


# Cord-Blood Engraftment Using an Enhanced Dual-Conditioning Regimen for Malignant Hematologic Diseases

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## Abstract

To explore a more effective conditioning regimen for umbilical cord blood transplantation (UCBT) to treat hematologic malignancies, we conducted a cohort study of a fludarabine/busulfan/cytarabine plus cyclophosphamide 200 mg/kg regimen. Forty-two consecutive patients with leukemia, myelodysplastic syndrome, or lymphoma received the regimen. The median number of infused total nucleated cells per kilogram was  $5.5 \times 10^7$  (1.81–20.6), the median number of infused CD34<sup>+</sup> cells per kilogram was  $1.58 \times 10^5$  (0.58–6.6), and the median follow-up for surviving patients was 37 months (4.0–79.5 months). The cumulative incidence of neutrophil engraftment at 31 days was 100% [95% confidence interval (CI): 0.9159–1.0], and the median time to neutrophil engraftment was 19 days. The cumulative incidence of nonrelapse mortality was 12.76% (95% CI: 0.0455–0.2356) at 180 days and 3 years. The 3-year overall survival (OS) and disease-free survival (DFS) rates were 71.6% and 59.6%, respectively. Especially in patients who received transplants in the early and intermediate stages, the 3-year OS and DFS rates were 90.3% (95% CI: 0.805–1.0) and 76.2% (95% CI: 0.608–0.956), respectively. The regimen significantly improved engraftment and survival, indicating that the high graft failure of UCBT was caused by rejection.

## Keywords

cord blood, hematopoietic stem cells, T cells, graft survival, malignant hematologic diseases

## Introduction

Graft failure (GF) and delayed engraftment restrict the application of umbilical cord blood transplantation (UCBT). Engraftment is successful in approximately 80%–90% of patients with UCBT<sup>1–10</sup>, and the remaining 10%–20% of patients do not become engrafted. It has always been believed that the limiting factor in UCBT is the low number of hematopoietic stem cells (HSCs) in cord blood units (CBUs); therefore, various strategies have been explored to increase the number of HSCs<sup>11–19</sup>. Although some methods have made progress, the clinical benefit remains to be further demonstrated.

However, there were not only fewer CD34<sup>+</sup> cells in umbilical cord blood but also fewer CD3<sup>+</sup> lymphocytes. The number of CD34<sup>+</sup> cells received by cord blood transplant recipients is approximately one-tenth the number in allogeneic bone marrow (BM) recipients, the median of which is  $0.2 \times 10^6$  cells per kilogram, and the content of CD3<sup>+</sup> T lymphocytes in cord blood is only 1% of the content in peripheral blood grafts<sup>20</sup> and is predominantly naïve<sup>21</sup>. The GF of T-cell-depleted (TCD) allogeneic donor transplantation was approximately 10%–20%<sup>22,23</sup>; similarly, the

GF of UCBT was also approximately 10%–20%. TCD allogeneic donor transplantation and UCBT GF may have similar mechanisms, and donor T cells are essential for engraftment to counterbalance the residual recipient T cells surviving after the conditioning regimen. If UCBT engraftment failure is mediated by recipient T cells, it may be overcome by an enhanced immunoablative conditioning regimen. To explore a more effective regimen to overcome rejection and improve UCBT for hematologic malignancy efficacy, on

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the basis of previous work<sup>24</sup> and the traditional BuCy200<sup>25</sup> regimen, we designed an enhanced dual-conditioning (EDCT) regimen consisting of a modified cyclophosphamide 200 mg/kg (CY200) regimen plus an fludarabine/busulfan/cytarabine (FBA) regimen. We evaluated the regimen based on safety, engraftment, and survival.

## Method

### Eligibility

Patients with leukemia, myelodysplastic syndrome (MDS), or lymphoma who were less than 38 years of age; an absent human leukocyte antigen (HLA)-matched sibling donor; and those with a Karnofsky score of  $\geq 80$  were included. In addition, one or two CBUs were available with adequate cell doses and were HLA-matched to both the patient and each other (i.e., a match score of 4/6, 5/6, or 6/6), HLA-A and HLA-B at the antigen level and HLA-DRB1 at the allele level. If the cell dose was insufficient with a single CBU, double CBU was used. Thirty-eight of the 42 patients used single cord blood, and 4 used double cord blood. UCBs were selected according to the criteria for cord blood donor choice<sup>20</sup>; patients who had prior allogeneic transplantation or other conditioning regimens (not EDCT regimen) were excluded. Infusion of one cord blood unit plus one haplo-identical graft was also excluded. This study was approved by the ethics committee of our institute. Written informed consent was provided in all cases, either by the parents or guardians for children younger than 18 years of age or by the patients if they were older than 18 years of age.

