Cord Blood Unit Dominance Analysis and Effect of the Winning Unit on Outcomes after Double-Unit Umbilical Cord Blood Transplantation in Adults with Acute Leukemia: A Retrospective Study on Behalf of Eurocord, the Cord Blood Committee of Cellular Therapy, Immunobiology Working Party, and the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation

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A B S T R A C T

Usually, after double umbilical cord blood transplantation (DUCBT), only 1 of the transplanted units persists in the long term. The characteristics of the winning cord blood unit (W-CBU) that determine unit dominance and how they influence the outcomes of DUCBT remain unclear. We retrospectively analyzed 347 patients with acute leukemia transplanted with a DUCBT (694 CBU) from 2005 to 2013 who had documented neutrophil engraftment and a W-CBU identified by chimerism analysis, to identify unit characteristics impacting on dominance. Median age at DUCBT was 40 years and median follow-up was 35 months. Among W-CBUs, 41% were ≥5/6 HLA matched to the recipient and 59% were ≤4/6. Multivariate analysis indicated that ≤4/6 HLA-matched W-CBUs led to lower leukemia-free survival (44% versus 56%; hazard ratio [HR], 1.5; $P = .032$) and overall survival (49% versus 62%; HR, 1.5; $P = .028$), increased nonrelapse mortality (26% versus 18%; HR, 1.9; $P = .027$), and acute graft-versus-host disease (46% versus 35%; HR, 1.7; $P = .013$). We were unable to predict unit dominance, but we demonstrated that outcomes were strongly influenced by the degree of HLA mismatch between W-CBU and recipient. Therefore, selection of both units with the lower number of HLA mismatches with the recipient is indicated.

INTRODUCTION

Umbilical cord blood transplantation (UCBT) is well established as a potentially curative treatment for life-threatening hematological diseases. However, despite cord blood advantages, namely the rapid availability and the more permissive degree of HLA mismatch (MM) compared with other stem cell sources, low cell dose remains a limiting factor. The amounts of total nucleated cells (TNCs), colony-forming units, and CD34$^+$ cells are strongly associated with transplant outcomes [1-8].

Since 2001, double UCBT (DUCBT) has been used in heavier patients to overcome the limitation of inadequate cell dose [7-11]. Sustained donor hematopoiesis following DUCBT usually shows dominance of 1 cord blood unit (CBU), and the winning CBU (W-CBU) is defined as the one contributing to the larger proportion of donor hematopoiesis in the host [12,13]. At day 100 after DUCBT, almost all patients will show a dominance pattern on chimerism [5]. Previous attempts have been made to better explain the dominance phenomenon assessing pretransplant characteristics, CBU features and immunological interactions such as host versus graft and graft versus graft (GVG) [3,5,8,9,13-17]. Several studies pointed out that T cell–mediated GVG effect is responsible for the rejection of the loser CBU (L-CBU) by the W-CBU [5,13,16,17]. Nevertheless, how to predict the W-CBU remains a matter of debate [18]. Also, previous analyses correlated W-CBU characteristics with DUCBT results, but some conflicting results were reported [12,19-21].

Determining which CBU characteristics, if any, are responsible for the dominance phenomenon could improve donor selection, as these specific characteristics could be included in the algorithms for CBU selection in DUCBT. The present study aims to identify which CBU features and clinical pretransplant factors may have a role on CBU dominance and to analyze the impact of the W-CBU characteristics on DUCBT outcomes in patients with acute leukemia (AL).

METHODS

Study Design

This is a multicenter retrospective study on adult patients with AL who received a DUCBT between 2005 and 2013 and were reported to Eurocord.

Inclusion criteria

Patients were eligible for this study if they met the following criteria: age ≥18 years at transplant; diagnosis of de novo or secondary acute myeloid leukemia or acute lymphoid leukemia according to the World Health Organization criteria of 2008 [22]; unrelated DUCBTs performed in an European Group for Blood and Marrow Transplantation center; and documented neutrophil engraftment and available chimerism information within 130 days after hematopoietic stem cell transplantation (HSCT). Patients were excluded if they had a previous allogeneic HSCT, or if the CBUs used in the transplantation were manipulated, given in association with another stem cell source, or infused by an intrabone route. All patients provided written informed consent for UCBT and data collection. The study was approved by the institutional review board of Eurocord scientific committee.

