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Optimizing selection of double cord blood units for transplantation of adult patients with malignant diseases

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Key Points

- Low TNC, HLA mismatching, and ABO incompatibility are associated with worse survival following DUCBT.
- Proper UCB unit selection is a key factor for improved outcomes in adult DUCBT recipients.

Double-unit unrelated cord blood transplantation (DUCBT) is an option in patients for whom a single unit is not sufficient to provide an adequate number of cells. As current guidelines on UCB unit selection are mainly based on single-unit UCB data, we performed a retrospective analysis of 1375 adult recipients of DUCBT for hematologic malignancies to determine optimal criteria for graft selection. Cryopreserved total nucleated cells (TNCs; ≤ 3.5 vs $> 3.5 \times 10^7$/kg: hazard ratio [HR], 1.53; 30% vs 45%; $P = .01$), number of HLA mismatches (≥2 vs 0-1: HR, 1.28; 42% vs 48%; $P = .01$), and ABO compatibility (minor/major ABO incompatibility vs compatibility: HR, 1.28; $P = .04$) were independent risk factors for OS. Cryopreserved $CD34^+$ cell dose $\geq 0.7 \times 10^5$/kg in the winning UCB was associated with improved OS (HR, 1.34; $P = .03$). Low TNC ($\leq 3.5 \times 10^7$/kg) and $CD34^+$ ($\leq 1.4 \times 10^5$/kg) cell doses were related to decreased neutrophil recovery (HR, 0.65 [$P = .01$] and HR, 0.81 [$P = .01$], respectively). DUCBT recipients with ≥2 HLA mismatches had a higher incidence of grade II-IV and III-IV acute graft-versus-host disease (HR, 1.26 [$P = .03$] and 1.59 [$P = .02$], respectively). Low TNC dose (HR, 1.57; $P = .02$) and receiving UCB with ≥2 HLA mismatches (HR, 1.35; $P = .03$) were associated with increased transplant-related mortality. Our data support selecting adequately HLA-matched UCB units with a double-unit cryopreserved TNC dose $> 3.5 \times 10^7$/kg and $CD34^+$ cell dose of $\geq 0.7 \times 10^5$/kg per unit in DUCBT candidates.

Introduction

Approximately one-fourth of White patients and an even larger number of individuals of racial/ethnical minorities lack an HLA-matched related or unrelated donor.[1] For these patients, unrelated cord blood (UCB) transplantation (UCBT) expands the donor pool by being readily available and allowing higher donor-recipient HLA disparity.[2,3] Despite the recent upward trend in the number of haploidentical-related transplants,[4] UCBT is still an option for patients with hematologic malignancies lacking a suitable donor.

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Cell dose is a critical determinant of outcomes in UCBT. Total nucleated cell (TNC) and $CD34^+$ cell content have been shown to significantly impact engraftment and survival in single-unit UCBT.[5,6] Double-unit UCBT (DUCBT) has been adopted to circumvent the issue of low numbers of cells delivered by a single UCB unit. Different studies have reported comparable overall survival (OS) between single- and double-unit UCBT, with the latter linked to more severe graft-versus-host disease (GVHD).[7-9] However, previous DUCBT studies addressing other UCB unit-related factors (eg, TNC, $CD34^+$ cell dose, UCB unit-recipient HLA and ABO match, or $CD34^+$ cell viability) were mainly from single centers, had relatively small numbers of patients, or examined only few factors and outcomes.[10-13] Current guidelines for UCB selection are also primarily based on criteria derived from single-unit UCBT studies and may not be directly transposable to DUCBT.[14,15] Therefore, in the present study, we investigated how UCB unit-related factors affected survival and other transplant outcomes in a large number of adult patients with hematologic malignancies receiving DUCBT. Our aim was to determine optimal criteria for cord blood unit selection in this setting.

Methods

Data collection, study design, and patient selection

The Eurocord registry collects data on UCBTs performed in Europe and other participating countries. Patients are followed longitudinally until death or loss to follow-up. In the present investigation, we performed a retrospective cohort study using the Eurocord database of consecutive DUCBT performed as first allogeneic transplant in adults (≥18 years) with hematologic malignancies at the European Society for Blood and Marrow Transplantation (EBMT) transplant centers between 2006 and 2017. DUCBT with missing data on TNC dose or unit-donor HLA match classification were excluded from the analysis. Patients provided informed consent for data entry into the EBMT and Eurocord registry database for observational studies. The study was performed in accordance with the Declaration of Helsinki. The institutional review board of Eurocord reviewed and approved this study.