### Conditioning Regimen and Graft-Versus-Host Disease Prophylaxis

The EDCT regimen consisted of the modified CY200 regimen plus the FBA regimen (Table 1). The FBA regimen included four doses of 0.8 mg/kg busulfan (BU) daily on days -9 to -6, for a total of 14 doses. Fludarabine (Flu) 30 mg/m<sup>2</sup>/d was administered on days -7 to -2 or -11 to -6. Cytarabine (Ara-C) 2–4 g/m<sup>2</sup>/d was administered intravenously for 2 days from days -10 to -9. CY200 regimen; cyclophosphamide 50 mg/kg (actual body weight or adjusted according to body surface area 1.5–1.2 g/m<sup>2</sup>/d, whichever was lower) was administered from days -5 to -2. Prophylaxis for graft-versus-host disease (GVHD) consisted of cyclosporine on days -1 through 180 and mycophenolate mofetil on days -1 through 28 (or longer if there was active GVHD).

### Supportive Care

Patients were kept in isolation with high-efficiency particulate air filtration. The prevention of bacterial and fungal infections was performed according to the strategy of each center. Broad-spectrum antibiotics and a mold-active azole

**Table 1.** EDCT Regimen, GVHD Prophylaxis.

Agent	Dose/d	Route	Day
Ara-C	1–2 g/m <sup>2</sup> q12h	IV 3 h	d -9 to -8
BU	0.8 mg/kg q6h	IV 2 h	d -8 to -5 (total 14 doses)
Flu	30 mg/m <sup>2</sup>	IV 1 h	d -7 to -2 or -11 to -6
CTX	50 mg/kg <sup>a</sup> qd	IV 1 h	d -5 to -2
Mesna	50 mg/kg q12h	IV 12 h	d -5 to -2
CSA	3 mg/kg	IV	d -1 to 180
MMF	14 mg/kg q12h	PO	d -1 to 28
G-CSF	5 μg/kg/d	SC	Day 7 to N > 0.5 × 10 <sup>9</sup> /L

EDCT: enhanced dual-conditioning; GVHD: graft-versus-host disease; Ara-C: cytarabine; IV: intravenously; BU: busulfan; Flu: fludarabine; CTX: cyclophosphamide; CSA: cyclosporine; MMF: mycophenolate mofetil; PO: orally; G-CSF: granulocyte-colony stimulating factor; SC: subcutaneous injection.

<sup>a</sup>Actual body weight or adjusted according to body surface area 1.5–1.2 g/m<sup>2</sup>/d, whichever was lower.

(itraconazole or voriconazole) are recommended during neutropenia. Granulocyte-colony stimulating factor (G-CSF) was administered subcutaneously at 5 mg/kg/d from day 6 until neutrophil engraftment. As cytomegalovirus (CMV) prophylaxis, all patients received intravenous ganciclovir (5 mg/kg q12h) from day -10 to day -2, followed by acyclovir from day -1 to 1 year. CMV viremia was monitored by polymerase chain reaction (PCR) and treated preemptively with ganciclovir or foscarnet.

### Definition and Chimerism

The time to neutrophil engraftment was defined as the first of three consecutive days with an absolute neutrophil count of  $0.5 \times 10^9$  per liter or higher, and the time to platelet engraftment was defined as the first of seven consecutive days with a platelet count of  $20 \times 10^9$  per liter or higher without platelet transfusion. Patients who survived >42 days after transplantation and failed to achieve neutrophil engraftment were defined as GF. Secondary GF was defined as the loss of engraftment.

Overall survival (OS) was measured from the date of transplantation to the date of death and was censored as of the date of the last follow-up visit for survivors. Disease-free survival (DFS) was measured from the date of transplantation to the date of relapse or death, whichever occurred first, and was censored at the date of the last follow-up.

Chimerism in peripheral blood or BM was tested by means of a PCR assay, with primer sets flanking microsatellite repeats, or by means of fluorescence in situ hybridization for transplants from the opposite sex at least once a month or several times as needed. Full donor chimerism was defined as the presence of >95% donor hematopoiesis.