Endpoints and statistical methods

Unit dominance

A W-CBU was defined as the CBU representing >50% of the total marrow hematopoiesis by day 130 after transplant. Chimerism was assessed mainly on peripheral blood or bone marrow and mostly by molecular biology techniques. Full donor chimerism (FDC) was defined as the detection of ≥95% hematopoietic cells derived from the donor (from 1 or both CBUs); mixed chimerism was defined as the presence of 5% to 94% of donor hematopoietic cells (from 1 or both CBUs); and autologous recovery was defined when <5% of total marrow chimerism derived from donor cells (from 1 or both CBUs). Dual chimerism was defined as a full donor chimerism with a minimum contribution to host hematopoiesis of at least 5% by each CBU. Unit-recipient HLA matching was defined, as previously described for UCBT, considering antigen level for loci A and B and allele level for locus DRB1. For unit dominance predicting factor analysis, the degree of HLA MM between W-CBU and recipient was compared with the degree of HLA MM between L-CBU and recipient. Inter unit matching was not analyzed in this study. CBU characteristics for the 2 units (W-CBU / L-CBU) were compared using chi-square statistics for categorical variables and the Mann-Whitney test for continuous variables. All clinical relevant variables were included in the general multivariate linear model to adjust for possible confounding factors. To consider the association between the 2 CBUs of each patient, we introduced a random effect or frailty for each patient into the model [23].

Patient outcomes

Primary endpoint was overall survival (OS). Secondary endpoints were leukemia-free survival (LFS), incidence of acute graft-versus-host disease (aGVHD), incidence of chronic GVHD (cGVHD), relapse incidence (RI), and nonrelapse mortality (NRM). OS was defined as the time from HSCT to death, regardless of the cause. LFS was defined as survival with no evidence of relapse or progression. Relapse was defined as morphological evidence of disease in bone marrow, blood, or extramedullary organs. NRM was defined as death without evidence of relapse or progression. aGVHD and cGVHD were defined according to standard criteria [24,25]. Neutrophil engraftment was defined as the first of 3 consecutive days with the neutrophil count ≥0.5×10^9/L, without evidence of autologous reconstitution or graft rejection within the first 100 days after UCBT. Relative frequencies and percentages were used to report categorical variables and median with ranges for continuous variables; for TNCs at cryopreservation and delay of neutrophil engraftment, ranges were reported in quartiles (25th to 75th).

To determine the impact of W-CBU characteristics on transplant outcomes, the degree of HLA MM between W-CBU and recipient was taken into consideration. Cumulative incidence curves were used for RI and NRM in a competing risk setting, because death and relapse are competing. Death was considered as a competing event for neutrophil engraftment. To study aGVHD and cGVHD, we considered relapse and death to be competing events. Probabilities of OS and LFS were calculated using the Kaplan-Meier method. Cumulative incidence was used to estimate the endpoints of NRM, RI, aGVHD, and cGVHD to accommodate for competing risks. Univariate analyses were done using the Gray's test for cumulative incidence functions and the log-rank test for OS and LFS. A Cox proportional hazards model was used for multivariate regression. All variables associated with the outcome or factors

Table 1
Patients, CBU, Disease, and Transplant Characteristics

	n = 347
Diagnosis (n = 347)	
AML	226 (65)
ALL	121 (35)
Sex (available n = 346)	
Female	158 (45)
Male	188 (55)
CMV status (available n = 336)	
Positive	196 (59)
Negative	140 (41)
Disease status at DUCBT (available n = 339)	
First CR	152 (45)
Second CR	150 (44)
Advanced disease	37 (11)
AL with high-risk cytogenetics (available n = 297)	97 (33)
TNCs at cryopreservation (10^7/kg)	5.1 (2.3-13.7)
HLA W-CBU/recipient (available n = 306)	
0-1 MM	126 (41)
2-3 MM	180 (59)
Conditioning regimen (available n = 347)	
RIC	180 (52)
MAC	167 (48)
ATG in the conditioning (available n = 320)	87 (25)
TBI-based conditioning (available n = 342)	285 (83)
GVHD prophylaxis	
CSA + MMF	258 (74)
Full donor chimerism at 100 days (n = 347)	
FDC with 1 W-CBU	325 (94)
Mixed	22 (6)