Definitions and end points

HLA matching was defined by the UCB unit with the worst match with the recipient considering the HLA antigen level for HLA-A and HLA-B and the allele level for HLA-DRB1. For example, HLA matching between DUCBT and the recipient was defined as 4 of 6 when 1 UCB unit was 5 of 6 or 6 of 6 and the other unit was 4 of 6. Likewise, ABO matching was defined by the UCB unit with highest ABO disparity (eg, if a patient with blood type O^+ received an O^+ and an A^+ UCB unit, the graft would be considered as bearing major ABO incompatibility). Myeloablative conditioning regimen (MAC) was defined as a regimen containing either total-body irradiation (TBI) with a dose of >6 Gy, a dose of oral busulfan of >8 mg/kg, or a dose of IV busulfan of >6.4 mg/kg.[16] Other conditioning regimens were defined as reduced-intensity conditioning (RIC). Patients were classified as having low-, intermediate-, or high-risk disease according to standard classification.[17] For exploratory analyses, HLA matching at high resolution considered HLA-A, HLA-B, HLA-C, and HLA-DRB1 at the allele level. Winning unit was defined as the cord unit representing >50% of the total marrow hematopoiesis by day 130 after transplant, as previously reported.[18]

The primary end point was OS. Secondary end points were relapse/progression, transplant-related mortality (TRM), neutrophil and platelet engraftment, and acute and chronic GVHD. The cumulative incidence of neutrophil recovery was defined as the first of 3 consecutive days of an absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L. The cumulative incidence of platelet recovery was defined as the first of 3 consecutive days achieving platelets $\geq 20 \times 10^9$/L for 3 consecutive days unsupported by platelet transfusions for 7 days. Diagnosis and grading of acute and chronic GVHD were performed according to standard criteria.[19,20]

Statistical analysis

Survival curves were constructed using the Kaplan-Meier method, and the log-rank test was used to assess differences between curves. Cumulative incidence function was estimated by the Kalbfleisch and Prentice method and compared using the Gray test. The effect of covariates (age, sex, weight, disease type, leukemia type, disease risk, ABO match, HLA match, conditioning intensity, TBI dose, antithymocyte globulin [ATG] use, cryopreserved TNC, cryopreserved $CD34^+$ cell dose, interunit sex match, year of transplant) on time to event end points were assessed by applying the Cox regression models or Fine-Gray regression models. Covariates with $P < .05$ in the univariate regression analysis were included in a multiple Cox regression analysis with backward elimination method ($P < .05$ to stay). The competing risk for relapse/progression was death without relapse and for TRM was relapse/progression. For acute and chronic GVHD, death without acute GVHD before 100 days and death without chronic GVHD were competing risks, respectively. Variables of interest (cryopreserved TNC, $CD34^+$ cell dose, HLA and ABO match) were forced in the final models for all outcomes, except for an exploratory analysis of a subset of winning UCB units. HLA and ABO matching was collapsed into dichotomous variables to avoid losing patients with missing information for 1 of the 2 UCB units and whose known UCB unit already had ≥2 HLA mismatches or a major ABO mismatch. Continuous covariates for OS were dichotomized to find an optimal cutoff value by using the Contal-O'Quigley method.[21] This method essentially calculates all possible splits and finds the one that maximizes the log-rank statistic. In Fine-Gray regression models, continuous covariates were categorized into terciles, except for cryopreserved TNC and $CD34^+$ cell dose, for which the cutoff values determined by the Contal-O'Quigley method for OS were kept. For exploratory subset analyses combining HLA match and cell dose, medians of cryopreserved TNC and $CD34^+$ cell doses were used instead of the above cutoff values due to lack of convergence. Unadjusted and adjusted hazard ratios (HRs) and their 95% confidence interval (CI) were reported. Median follow-up time was computed using the reverse Kaplan-Meier estimator. Multicollinearity was checked by calculating the variance inflation factor for linear regression of time to event on the covariates with $P < .05$ in the univariate candidates to a multivariate model. A variance inflation factor of ≥10 indicated multicollinearity.[22] Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, NC). All tests were 2-sided, and $P < .05$ was considered significant.

Results

Patients, disease, and graft characteristics

The median age at transplantation was 48 years (18-73 years). Acute leukemia and myelodysplastic syndrome accounted for 67% of the cases. The median cryopreserved TNC count was 5.1×10^7

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Table 1. Patient, cord blood, and transplant characteristics of 1375 patients with hematologic malignancies undergoing DUCBT from 2006 to 2017

Characteristics	Values (N = 1375)
Male, n (%)	805 (59)
Median age (range), y	48 (18-73)
Median body weight (range), kg*	71 (40-150)
Positive cytomegalovirus serology, n (%)	776 (59)
Disease type, n (%)	
Acute leukemia	791 (57)
Lymphoma	270 (20)
Myelodysplastic syndrome	143 (10)
Myeloproliferative disorder	90 (7)
Others†	81 (6)
Disease status, n (%)	
Early	420 (31)
Intermediate	426 (31)
Advanced	400 (29)
Not reported	129 (9)
Median cryopreserved cells (IQR)	
TNCs, 10^7/kg	5.1 (4.3, 6.0)
Total $CD34^+$ cells, 10^5/kg‡	1.9 (1.4, 2.7)
Median infused cells (IQR)	
TNCs, 10^7/kg	3.9 (3.0, 4.8)
Total $CD34^+$ cells, 10^5/kg‡	1.4 (0.9, 2.0)
HLA compatibility, n (%)§	
6 of 6	18 (1)
5 of 6	373 (27)
4 of 6	851 (62)
3 of 6	91 (7)
2 of 6	6 (0)
Not reported	36 (3)
ABO compatibility, n (%)‖	
Compatible	224 (16)
Minor incompatibility	394 (39)
Major incompatibility	654 (48)
Not reported	103 (7)
Interunit ABO match, n (%)	
Matched	565 (41)
Interunit sex match, n (%)	
Matched	703 (51)
GVHD prophylaxis, n (%)	
Cyclosporine and mycophenolate	1045 (76)
Others	330 (24)
Conditioning intensity, n (%)	
Reduced-intensity conditioning	929 (68)
Myeloablative conditioning	408 (30)
Not reported	38 (3)
Conditioning regimens, n (%)	
Cyclophosphamide + fludarabine + TBI, low dose	728 (53)
Cyclophosphamide + fludarabine + TBI, high dose	181 (13)
Cyclophosphamide + TBI, high dose	66 (5)
Others	342 (25)
Not reported	58 (4)
Use of antithymocyte globulin, n (%)	316 (28)
Year of transplant, n (%)	
≥2012	552 (40)