### Statistical Considerations

The analysis included 42 consecutive patients who underwent UCBT between May 2014 and October 2020 in this

retrospective multicenter cohort study, and the data were collected as of February 2021. The primary end point was the cumulative incidence of neutrophil engraftment at 42 days. The secondary end points were the cumulative incidence of platelet engraftment, the recovery time of neutrophils and platelets of all patients and its subgroups, OS and DFS rates for all patients and subgroups based on different stages of disease, nonrelapse mortality (NRM), disease relapse, acute GVHD (aGVHD), and chronic GVHD (cGVHD). The probabilities of neutrophil and platelet engraftment, aGVHD, cGVHD, NRM, and relapse were generated by the cumulative incidence method. Among them, competing risks for engraftment were death and relapse; for GVHD, competing risks were death, neutrophil engraftment failure, and relapse; for NRM, competing risk was relapse; and for relapse rate (RR), competing risk was death. The survival rate was estimated by the Kaplan–Meier method. The Wilcoxon rank-sum test was used in analyses of cell dose and HLA match and time to neutrophil and platelet recovery. All reported *P* values are two-sided, and the significance level was 0.05. A *P* value of 0.05 or less was considered to be statistically significant. All analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC, USA). Figures were generated using R3.6.

## Results

### Patient and Graft Characteristics

Table 2 shows the characteristics of the 42 consecutive patients who received the EDCT regimen for UCBT. Thirty-eight patients had high-risk leukemia or leukemia with high risk of recurrence<sup>26–30</sup>. Of the 19 acute myeloid leukemia (AML) patients, 4 had complex karyotypes, 2 had MLL rearranged, 1 had ASXL1 and 2 had FLT3-ITD mutations, 4 had persistent minimal residual disease, 3 had second complete remission (CR2), and 3 had no remission (NR). Of the 17 acute lymphocytic leukemia (ALL) patients, 3 had B-ALL and 2 had T-ALL with hyperleukocytosis, 4 with Ph chromosome, 3 with CR2, and 5 with NR. One juvenile myelomonocytic leukemia (JMML) patient had KRAS mutation and 1 JMML patient had PTPN11 mutation. Disease status at the time of transplantation was classified into the following two categories: (a) 31 patients in early and intermediate (EI) stage—among them, 29 with high-risk acute leukemia in first, second, or further complete remission (CR1, CR2, CR3) and 2 untreated MDS with <5% blasts; (b) 11 patients were in an advanced disease (AD) stage—among them, 7 cases of acute leukemia, 2 cases of lymphoma, and 2 cases of JMML were not in CR (NR). The median age at transplantation was 9 years (range, 1.0–38.0 years). The median follow-up for surviving patients was 37 months (range, 4.0–79.5 months). The median number of infused total nucleated cells (TNCs) per kilogram was  $5.5 \times 10^7$  (1.81–20.6), and the median number of infused CD34<sup>+</sup> cells per kilogram was  $1.58 \times 10^5$  (0.58–6.6).

**Table 2.** Patient and Graft Characteristics (N = 42).

Characteristics	N = 42
Median age (range), years	9 (1–38)
Sex, n (%)	
Female	21 (50)
Male	21 (50)
Median weight (range), kg	25.5 (9.3–85.0)
Diagnosis, n (%)	
Acute myeloid leukemia	19 (45.24)
First complete remission (CR1)	13 (68.4)
Second complete remission (CR2)	3 (15.8)
No remission (NR)	3 (15.8)
Acute lymphocytic leukemia	17 (40.48)
First complete remission	9 (52.9)
Second complete remission	3 (17.6)
Not remission	5 (29.4)
Lymphoma	2 (4.76)
Myelodysplastic syndrome	2 (4.76)
Juvenile myelomonocytic leukemia	2 (4.76)
Disease status	
Early and intermediate (EI) stage	31 (73.8)
Advanced disease (AD) stage	11 (26.2)
HCT-CI score	
0	37 (88.1)
1–2	5 (11.9)
HLA compatibility, n (%)	
4/6	11 (26.19)
5/6	18 (42.86)
6/6	13 (30.95)
TNC cells, $\times 10^7$ /kg, median (range)	
Cryopreserved TNC dose	5.8 (2.5–19.3)
Infused TNC dose	5.5 (2.3–15.1)
CD34 cells, $\times 10^5$ /kg, median (range)	
Cryopreserved CD34 cell dose	2.16 (0.68–14.30)
Infused CD34 cell dose	1.58 (0.58–11.00)
CD34 cells lower than median <sup>a</sup> , $\times 10^5$ /kg	
Median (range)	1.26 (0.58–1.55)
Interquartile range	1.15–1.32
CD34 cells higher than median, $\times 10^5$ /kg	
Median (range)	2.96 (1.60–11.00)
Interquartile range	2.42–4.59

HCT-CI: hematopoietic cell transplantation-comorbidity index;