Data are presented as n (%) or median (range).
ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; CMV, cytomegalovirus; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; TBI, total body irradiation; GVHD, graft-versus-host disease; CSA, cyclosporine; MMF, mycofenolate mofetil.

known to influence outcomes were included in the Cox model. All tests were 2 sided. The type I error rate was fixed at .05 for the determination of factors associated with time-to-event outcomes. Statistical analyses were performed with SPSS 22.0 (IBM, Armonk, NY) and R 3.2.3 5 (R Core Team, Vienna, Austria)

RESULTS

Population

Of 706 patients identified in the Eurocord database as receiving DUCBT for AL during the study period, 591 (84%) engrafted, and chimerism data was available for 347 of them. The main characteristics of the 347 patients are shown in Table 1. Median age at DUCBT was 40 years (range, 18 to 76 years), disease status at DUCBT was first complete remission (CR) in 152 (45%) patients, second CR in 150 (44%), and advanced disease in 37 (11%). Reduced-intensity conditioning was administered in 180 (52%) patients. Antithymocyte globulin (ATG) was used in 25% of the cases (n = 87 of 320 with available information). The median number of TNCs at cryopreservation was 5.1×10^7/kg (range, 2.3 to 13.7). Considering the degree of MM between W-CBU and recipient, 41% of W-CBU had 0 to 1 MM and 59% had 2 to 3 MM (n = 306). Neutrophil engraftment occurred at a median time of 25 days (range, 18 to 32) after DUCBT, with no autologous hematological reconstitution. Twenty patients had mixed and 327 FDC. Among the patients with FDC, 40 had dual chimerism. Median follow-up for survivors was 35 months (range, 3 to 99 months).

Predicting factors for unit dominance

Table 2 shows the characteristics of W-CBU and L-CBU. No significant differences were identified between W-CBUs and L-CBUs characteristics. In addition, after adjusting for CBU (length of storage, number of TNCs, and CD34$^+$ cells at cryopreservation) and unit-host (degree of HLA MM, occurrence of MM in each individual locus, gender match, and ABO compatibility) characteristics, no statistically significant factors predicting the W-CBU were identified (Table 3).

Table 2
W-CBU and L-CBU Characteristics

	W-CBU	L-CBU	*P* value
Median length of storage (n)	3.4 (299)	3.7 (294)	.066
Median infused TNCs (n)	1.92 (319)	1.92 (315)	.945
Median TNCs at cryopreservation (n)	2.56 (319)	2.54 (315)	.990
ABO match			.730
Compatible	114 (38)	115 (39)	
Minor	76 (25)	80 (27)	
Major	109 (37)	98 (34)	
HLA MMs			.419
2-3 MM	180 (59)	183 (62)	
2 MM	167 (55)	176 (60)	
3 MM	13 (4)	7 (2)	
0-1 MM	126 (41)	113 (38)	
0 MM	12 (4)	13 (4)	
1 MM	114 (37)	100 (34)	
Sex match			.450
Match	182 (54)	170 (51)	
Mismatch	154 (46)	162 (49)	

Data are presented as n (%) unless otherwise indicated.
CBU, cord blood unit; W-CBU, winning cord blood unit; L-CBU, losing cord blood unit; TNC, total nucleated cells; HLA, human leukocyte antigen; MM: mismatch.

Outcomes by W-CBU characteristics and other clinical variables

Acute and chronic GVHD

In univariate analysis, 100-day cumulative incidence (CI) of aGVHD was higher in patients whose W-CBU had 2 to 3 HLA MM (46%; 95% CI, 38% to 53%) than in those who had a W-CBU with 0 to 1 HLA MM (35%; 95% CI, 27% to 42%; $P = .040$). The CI of aGVHD was also higher in recipients given W-CBU stored longer than 3 years (48%; 95% CI, 40% to 56%) compared with those who received W-CBU stored for a shorter period (35%; 95% CI, 27% to 43%; $P = .049$). The effect of HLA MM and length of storage on aGVHD was confirmed in multivariate analyses (Table 4; HR, 1.73; 95% CI, 1.12 to 2.67, $P = .013$; and HR, 1.61; 95% CI, 1.09 to 2.39, $P = .018$, respectively). In addition, the use of ATG was independently associated with lower risk of aGVHD in multivariate analysis (HR, .31; 95% CI, .17 to .55; $P < .001$). There was no association among W-CBU characteristics and risk of cGVHD in univariate or in multivariate analyses.