GVHD, graft-versus-host disease; IQR, interquartile range; TBI, total-body irradiation.
*Information missing for 2 cases.
†Plasma cell and myelodysplastic/myeloproliferative disorders.
‡Information was missing for 69 cases.
§HLA-A and B at low and HLA-DRB1 at high-resolution levels. Cord blood unit with the highest HLA disparity in relation to the patient. Information on the number of mismatches (ie, ≤1 or >1) was available for all patients.
‖Cord blood unit with the highest ABO disparity in relation to the patient.

(interquartile range [IQR], 4.3-6.0) per kg of the recipient. Seventy-two percent of patients (n = 985) received at least 1 cord blood unit bearing 2 or more HLA mismatches. Eighty-seven percent of cases (n = 1048) were given at least 1 cord blood unit with a major or minor ABO incompatibility. Median follow-up time was 4.5 years (95% CI, 4.1-4.9). Other characteristics of the patients, diseases, and cord blood units are summarized in Table 1. Considering temporal trends in DUCBT practice, cryopreserved TNC (<2012 vs ≥2012: median, 4.9 [IQR, 4.1-5.8] vs 5.5 × 10^7/kg [IQR, 4.7-6.3]; $P = <.001$) and $CD34^+$ cell doses (<2012 vs ≥2012: median, 1.8 [IQR, 1.2-2.6] vs 2.2 × 10^5/kg [IQR, 1.7-3.0]; $P = <.001$) were significantly higher in recent years. In addition, <5% of patients transplanted in the 2012 to 2017 period received a TNC dose below 3.5 × 10^7/kg, and a lower frequency of patients with active disease (22%) was observed in this subgroup when compared with those transplanted before 2012 (38%; $P = .03$).

Hematologic recovery

The median time to neutrophil recovery was 27 days (95% CI, 26-28), whereas day +28 and day +42 neutrophil recovery cumulative incidences were 56% (95% CI, 53-58) and 82% (95% CI, 80-85), respectively. The median time to platelet recovery was 48 days (95% CI, 47%-50%). Cryopreserved TNC dose ≤3.5 × 10^7/kg and $CD34^+$ cell dose ≤1.4 × 10^5/kg were independent risk factors associated with decreased neutrophil recovery (HR, 0.65 [95% CI, 0.50-0.85], $P = .01$, and HR, 0.81 [95% CI, 0.69-0.94], $P = .01$, respectively; Table 2) after adjusting for HLA match, ABO match, sex and disease risk. In turn, disease risk (very high/high vs intermediate/low risk: HR, 0.70 [95% CI, 0.57-0.86]; $P = <.001$), recipient's age (39-55 years vs <39 years: HR, 0.82 [95% CI, 0.68-0.98]; $P = .03$), and year of transplant (>2012 vs <2009: HR, 1.32 [95% CI, 1.10-1.60]; $P = .01$) were significant predictors for platelet recovery, whereas HLA and ABO matching and TNC and $CD34^+$ cell doses were not.

TRM and relapse

The cumulative incidence of TRM at 100 days and 4 years was 14.5% (95% CI, 13-17) and 32.6% (95% CI, 30-35), respectively.

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Table 2. Independent predictors for transplant-related outcomes among patients with hematologic malignancies undergoing double cord transplantation

	Multivariate HR (95% CI)					
Transplant predictors*	**OS†**	**Neutrophil recovery‡**	**TRM§**	**Relapse/ Progression‖**	**aGHVD¶**	**cGHVD#**
TNCs, cryopreserved						
≤3.5 vs >3.5 × 10^7/kg**	1.53 (1.16-2.03) ***P* = .01**	0.65 (0.5-0.85) ***P* = .01**	1.57 (1.07-2.31) ***P* = .02**	0.97 (0.63-1.47) *P* = .87	1.08 (0.76-1.55) *P* = .66	0.85 (0.53-1.36) *P* = .49
Total CD34$^+$ cells, cryopreserved						
≤1.4 vs >1.4 × 10^5/kg**	0.96 (0.79-1.16) *P* = .65	0.81 (0.69-0.94) ***P* = .01**	1.03 (0.79-1.34) *P* = .83	1.04 (0.81-1.34) *P* = .76	0.82 (0.65-1.03) *P* = .08	0.96 (0.74-1.24) *P* = .76
HLA mismatches						
≥2 vs 0-1	1.28 (1.06-1.56) ***P* = .01**	1.02 (0.88-1.16) *P* = .89	1.35 (1.04-1.77) ***P* = .03**	0.90 (0.71-1.14) *P* = .39	1.26 (1.02-1.57) ***P* = .03**	0.79 (0.63-0.99) ***P* = .04††**
ABO compatibility						
Minor or major incompatibility vs compatible	1.28 (1.02-1.62) ***P* = .04**	1.02 (0.86-1.21) *P* = .81	1.34 (0.96-1.86) *P* = .09	1.03 (0.77-1.37) *P* = .86	1.04 (0.82-1.31) *P* = .76	0.91 (0.71-0.20) *P* = .53

Bold *P* values denote statistical significance ($P < .05$).

aGVHD, acute GVHD; cGVHD, chronic GVHD; CI, confidence interval; HR, hazard ratio; OS, overall survival.