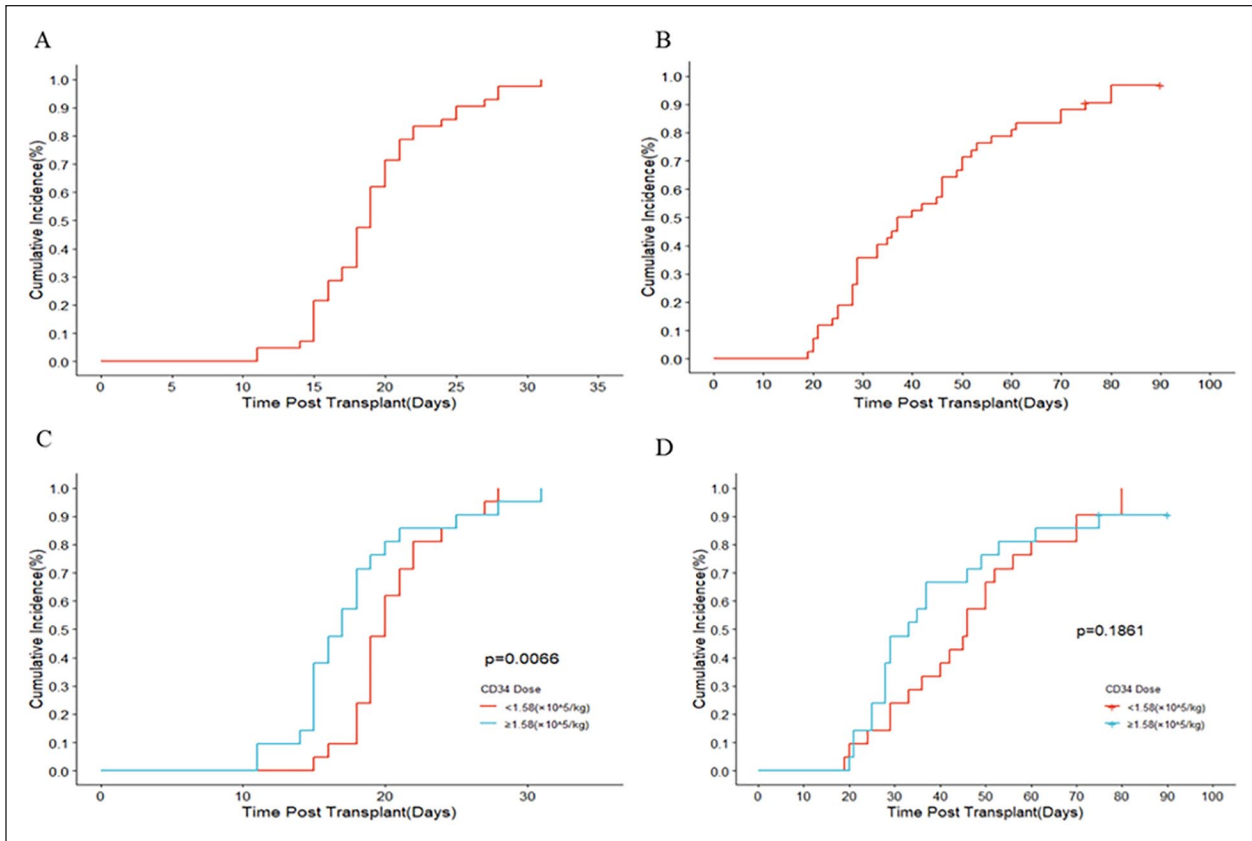
HLA: human leukocyte antigen; TNC: total nucleated cells.

<sup>a</sup>Patients with infused CD34 cells  $< 1.58 \times 10^5$ /kg.

### Neutrophil Engraftment and Donor Chimerism

The cumulative incidence of neutrophil engraftment at 31 days after transplantation was 100% [95% confidence interval (CI): 0.9159–1.0], the median time to neutrophil recovery was 19 days (range, 7–31 days) (Fig. 1A), and no patient developed secondary GF.

In the univariate analysis, the results of the subgroups showed that the median time of neutrophil engraftment in the CD34<sup>+</sup> cell  $> 1.58$  group [median, 2.96; interquartile range (IQR), 2.42–4.59] and  $< 1.58$  group (median, 1.26;



**Figure 1.** Neutrophil and platelet engraftment. (A) Neutrophil engraftment of all the patients. (B) Platelet engraftment of all the patients. (C) Neutrophil engraftment according to CD34 dose. (D), Platelet engraftment according to CD34 dose.

IQR, 1.15–1.32) was 17 days versus 20 days, respectively ( $P = 0.0066$ ), and CD34+ cell doses above the median were associated with faster neutrophil engraftment (Fig. 1C). HLA compatibility, body weight  $< 40$  kg, age  $< 12$  years at transplantation, and infused TNC dose higher than the median were not significantly associated with faster neutrophil engraftment (Fig. S1 in the Supplemental Appendix).

