
11. Cox DR. Regression Models and Life-Tables. J R Stat Soc Series B Stat Methodol. 1972;34(2):187-220.
12. Andersen PK, Klein JP, Zhang MJ. Testing for centre effects in multi-centre survival studies: a Monte Carlo comparison of fixed and random effects tests. Stat Med. 1999; 18(12):1489-1500.
13. Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med. 2014;371(4):339-348.
14. Luger SM, Ringden O, Zhang MJ, et al. Similar outcomes using myeloablative vs reduced-intensity allogeneic transplant preparative regimens for AML or MDS. Bone Marrow Transplant. 2012;47(2):203-211.
15. Marks DI, Wang T, Perez WS, et al. The outcome of full-intensity and reduced-intensity conditioning matched sibling or unrelated donor transplantation in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia in first and second complete remission. Blood. 2010; 116(3):366-374.
16. Cutler C, Kim HT, Sun L, et al. Donor-specific anti-HLA antibodies predict outcome in double umbilical cord blood transplantation. Blood. 2011;118(25):6691-6697.
17. Eapen M, Klein JP, Ruggeri A, et al. Impact of allele-level HLA matching on outcomes after myeloablative single unit umbilical cord blood transplantation for hematologic malignancy. Blood. 2014;123(1):133-140.
18. Eapen M, Klein JP, Sanz GF, et al. Effect of donor-recipient HLA matching at HLA A, B, C, and DRB1 on outcomes after umbilical-cord blood transplantation for leukaemia and myelodysplastic syndrome: a retrospective analysis. Lancet Oncol. 2011;12(13):1214-1221.
19. Sanz J, Sanz MA, Saavedra S, et al. Cord blood transplantation from unrelated donors in adults with high-risk acute myeloid leukemia. Biol Blood Marrow Transplant. 2010;16(1):86-94.
20. Wagner JE, Barker JN, DeFor TE, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood. 2002;100(5):1611-1618.
21. Brunstein CG, Petersdorf EW, DeFor TE, et al. Impact of Allele-Level HLA Mismatch on Outcomes in Recipients of Double Umbilical Cord Blood Transplantation. Biol Blood Marrow Transplant. 2016;22(3):487-492.
22. Brunstein CG, Noreen H, DeFor TE, Maurer D, Miller JS, Wagner JE. Anti-HLA antibodies in double umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2011;17(11):1704-1708.
23. Dahi PB, Ponce DM, Devlin S, et al. Donor-recipient allele-level HLA matching of unrelated cord blood units reveals high degrees of mismatch and alters graft selection. Bone Marrow Transplant. 2014;49(9):1184-1186.
24. Ballen KK, Logan BR, Laughlin MJ, et al. Effect of cord blood processing on transplantation outcomes after single myeloablative umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2015; 21(4):688-695.