*Other predictor variables according to outcomes:

†OS: Adjusted for age, disease status, ATG use, and year of transplant (n = 949): risks were higher in patients older than 43 years (HR, 1.29; 95% CI 1.09-1.54; $P = .01$); intermediate-risk (HR, 1.30; 95% CI, 1.04-1.61; $P = .02$) and high-risk (HR, 1.5; 95% CI, 1.22-1.87; $P < .001$) disease; and with use of ATG in the conditioning regimen (HR, 1.74; 95% CI, 1.45-2.10; $P = <.001$). Risks were lower in transplants performed after 2012 (HR, 0.80; 95% CI, 0.65-0.98; $P = .03$).

‡Neutrophil recovery: Adjusted for sex and disease risk at transplant (n = 1109): neutrophil recovery was higher in women (1.17; 95% CI, 1.03-1.33; $P = .02$); intermediate-risk (HR, 1.21; 95% CI, 1.05-1.42; $P = .01$) compared with low-risk disease.

§TRM: Adjusted for age, disease risk, TBI-based conditioning, and ATG use (n = 949). Risks of TRM were higher in patients older than 39 years of age (>39 and <56 years old: HR, 1.37; 95% CI, 1.02-1.84; $P = .04$; ≥56 years old: HR, 1.89; 95% CI, 1.40-2.55; $P < .001$); intermediate-risk (HR, 1.47; 95% CI, 1.10-1.98; $P = .01$) and high-risk (HR, 1.45; 95% CI, 1.07-1.95; $P = .02$) disease; high-dose TBI (≥8 Gy) compared with those without TBI (1.51; 95% CI, 1.03-2.21; $P = .04$); and ATG use (HR, 1.39; 95% CI, 1.04-1.85; $P = .03$).

‖Relapse/progression: Adjusted for TBI-based conditioning regimen, disease risk, and year of transplantation (n = 1105): risks were lower in patients receiving high-dose TBI (≥8 Gy) compared with those without TBI (HR, 0.57; 95% CI, 0.38-0.85; $P = .01$); and in transplants performed after 2012 (HR, 0.65; 0.49-0.87; $P = .01$).

¶aGVHD: Adjusted for TBI-based conditioning regimen (n = 1165). Risks were higher in patients receiving high-dose TBI (≥8 Gy; HR, 1.83; 95% CI, 1.35-2.47; $P < .001$).

#cGVHD: Adjusted for UCB-recipient sex match and TBI-based conditioning regimen (n = 1167): risks were higher for patients receiving TBI-based conditioning (<8 Gy [HR, 1.53; 95% CI, 1.11-2.11; $P = .01$]; ≥8 Gy [HR, 1.85; 95% CI, 1.29-2.65; $P < .001$]). Risks were lower in patients with UCB-recipient sex mismatch (HR, 0.68; 95% CI, 0.54-0.84; $P < .001$).

**Defined by the Contal-O'Quigley method for OS (see "Methods").

††Landmark analysis at day +120 did not confirm this association (GVHD, see "Results").

In multivariate analysis, TNC dose ≤3.5 × 10^7/kg and having ≥2 unit-recipient HLA mismatches were independent risk factors for increased TRM (HR, 1.57 [95% CI, 1.07-2.31), $P = .02$ and HR, 1.35 [95% CI, 1.04-1.77], $P = .03$, respectively) after adjusting for age, disease risk, TBI-based conditioning, and ATG use. ABO compatibility and CD34$^+$ cell dose were not statistically significant factors.

The 4-year cumulative incidence of relapse/progression was 31% (95% CI, 28-34). TNC, CD34$^+$ cell dose, HLA mismatch, and ABO compatibility were not predictive of relapse/progression after adjusting for TBI-based conditioning regimen, disease risk, and year of transplantation (Table 2).

GVHD

The 100-day cumulative incidence of grade II-IV acute GVHD was 39% (95% CI, 37-42), whereas the 100-day grade III-IV acute GVHD was 17% (95% CI, 15% to 19%). Patients receiving UCB units with ≥2 HLA mismatches had a 100-day cumulative incidence of grade III-IV acute GVHD of 19% (95% CI, 16-21) compared with 12% (95% CI, 9-16) in those with 1 HLA-mismatch or HLA-matched grafts ($P = .01$). After adjusting for TBI-based conditioning, recipients of grafts with ≥2 HLA-mismatches had a higher incidence of grade II-IV acute GVHD compared with 1 HLA mismatch or HLA-matched grafts (HR, 1.26 [95% 1.02-1.57]; $P = .03$), whereas TNC dose, CD34$^+$ cell dose and ABO incompatibility were not significantly related to grade II-IV acute GVHD. Moreover, after adjusting for ATG use, recipients of UCB units with ≥2 HLA-mismatches and a TNC dose ≤3.5 × 10^7/kg had a higher incidence of grade III-IV acute GVHD (HR, 1.59 [95% CI, 1.09-2.32], $P = .02$, and HR, 1.86 [95% CI, 1.13-3.06], $P = .01$, respectively), whereas CD34$^+$ cell dose and ABO match were not significant risk factors.