Platelet engraftment was achieved in 40 patients, the cumulative incidence of platelet engraftment was 95.24% (95% CI: 0.7953–98.97), and the median time to platelet recovery was 33.5 days (range, 12–80 days) (Fig. 1B).

The infused CD34+ cell doses (Fig. 1D) and TNC doses above the median were not significantly different from the faster platelet engraftment.

### Chimerism

After successful engraftment of neutrophils, all the patients were evaluated for whole-blood chimerism. All the patients showed complete donor chimerism. Two of the four patients who received double UCBT showed double donor chimerism in the early stage, and then all converted to single donor chimerism. Among them, 10 patients were tested for chimerism as early as +1 day after transplantation, and all patients achieved complete donor chimerism on +10 days after

transplantation, which was approximately a week earlier than neutrophil engraftment.

### Survival

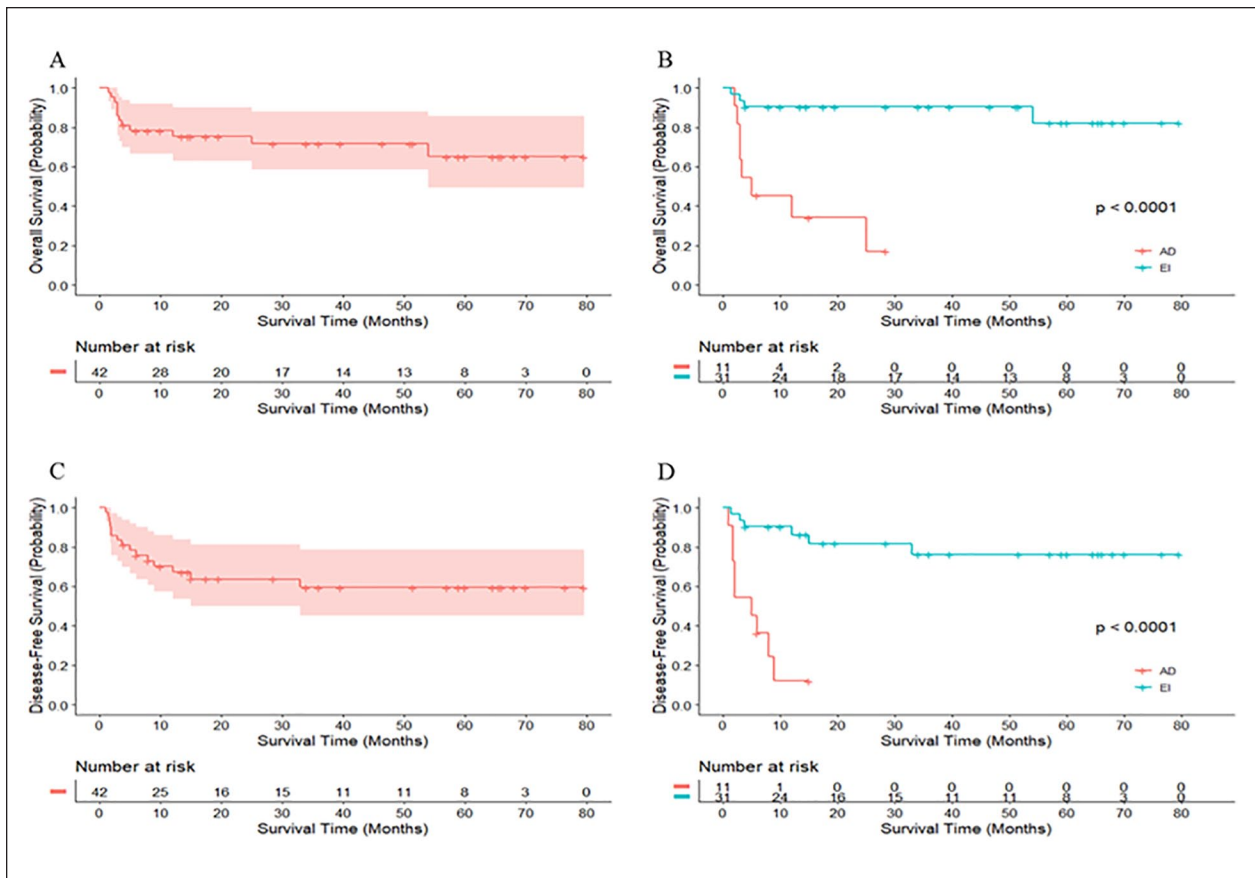
The 1- and 3-year OS rates for the 42 patients were 75.3% (95% CI: 0.63–0.9) and 71.6% (95% CI: 0.583–0.878), respectively (Fig. 2A). The 1- and 3-year DFS rates for the 42 patients were 75.30% (95% CI: 0.5883–0.8592) and 67.53% (95% CI: 0.4897–0.8058), respectively (Fig. 2C).