Table 3
Predicting Factors for W-CBU: MVA by General Linear Model

CBU variable	*P* value	Odds ratio	95% CI	
			Lower	Upper
2-3 HLA MM versus 0-1 HLA MM CBU—recipient	.267	.81	.57	1.16
Sex match versus sex mismatch CBU—recipient	.264	.82	.58	1.15
ABO match versus ABO MM CBU—recipient	.851	.95	.56	1.59
Collected CD34$^+$ cells >0.95 versus ≤0.95 × 10^5/kg	.269	1.12	.92	1.37
Length of storage >3 yr versus ≤3 yr	.135	.95	.90	1.01

MVA, multivariate analysis.

Table 4
Multivariate Analysis General/ W-CBU Factors and Outcomes

Outcomes	Variables	*P*-value	HR	95% CI	
				Lower	Upper
LFS	0-1 HLA MM W-CBU versus 2-3 MM W-CBU	.032	1.49	1.03	2.15
	Disease status	.006			
	Intermediate versus early	.813	1.05	.69	1.61
	Advanced versus early	.003	2.21	1.31	3.73
	ATG versus no ATG	<.001	2.14	1.48	3.09
RI	$>2.5 \times 10^7$/kg TNC W-CBU versus $\leq 2.5 \times 10^7$/kg TNC W-CBU	.049	1.32	1.00	1.74
	Disease status	.001			
	Intermediate versus early	.841	.94	.53	1.68
	Advanced versus early	.001	3.21	1.61	6.38
NRM	0-1 HLA MM W-CBU versus 2-3 MM W-CBU	.027	1.89	1.07	3.33
	ATG versus no ATG	<.001	3.05	1.79	5.20
OS	0-1 HLA MM W-CBU versus 2-3 MM W-CBU	.028	1.53	1.05	2.25
	Disease status	.003			
	Intermediate versus early	.483	1.17	.75	1.82
	Advanced versus early	.001	2.49	1.45	4.28
	ATG versus no ATG	<.001	2.10	1.43	3.08
aGVHD	0-1 HLA MM W-CBU versus 2-3 MM W-CBU	.013	1.73	1.12	2.67
	ATG versus no ATG	<.001	.31	.17	.55
	Length of storage >3 yr versus ≤3 yr	.018	1.61	1.08	2.39

NRM and RI

In univariate analysis, patients whose W-CBU had 2 to 3 HLA MM showed a trend for a higher 3-year CI of NRM (26%; 95% CI, 19% to 34%) than did those whose W-CBU had 0 to 1 HLA MM (18%; 95% CI, 11% to 25%; $P = .071$). In multivariate analysis, NRM was significantly increased in the W-CBU 2 to 3 HLA MM group (HR, 1.89; 95% CI, 1.07 to 3.33; $P = .027$). The use of ATG was also independently associated with higher NRM in multivariate analysis (HR, 3.05; 95% CI, 1.79 to 5.21; $P < .001$). In multivariate analysis, 3-year RI was higher in patients who received a W-CBU with $<2.5 \times 10^7$/kg TNCs at cryopreservation (30% versus 26%; HR, 1.32; 95% CI, 1.00 to 1.74; $P = .049$). Also, advanced disease status was an independent risk factor for RI compared with early disease status (HR, 3.21; 95% CI, 1.61 to 6.38; $P = .001$).