The 4-year cumulative incidence of chronic GVHD was 31% (95% CI, 29-34) for the whole cohort. Grafts with 2 or more HLA-mismatches had lower incidence of chronic GVHD (HR, 0.79 [95% CI, 0.63-0.99]; $P = .04$) after adjusting for sex match and TBI-based conditioning. However, in a landmark analysis at day +120, the effect of HLA disparity on chronic GHVD did not achieve statistical significance in the final model (HR, 0.84 [95% 0.67-1.06]; $P = .14$).

Overall survival

The median OS at 4 years was 43% (95% CI, 41-46) (Figure 1A). The optimal TNC and CD34$^+$ cell cut points for OS were 3.5 × 10^7/kg and 1.4 × 10^5/kg, respectively (supplemental Table 1). At 4 years, 45% of patients (95% CI, 42% to 48%) receiving a TNC dose >3.5 × 10^7/kg were alive compared with 30% (95% CI, 22% to 39%) of those given ≤3.5 × 10^7/kg ($P < .001$) (Figure 1B). Four-year OS was 50% (95% CI, 43% to 57%) for patients receiving ABO-compatible UCB units compared with 43% (95% CI, 39% to 46%) with at least a cord blood unit bearing a minor or major ABO incompatibility ($P = .04$) (Figure 1C). Similarly, 4-year OS was 48% (95% CI, 42% to 53%) for recipients of 6 of 6 or 5 of 6 HLA-matched UCB units compared with 42% (95% CI, 38% to

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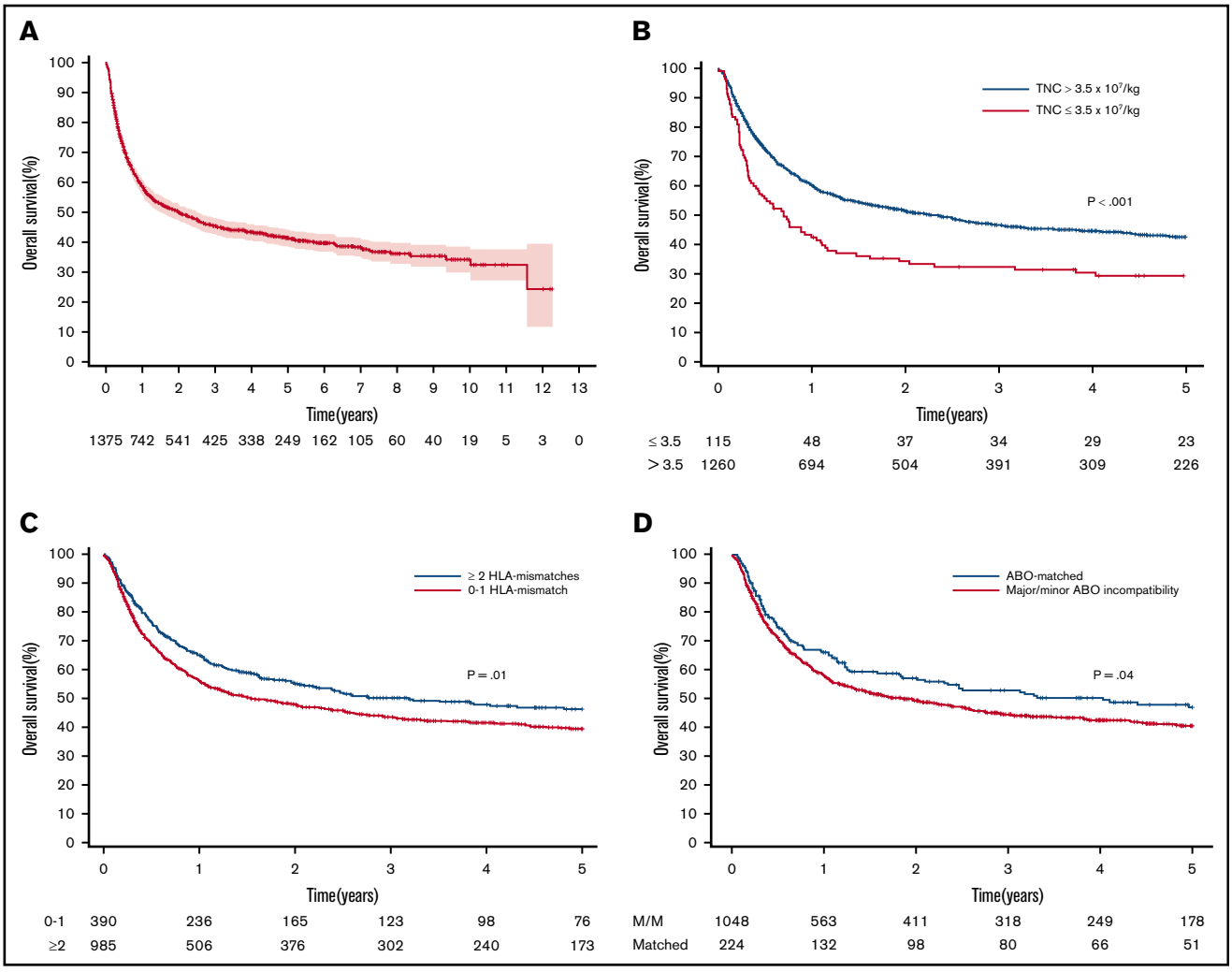