The 3-year OS and DFS rates were 90.3% (95% CI: 0.805–1.0) and 76.2% (95% CI: 0.608–0.956) in the EI stage, respectively, which were significantly better than those in the AD stage (Fig. 2B, D).

The univariate analysis results for the subgroups showed that there were no associations between OS and DFS and infused CD34+ cell dose and TNC dose above the median, HLA compatibility, body weight  $> 40$  kg, ABO incompatibility, and age  $> 12$  years at the time of transplantation. Disease stage was related to OS and DFS (Table 3).

### NRM and Relapse

Five patients died of transplant-related complications, including aGVHD ( $n = 2$ ), cardiac dysfunction ( $n = 1$ ), central



**Figure 2.** K-M curves in different groups. (A) Overall survival of all the patients. (B) Overall survival according to disease status. (C) Disease-free survival of all the patients. (D) Disease-free survival according to disease status. AD: advanced disease; EI: early and intermediate; K-M: Kaplan-Meier.

**Table 3.** Univariate Analyses for Survival and NRM in Patients Who Received CBT.

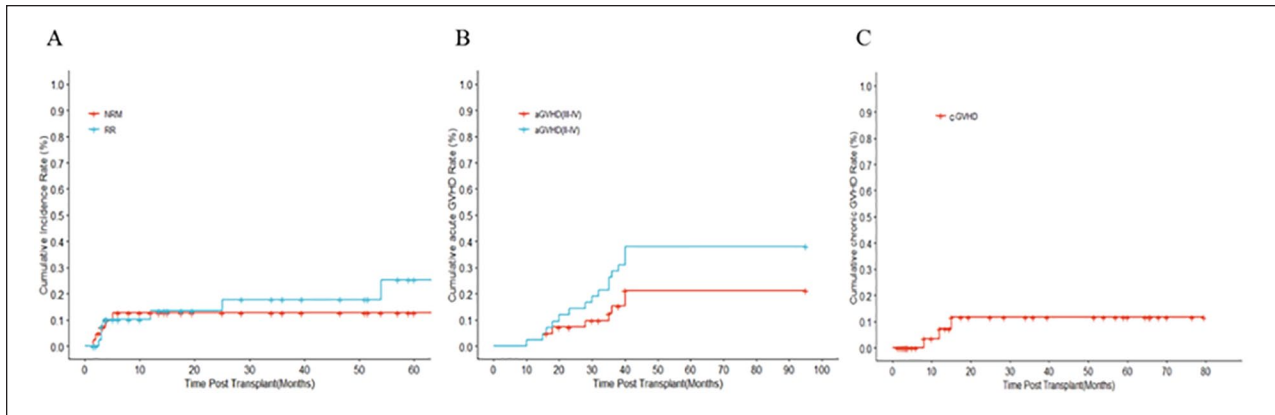
Patients	Overall survival		Disease-free survival		NRM	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age ( $\geq 12$ years)	1.493 (0.473–4.716)	.494	1.853 (0.672–5.114)	.233	3.204 (0.535–19.179)	.202
Weight ( $\geq 40$ kg)	1.321 (0.417–4.179)	.636	1.593 (0.577–4.396)	.369	2.837 (0.474–16.986)	.253
HLA (not fully matched)	1.440 (0.455–4.551)	.535	1.850 (0.668–5.118)	.236	37.300 (0.014–98,862.216)	.368
ABO (not matched)	2.923 (0.899–9.502)	.075	2.354 (0.844–6.562)	.102	1.160 (0.193–6.967)	.871
TNC ( $< 5.50 \times 10^7/\text{kg}$ )	1.280 (0.405–4.041)	.674	1.783 (0.610–5.219)	.291	3.661 (0.409–32.775)	.246
CD34 ( $< 1.58 \times 10^5/\text{kg}$ )	1.145 (0.368–3.566)	.815	0.743 (0.264–2.090)	.574	1.427 (0.238–8.550)	.697
Disease status (AD)	10.94 (2.856–41.93)	<.001	10.49 (3.317–33.17)	<.001	1.521 (0.618–3.740)	.361

CBT: cord blood transplantation; NRM: nonrelapse mortality; CI: confidence interval; HLA: human leukocyte antigen; TNC: total nucleated cells; AD: advanced disease.

nervous system human herpes virus 6 (HHV6) infection ( $n = 1$ ), and hematopoietic stem cell transplantation (HSCT)-associated thrombotic microangiopathy (TMA) ( $n = 1$ ). The cumulative incidence of NRM was 12.76% (95% CI: 0.0455–0.2356) at 180 days and 3 years. Thirty-eight patients had episodes of CMV infection. Thirty-six patients had CMV viremia alone, and two patients had CMV

viremia with CMV retinitis. Thirty-eight CMV viremia and two CMV retinitis cases were treated successfully with induction therapy of ganciclovir or foscarnet.