OS and LFS

In univariate analysis, W-CBUs with 2 to 3 HLA MM were associated with worse 3-year OS and LFS. OS was 62% (95% CI, 53% to 71%) in the W-CBU 0 to 1 HLA MM group and 49% (95% CI, 41% to 56%) in the W-CBU 2 to 3 HLA MM group ($P = .015$), while LFS was 56% (95% CI, 46% to 65%) in the W-CBU 0 to 1 HLA MM group and 44% (95% CI, 36% to 52%) in the W-CBU 2 to 3 HLA MM group ($P = .034$). In multivariate analyses, patients with 2 to 3 HLA MM with the W-CBU had significantly lower OS (HR, 1.53; 95% CI, 1.05 to 2.25; $P = .028$) and LFS (HR, 1.49; 95% CI, 1.04 to 2.15; $P = .032$). Other factors independently associated with lower OS and LFS in multivariate analysis were the use of ATG (HR, 2.10; 95% CI, 1.43 to 3.08; $P < .001$ for OS; and HR, 2.14; 95% CI, 1.48 to 3.09, $P < .001$ for LFS) and advanced disease status (HR, 2.49; 95% CI, 1.45 to 4.29; $P = .001$ for OS; and HR, 2.21; 95% CI, 1.31 to 3.73; $P = .003$ for LFS). Overall, 153 patients died. Causes of death were disease relapse in 45% (n = 69) and transplant related in 55% of the cases (n = 84), mainly infection (n = 45) and GVHD (n = 17).

DISCUSSION

This study analyzed the influence of W-CBU characteristics on unit dominance and on outcomes in 347 patients with donor engraftment after a DUCBT for acute leukemia. A previous study with a smaller cohort showed that order of infusion and post-thaw cell dose are independent predicting factors for W-CBU, whereas no influence of ABO, sex, and HLA match unit-recipient was observed [8]. Nevertheless, more recent evidences did not find any association among unit dominance and order of infusion [17,26]. A different study also failed to show any association between HLA matching unit-recipient and unit dominance [27]. Considering immunological aspects, it was hypothesized that the GVG effect, mediated by $CD34^-$ cells in the CBUs, might define the W-CBU [13,28]. CBU dominance is a T cell–mediated phenomenon, driven by factors as increased cytotoxic T cell activity from W-CBU against the L-CBU, higher number of $CD3^+$ cells in the W-CBU, $CD34^+$ and $CD3^+$ cells viability, and more recently, higher $TCD4^+$ response from W-CBU toward mismatched HLA class II alleles in the L-CBU [5,15,29,30]. Also, the role of killer cell immunoglobulin-like receptor–HLA interactions on unit dominance is a matter of debate. Although an in vitro model suggested that unidirectional potential natural killer alloreactivity might play a role in unit dominance, 2 studies that evaluated the association of killer cell immunoglobulin-like receptor/HLA GVG mismatches and unit dominance in patients showed conflicting results [5,31,32]. In our study, we did not compare $CD34^+$ cells viability between CBUs. Also, median TNC doses in both the W-CBU and L-CBU groups were greater than 2.5×10^7/kg, which prevented us from further analyzing the impact of lower TNC dose on unit dominance. Unit dominance remains a very complex phenomenon, modulated not only by CBU features, but also by complex immunological interactions, both interunits and unit and recipient.

We have demonstrated that a higher degree of HLA MM between the W-CBU and the recipient leads to worse outcomes. The increase in NRM without any beneficial effect on RI, together with the higher incidence of aGVHD, might explain, in part, the decreased OS and LFS observed in the W-CBU 2-3 HLA MM group. Because cord blood lymphocytes are less reactive, a higher degree of HLA MM is better tolerated in cord blood than in other graft sources. Previously, it has been proposed that a higher HLA MM in UCBT might increase graft-versus-leukemia effect, contributing to diminish RI [28,33]. Nevertheless, recent evidences failed to show any benefits of a higher degree of HLA MM in

UCBT. In single UCBT (SUCBT) settings, it was demonstrated that a higher degree of HLA MM increases the risk of NRM without decreasing RI [34,35]. More recently, in DUCBT, Oran et al. [12] retrospectively analyzed the impact of HLA matching between W-CBU and recipient on outcomes of 133 DUCBTs for hematological malignancies. Patients older than 32 years of age with higher HLA MM W-CBU recipient had higher TRM and lower OS than did younger or better HLA matched patients, but progression-free survival was not impacted by the degree of HLA MM [12]. On the other hand, Brunstein et al. [19], in a retrospective single-center study measuring the impact of HLA matching on DUCBT outcomes, demonstrated in a subset of 174 AL patients that the W-CBUs matching 2-5/10 to the recipient had significantly lower RI and treatment failure rates when compared with matching 9-10/10 matching [19]. However, their cohort was considerably smaller than ours, and the best matched group had a low number of patients.