Figure 1. OS of 1375 patients undergoing DUCBT. (A) Whole cohort. (B) By TNCs. (C) By number of HLA mismatches. (D) By ABO compatibility. M/M, major/minor.

45%) of those ≤4 of 6 HLA-matched UCB units (P = .01) (Figure 1D). In multivariate analysis, cryopreserved TNC (≤3.5 × 10^7/kg vs >3.5 × 10^7/kg: HR, 1.53 [1.16-2.03]; P = .01), ABO compatibility (minor or major incompatibility vs compatibility: HR, 1.28 [1.02-1.62]; P = .04) and number of HLA mismatches (≥2 vs 0-1: HR, 1.28 [1.06-1.56]; P = .01) were significant risk factors for OS after adjusting for $CD34^+$ cell dose, age, disease status, ATG use, and year of transplantation (Table 2). As ATG was associated with worse survival and the impact of the above-mentioned graft variables could vary according to whether the graft is T-cell replete, we performed a multivariate analysis restricted to a subset of patients not receiving ATG and found that TNC dose and HLA matching remained significant factors for survival (data not shown).

The association between HLA matching and OS likewise remained significant even when comparing only recipients of 5 to 6 of 6 vs 4 of 6 HLA-matched UCB units (4-year OS: 42% [95% CI, 38% to 45%] vs 48% [42% to 53%], P = .03; HR, 1.24 [95% CI, 1.03-1.49], P = .02, after adjusting for TNC, age at transplant, disease risk and ATG use). There were not significant differences in OS when combining different sets of 2 UCB units according to HLA matching (supplemental Table 2). When ABO matching was broken down into major and minor incompatibilities, the HRs were directionally comparable to the collapsed covariate (ie, minor plus major ABO incompatibility) yet no longer significant (minor ABO incompatibility vs ABO compatible, multiple HR, 1.30 [1.0-1.68], P = .05; major ABO incompatibility vs ABO compatible, multiple HR, 1.23 [0.96-1.58], P = .09).

With the aim to evaluate whether increasing TNC or $CD34^+$ cell dose could harness the negative impact of HLA mismatch on OS, we performed exploratory subset analyses combining these variables (supplemental Table 3). In multivariate analyses, receiving one or two 4 of 6 HLA-mismatched UCB units with lower total $CD34^+$ cell dose was significantly associated with worse OS compared with receiving one or two 5 of 6 HLA-mismatched or HLA-matched UCB units with higher total $CD34^+$ cell dose (HR, 1.36 [95% CI, 1.01-1.84]; P = .045). Also, a trend for decreased survival rate was observed in those receiving one or two 4 of 6 HLA-mismatched UCB units with higher total $CD34^+$ cell dose (HR, 1.33 [95% CI, 0.98-1.80]; P = .07). We also performed exploratory analyses of the effect of interunit sex and ABO matching on survival and found no statistically significant association (data not shown).

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There were 692 deaths in the cohort: 273 (40%) due to disease relapse/progression, 397 (57%) TRM, and 22 other causes or unknown. Among those succumbing to TRM, 180 deaths (42%) were due to infectious complications, 101 (23%) GVHD, and 40 (9%) multiorgan failure.

Critical cryopreserved TNC and CD34$^+$ cell counts in winning UCB units

We had information on the winning UCB unit from 683 patients of this cohort. In this subset, we determined that the best cut points for OS were a TNC of 3.4 $\times$ 10^7/kg and CD34$^+$ cell count of 0.7 $\times$ 10^5/kg in the winning unit. Considering TNC, CD34$^+$ cell dose, ABO and HLA matching of both UCB units vs the winning unit, we found the CD34$^+$ cell count of the winning unit was the only significant factor for OS in this subgroup after adjusting for disease risk ($<$0.7 vs $\geq$0.7 $\times$ 10^5/kg, 4-year OS: 47% [95% CI, 42% to 51%] vs 56% [95% CI, 48% to 63%], multivariate HR, 1.34 [95% CI, 1.03-1.75], $P = .03$).