Seven patients relapsed; five ALL patients relapsed (one transplanted in EI, four transplanted in AD), one AML patient transplanted in AD relapsed, and one lymphoma patient transplanted in AD relapsed. The cumulative incidence of



**Figure 3.** Cumulative incidence of NRM, RR and GVHD. NRM: nonrelapse mortality; RR: relapse rate; GVHD: graft-versus-host disease.

RR was 13.53% (0.0476–0.2686), 17.85% (0.0677–0.3319) and 25.32% (0.0918–0.4538) at 1, 3, and 5 years, respectively (Fig. 3A).

### GVHD

The cumulative incidence of grade II to IV and grade III to IV aGVHD by 100 days after transplantation was 38% (95% CI: 0.23.51–0.5256) and 21% (95% CI: 0.0973–0.355), respectively (Fig. 3B)<sup>31,32</sup>. For patients who survived for at least 100 days after transplantation, the cumulative incidence of limited cGVHD was 11.72% (95% CI: 0.0281–0.2764), and no patient developed moderate to extensive cGVHD at 24 months (Fig. 3C).

### Discussion

The FB regimen successfully used for peripheral blood stem cell transplantation (PBSCT) and bone marrow transplantation (BMT) cannot enable reliable engraftment of umbilical cord blood grafts<sup>33</sup>. Adding TT to FB improved the engraftment and survival of UCBT<sup>10,34</sup>. We added CY200 to FB to form a more enhanced immunoablative regimen. All 42 patients (100%, 95% CI: 0.9159–1.0) exhibited uniform neutrophil engraftment within 1 month (31 days) regardless of whether they were in the high cell-dose group or the low cell-dose group, which was better than the 80%–90% engraftment rate reported. The low boundary engraftment rate of 91.59% was equal to or superior to the 91% engraftment rate of BMT<sup>35</sup>. The median time to neutrophil recovery was 19 (7–31) days, almost equal to the reported recovery time in BMT<sup>2,4</sup>. The 3-year OS and DFS rates of the 42 patients were 71.6% and 67.53%, respectively. In particular, the OS and DFS rates of the patients with hematologic malignancies who received a transplant in the EI stage reached 90.3% and 76.2% at 3 years, respectively, and UCBT using the EDCT regimen is very promising. However, for AD stage patients, the RR is high, which is in line with Baron et al<sup>36</sup>.

A series of studies have shown that TT is added to modified BuCy2<sup>37</sup>, MEL is added to FB<sup>38</sup>, Flu or Ara-C is added to the CyTBI regimen<sup>1,5,11,39,40</sup>, and a conditioning regimen composed of TT, TBI, and fludarabine<sup>9</sup> shows improved engraftment and survival in UCBT. These alternative enhanced conditioning regimens have become preferable protocols for UCBT. It is worth noting that many other reports have shown that the use of less intensive conditioning regimens yields excellent engraftment rates, which may be due to the use of an intensified immunoablative regimen rather than MA regimens to overcome rejection. These low-intensity regimens show a positive correlation between the intensity of immunosuppression and engraftment and survival; the cumulative incidence of sustained donor chimerism with the Cy/Flu/TBI regimen was significantly higher than with the Bu/Flu/TBI regimen<sup>41</sup>, the engraftment rates of Cy/Flu/TBI and Flu/MEL<sup>42</sup> are comparable to those of traditional MA regimens, and thiotepa-based Intensified Reduced-Intensity Cy/Flu/TBI yielded a better survival rate than Cy/Flu/TBI<sup>43</sup>. Although these enhanced immunoablative regimens increase the engraftment rate compared with the myeloablative regimens, the engraftment rate is still lower than BMT and PBSCT. To the best of our knowledge, EDCT is currently one of the strongest immunoablative conditioning regimens available. The use of this regimen to achieve such a high engraftment rate and survival rate indirectly confirms our speculation that this protocol can compensate for the rejection of the grafts due to the insufficient number of cord blood CD3+ cells. For grafts with different characteristics, the conditioning regimen should be different.