The incidence of aGVHD has been previously reported to be slightly higher after DUCBT than SUCBT, although specific risk factors associated with this increase are yet to be determined [10,20,21]. A previous single-center prospective study showed no effect of W-CBU HLA MM on aGVHD incidence or severity [21]. More recently, Ponce et al [20] demonstrated that W-CBUs with 0 or 1 HLA MM were associated to lower risk of grade III to IV aGVHD. We demonstrated that lower degree of HLA MM between W-CBU and recipient leads to a lower incidence of aGVHD. In addition, the length of W-CBU storage was associated with aGVHD, with recipients of units cryopreserved and stored for a shorter period experiencing less aGVHD. The mechanism involved is unknown.

One may argue that both allele level typing for class I loci and locus C matching should have been taken into consideration for HLA analyses. The knowledge about allele level typing for class I loci and locus C matching and their impact on CBT outcomes were greatly improved in the past decade, and recent studies considered these factors in their analyses [12,19,35]. However, most studies targeting HLA were performed in SUCBT settings [36]. Guidelines to HLA matching for DUCBT were established based on the previous experience with SUCBT [12]. Allele level HLA typing has been established for SUCBT since 2014 [35]. Furthermore, in DUCBT settings, previous studies assessing the impact of allele level resolution match on outcomes have shown controversial results [12,19]. In addition, most studies that analyze HLA in the context of DUCBT consider MM degree based on the less matched CBU, not on the W-CBU. Therefore, because our study was registry-based and covered a wide range period, high resolution HLA typing was not available for most patients. Although this has restricted deeper evaluation of our HLA findings, it has not prevented us from demonstrating significant results using low resolution typing. Even if low resolution in class I loci may subestimate the real degree of mismatch between unit and recipient, we were still able to demonstrate that greater degree of mismatch leads to worse results, underlining the importance of HLA matching in this setting [36].

We could demonstrate that lower degree of HLA MM between W-CBU and recipient positively impacts DUCBT outcomes, but our results did not single out any characteristic of the CBU that would predict the W-CBU. Therefore, we suggest that matching criteria for DCBU should be based on best HLA match between CBU and recipient for both units. This recommendation should not have a great impact on donor availability once there is no evidence indicating that HLA match interunit is necessary [18,27,37]. Of course, other aspects that were demonstrated to influence survival, such as TNC or $CD34^+$ cell dose, must be taken into consideration together with HLA match [34,37,38]. Finally, although CBU length of storage is not a current criterion for CBU choice, it might be of interest to further evaluate this aspect in DUCBT settings.

The negative effect of ATG on OS and NRM has been previously described by our group in a study of patients with hematological malignancies, including AL, receiving single or DUCBT after reduced-intensity conditioning with total body irradiation, cyclophosphamide, and fludarabine [39]. However, conditioning regimen in our cohort was heterogeneous and the population analyzed was selected (only engrafted patients), which precludes further investigation on the ATG effect.

Our report has some limitations. It is a retrospective study in which the chimerism assessment methods and techniques may have varied according to transplant center. CBU collecting and infusing conditions might also have changed throughout the transplant period considered. Also, the host immune environment may be altered by the specific conditioning regimen and GVHD prophylaxis. Also, because of the main objectives of the study, the population was selected, as only engrafted patients with a defined W-CBU could be taken into consideration for the analyses. Finally, missing chimerism data in the registry precluded the inclusion of some patients with reported neutrophil engraftment and meeting other study criteria.

The strength of our study relies on the size of the cohort included in the analyses on unit dominance and W-CBU risk factors, larger than in previous studies, justifying the relevance of our findings.

Although we were unable to determine the characteristics of the CBU that may predict the W-CBU, we have, successfully, demonstrated that some specific W-CBU characteristics, namely degree of HLA matching with the recipient, influence transplant outcomes, contributing to improve donor selection criteria in DUCBT.

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