Impact of high-resolution HLA typing on OS

We analyzed 337 DUCBT for which we had allele-level HLA typing at loci HLA-A, B, C, and DRB1 for both UCB units and the recipient. We determined that 4 allele-level HLA mismatches were the most predictive cutoff value for OS in order to dichotomize this subgroup (eg, 0-3 vs 4-7 mismatches or 5-8 of 8 vs 1-4 of 8 HLA matching). Considering the worst matched UCBT unit at the allele level, 5 to 8 of 8 HLA-matched DUCBT recipients were more likely to be transplanted after 2012 in comparison with 1 to 4 of 8 HLA-matched DUCBT recipients (data not shown). Seventy-five percent of the 5 to 6 of 6 HLA-matched DUCBT recipients at lower resolution had up to 3 allele-level mismatches in contrast to 41% of the 4 of 6 HLA-matched DUCBT recipients (supplemental Table 4). Of note, 19% of the 4 of 6 HLA-matched UCBT recipients received at least 1 UCB unit with 5 to 7 allele-level mismatches. The number of HLA mismatches considering high-resolution typing had no significant impact on survival in multivariate analysis (1-4 of 8 vs 5-8 of 8 HLA-matched units at the allele level, multiple HR, 1.22 [0.85-1.75], $P = .28$; supplemental Table 5).

Discussion

Cord blood unit selection is a key modifiable factor for improving outcomes of UCBT recipients. In this analysis, we found that higher double-unit cryopreserved TNC dose and optimized HLA and ABO compatibility were independently associated with superior OS in DUCBT. Of these 3 factors, the TNC dose had the highest impact on survival, similar to previous single-unit UCBT studies[6,23] but not yet shown in DUCBT. Higher winning-unit cryopreserved CD34$^+$ cell dose was also found to be significantly associated with improved OS. We also determined the critical cutoff values of double-unit TNC dose $\leq$3.5 $\times$ 10^7/kg and winning-unit CD34$^+$ cell dose $<$0.7 $\times$ 10^5/kg as having the highest predictability of adverse effect on OS. The difference in survival seemed to be mediated by improved neutrophil recovery and TRM as cryopreserved TNC and CD34$^+$ cell doses increased, which has been reported in UCBT and other stem cell sources.[6,10,23-25]

In parallel with previous UCBT studies,[6,13,23,26] HLA matching was also a significant factor for OS in our study. The association of HLA-mismatch with higher incidences of grade II-IV and III-IV acute GVHD and TRM appears to be linked to this finding. We recently reported, in the setting of DUCBT for acute leukemia, increased TRM and acute GVHD and worse OS when the winning cord unit had higher HLA disparity,[18] yet this was not confirmed in this cohort comprising various hematologic malignancies. The present study only focused on UCB unit-recipient HLA disparity as interunit HLA disparity was not found to affect transplant outcomes following DUCBT.[27] Similar to a previous report in DUCBT[13] and contrasting with more robust data on single-unit UCBT,[23,26] we did not find that HLA typing at high resolution was associated with OS, yet the small number of cases with data on HLA at the allele level may have underpowered the analysis.

Conversely, the association of ABO matching with OS has not been previously reported in UCBT to our knowledge, but previous studies included none or only few DUCB transplants.[28-30] In the present analysis, ABO matching remained a significant risk factor for OS after adjusting for potential confounders, although it is important to note that its effect size was small with borderline significance. We also looked at the influence of interunit ABO match on survival but did not find any association. A few studies with different graft types have showed that ABO incompatibility might be a risk factor for higher incidence of overall mortality, TRM, and GVHD.[31-33] A small single-unit UCBT study showed that ABO incompatibility was associated with delayed platelet engraftment and higher transfusion requirement of red blood cells and platelets, though with no effect on survival.[34] The explanation for the effect of ABO matching on OS in the present cohort is unclear as it did not significantly affect neutrophil recovery, TRM, or GVHD and thus needs to be taken cautiously. Unfortunately, given the retrospective analysis of our study, we could not retrieve information on recipients' anti-ABO isoagglutinin titers (anti-A, anti-B), red cell engraftment, time to transfusion independence or duration of systemic immunosuppression to evaluate these hypotheses in our cohort. Therefore, ABO might be considered in the algorithm of UCB selection only in case of many possible UCB units due to its borderline statistical significance and lack of biological reasons to explain the effect of ABO incompatibility on survival rates.

Importantly, the negative effect of HLA disparity on OS seemed to persist despite higher CD34$^+$ or TNC cell doses. As $<$10% of this cohort received a double-unit cryopreserved TNC dose equal to or below the critical cutoff value of 3.5 $\times$ 10^7/kg and this figure was even lower in recent years, finding well-HLA-matched UCB units relative to recipients receiving DUCBT will probably be more challenging than achieving that critical TNC dose value. This TNC threshold is higher than the value of $>$3.0 $\times$ 10^7/kg recommended in current guidelines for DUCBT.[14,15] On the other hand, the optimal cut point for cryopreserved CD34$^+$ cell dose most predictive of OS was 0.7 $\times$ 10^5/kg in the winning cord unit, lower than the value of $\geq$ 1.0-2.0 $\times$ 10^5/kg currently recommended.[14,15] As there are not predictive factors for which cord unit will engraft and be dominant at the time of UCB selection,[18] one could argue that 0.7 $\times$ 10^5/kg is the minimal cryopreserved CD34$^+$ cell dose per unit in DUCBT. These findings suggest that selecting better HLA-matched UCB units should be prioritized in DUCBT even at the cost of somewhat lower TNC and CD34$^+$ cell doses as long as they are kept above those relatively low critical cutoff values.