The presence of recipient cells in the blood and BM and the absence of donor cells support the hypothesis that rejection was the main cause of GF<sup>24,44–46</sup>. Chimerism monitoring confirmed from another perspective that with the use of the EDCT regimen for UCBT, all the patients showed full donor chimerism after successful engraftment of neutrophils. All 10 patients who were monitored as early as +1–+10 days after transplantation achieved full donor chimerism approximately

1 week before neutrophil engraftment (10 days after transplantation). This is different from the myeloablative conditioning regimen for BMT; mixed chimerism was found in 17% of patients 14 days after transplantation, and even this mixed chimerism persisted for a long time<sup>47</sup>. Obviously, the earlier full donor chimerism came from the EDCT regimen, which successfully overcame the rejection reaction.

This study has several limitations. One of the limitations of our study is the inclusion of adult and pediatric patients. Although it contains many young patients, previous reports showed that similar to adult patients, the GF of UCBT was also 10%–20%<sup>1,2,6–8</sup> or even higher among children or young people<sup>48</sup>. The median CD34+ cell dose we used was the same as that reported in these studies for children, indicating that the high engraftment rate comes from a more effective EDCT regimen. Subgroup analysis showed that neutrophil engraftment times were faster with CD34+ cells above the median, but eventually, neutrophil engraftment was achieved in all patients within 31 days. This indicates that as long as the graft was not rejected, although the engraftment of the lower cell number group was slower, stable engraftment could eventually be obtained, and there was no difference in OS between the two groups. Other indirect evidence supporting rejection as the cause of GF is that patients who receive fully matched CBUs have superior engraftment and survival, even regardless of the cell dose<sup>3,6</sup>, and TNC in excess of the minimum required dose does not lower mortality<sup>49</sup>.

Another limitation is that this study included several different malignant diseases, including 38 cases of high-risk leukemia, 2 cases of refractory relapsed malignant lymphoma, and 2 cases of MDS. Although the increased intensity of the conditioning regimen resulted in excellent results for patients in the EI stage, 8 of 11 AD patients died from recurrence or transplant-related complications, which significantly reduced the survival rate calculated in this study. It should be considered whether the antitumor effect of CY on patients not in remission is limited, or it should be considered improving the prevention of GVHD after transplantation to enhance the graft versus leukemia (GVL) effect. The other limitations are that the cohort was small and that this was a retrospective study; thus, the results need to be further validated.

The patients showed good tolerance to EDCT, and the NRM rate at 180 days and 2 years was only 12.76%. From another perspective, the regimen is based on the traditional BuCy200 with two fewer doses of BU, six more doses of Flu, and four doses of Ara-C. The safety of the BuCy4 regimen has undergone long-term clinical testing. To reduce the toxicity of the regimen, we adjusted the dosage of CY according to the surface area of the patients, which was another important reason for the good tolerance. In addition, most of the subjects in this study were younger and had disease at the EI stage at transplantation; therefore, the good disease status at transplantation may have influenced the

results. Although the incidence of CMV viremia was relatively high, it was controlled through timely detection and preemptive treatment. The infection mortality rate of this group of patients is relatively low, mainly due to the absence of antithymocyte globulin (ATG)<sup>24</sup>.

In conclusion, the EDCT regimen can overcome GF and delayed engraftment and achieve excellent OS and DFS rates. Our findings reveal that UCBT requires a deeper immunoablative regimen. A possible mechanism is to enhance the suppression of the immune system to compensate for the insufficiency of T cells in cord blood to ensure that the graft is not rejected, and the strong expansive ability of cord blood stem cells compensates for the lack of quantity. Therefore, we can at least cautiously propose here that according to the current cell dose used, the main reason for the 10%–20% GF of UCBT is rejection, not the CD34+ dose lower than of BMT and PBSCT.

### Author Contributions

JD analyzed and interpreted the data, performed research, wrote the manuscript, and designed the EDCT regimen; YF performed research and wrote the manuscript; RZ performed the research; YG analyzed and interpreted data and wrote the manuscript; YG, SD, QL, and QY collected the data and performed statistical analyses.

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Ethical Approval

This study was approved by the ethics committee of ZhongDa Hospital of Southeast University (ZDLY[2010]108)

### Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the ethics committee of ZhongDa Hospital of Southeast University (ZDLY[2010]108) approved protocols. This article does not contain any studies with animal subjects.

### Statement of Informed Consent

Written informed consent was obtained from the patients for their anonymized information to be published in this article.

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### Supplemental Material

Supplemental material for this article is available online.

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