Despite the reasonable 4-year OS of 42% among 4 of 6 HLA-matched DCUBT recipients, the survival of these patients was significantly inferior compared with 5 to 6 of 6 HLA-matched

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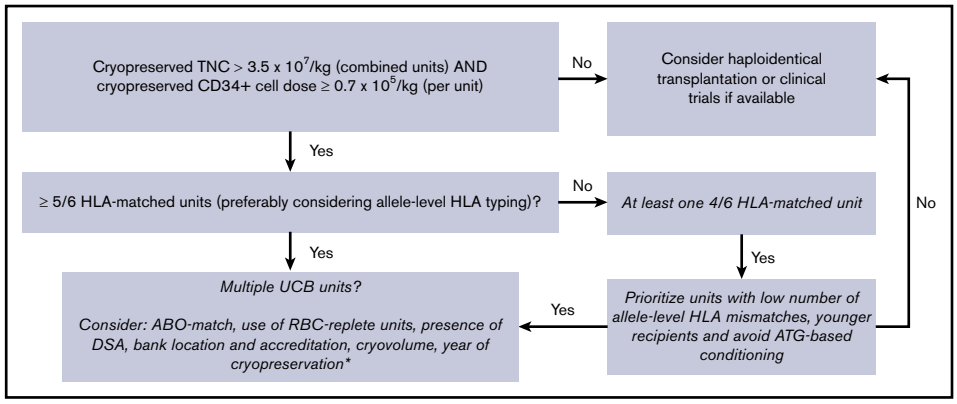

Figure 2. Suggested algorithm for UCB unit selection for DUCBT. *Based on current guidelines (see Barker et al[14] and Dehn et al[15] for further information). ATG, antithymocyte globulin; DSA, donor-specific antibody; RBC, red blood cell.

DUCBT. We are aware that excluding 4 of 6 units is not always feasible. In order to improve the selection of UCB units in this particular subset, we strongly recommend prioritizing younger transplant candidates and avoiding highly allele-level HLA-mismatched UCB units and use of ATG. Emerging data prompt more stringent UCB unit selection for those who have other alternative grafts available, especially haploidentical related donors. The phase 3 BMT CNT 1101 trial recently showed significantly lower OS and higher TRM in DUCBT recipients (with a minimum TNC dose of 3.0 $\times$ 10^7/kg and 53.2% receiving at least one 4 of 6 HLA-matched UCB unit [Ephaim Fuchs, Sidney Kimmel Cancer Center at Johns Hopkins, Baltimore, MD, written communication, 9 September 2020]) compared with haploidentical transplantation.[35] Moreover, a recent CIBMTR-EBMT-Eurocord joint study also evidenced worse OS in UCBT recipients (66% of whom received at least one 4 of 6 HLA-matched UCB unit, all with TNC dose >3.0 $\times$ 10^7/kg) compared with haploidentical transplantation recipients attributable to higher rates of acute and chronic GVHD and TRM.[36] Hence, if UCB units meeting these criteria cannot be found, one might consider haploidentical transplantation, particularly in case of availability of haploidentical donors who are young and the recipient's siblings/children.[37]

Our study has other relevant limitations, the first being that it was a retrospective cohort analysis in which UCB unit choice was physician dependent and/or institutional preference and possibly dependent on unmeasured factors. Second, we did not have data on progenitor cell viability, which was found to be a better predictor of engraftment and unit dominance than TNC and $CD34^+$ cell count.[38] Third, one could argue that the present study should have been restricted to more recent years to reflect the results of and advances in DUCBT using current guidelines. Nevertheless, keeping the entire cohort of DUCBT from 2006 to 2017 allowed us to perform robust analyses exploring key covariates and detail trends observed over time using this graft source. In fact, we have found that patients transplanted after 2012 had better outcomes when compared with those transplanted before 2012. Higher TNC and $CD34^+$ cell doses and better selection of transplant candidates (fewer cases with active disease at the time of transplant) may explain the better outcomes in recent years, and these differences were carefully accounted for in the final multivariate models.

Taken together, our data support that UCB unit selection for DUCB transplantation should take into account HLA, cryopreserved TNC, and $CD34^+$ dose. A suggested algorithm for UCB unit selection is summarized in Figure 2. In patients lacking an optimal single UCB unit, we suggest that transplant clinicians aim for a double-unit cryopreserved TNC dose above 3.5 $\times$ 10^7/kg and $CD34^+$ cell dose equal to or above 0.7 $\times$ 10^5/kg per unit and, if 2 adequately dosed 5 to 6 of 6 HLA-matched units are not found, select 4 of 6 HLA-matched units prioritizing younger transplant candidates, better allele-level HLA matching, and no use of ATG. In case these criteria are not met, one might consider haploidentical transplantation or clinical trials using alternative GVHD prophylaxes for UCBT.

Authorship

Contribution: V.R., A.R., E.G., and G.F. designed the study; F.V. and F.M. collected and prepared data; F.M., F.V., G.F., and V.R. analyzed data; L.M. classified HLA matching at high resolution; G.F., F.M., A.R., E.G., and V.R. wrote the manuscript; P.C., S.F., H.L.-W., R.P.d.l.T., E.D., T.C., N.R., D.K., and E.F. provided cases for the study; and all authors edited and approved the manuscript.

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