Biol Blood Marrow Transplant 25 (2019) 121–128

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Cord Haploidentical Non-In Vitro T Cell Depletion Allogeneic Hematopoietic Stem Cell Transplantation Reduces Relapse of Refractory Acute Leukemia

Jingbo Wang[1,*], Zheng Wang[1], Wei Wei[1], Weijie Zhang[1], Tao Zhang[1], Haoyu Cheng[1], Xinhong Fei[1], Yuming Yin[1], Jiangying Gu[1], Lei Yuan[2,*]

[1] *Department of Hematology, China Aerospace Center Hospital, Beijing, China*
[2] *Department of Hematology, Peking University Third Hospital, Beijing, China*

Article history:
Received 2 May 2018
Accepted 4 September 2018

Key Words:
Acute leukemia
Cord blood transplantation
Chimerism
Haploidentical cord
Refractory
Relapse

A B S T R A C T

Whether a graft-versus-graft (GVG) response in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) is associated with an enhanced graft-versus-leukemia (GVL) effect remains highly controversial. Furthermore, it is unknown if the GVG response overwhelms the impact of refractory acute leukemia. We aimed to compare the characteristics and therapeutic outcomes between patients undergoing a modified haploidentical cord blood (cord-haplo) HSCT protocol (n = 97) and those undergoing haploidentical HSCT (n = 42) for refractory acute leukemia. A reliable and stable predominant haploidentical donor chimerism was established. The 2-year relapse rate was more favorable in patients undergoing cord-haplo HSCT than in those undergoing haploidentical HSCT (25.9% versus 53.2%; $P = .007$), as was progression-free survival (PFS; 35.5% versus 17.9%; $P = .049$). Meanwhile, nonrelapse mortality at 2 years was not significantly different (38.0% versus 24.6%; $P = .367$). We also found that a higher number of mutual haploidentical donor-mismatched antigens, a concept similar to HLA mismatching, was associated with better disease control. Multivariate analysis identified cord-haplo HSCT as an independent significant predictor of reduced relapse (hazard ratio [HR], .44; $P = .028$) and improved PFS (HR, .58; $P = .033$), as was chronic graft-versus-host disease (GVHD) (relapse: HR, .42; $P = .013$; PFS: HR, .63: $P = .052$). However, the incidences of neutrophil and platelet engraftment, GVHD, and virus reactivation were comparable in the 2 groups. This study demonstrates that cord-haplo HSCT significantly enhances the GVL effect and improves PFS, providing a reliable and efficient therapeutic platform for patients with refractory acute leukemia.

INTRODUCTION

Refractory acute leukemia has a dismal prognosis and represents a tremendous therapeutic challenge. Conventional allogeneic hematopoietic stem cell transplantation (HSCT) yields long-term survival in only 7% to 24% of patients with refractory acute lymphoblastic leukemia and in only 10% to 40% of those with late-stage acute myelogenous leukemia (AML) [1-6]. Although several recent studies of allogeneic HSCT using 2 grafts (ie, double-umbilical cord blood [UCB] transplantation [dUCBT]) and haploidentical cord blood (haplo-cord) HSCT have shown an enhanced graft-versus-leukemia (GVL) effect along with a graft-versus-graft (GVG) response [4,7-17], this finding remains controversial [9,14,18,19]. The outcomes of previous studies were strongly influenced by the extent of HLA mismatching and patient selection [20,21].

Several studies of dUCBT have concluded that the immunologic interaction between the 2 grafts yields an enhanced GVL effect favoring disease control while inducing the GVG response [7,8,12,22]. Subsequently, other investigators found that the "loser" graft-specific $CD4^+$ and $CD8^+$ T cells were both responsible for the predominance of the chimerism and reduced rate of relapse [7,8,23]. However, the most recently published population-based clinical study, which was based on more substantial evidence, suggested no correlation between dUCBT and an enhanced GVL effect [18]. Furthermore, the assumption of fewer relapses in UCBT recipients has been disputed, given the previously revealed association between high-degree HLA disparity and reduced relapse [24]. Because the majority of transplant recipients enrolled in these studies were in complete remission (CR) while undergoing

Financial disclosure: See Acknowledgments on page 127.

* Correspondence and reprint requests: Jingbo Wang, Department of Hematology, Aerospace Central Hospital, No. 15 Yu Quan Road, Beijing 100049, China and Lei Yuan, Department of Hematology, Peking University Third Hospital, No. 49 North Garden Road, Beijing, 100191, China.
E-mail address: wangjingbo@asch.net.cn (J. Wang), yuanlei@bjmu.edu.cn (L. Yuan).

https://doi.org/10.1016/j.bbmt.2018.09.002
1083-8791/

transplantation, the power of GVL might have been underestimated, given the fewer incidences of relapse. In light of these possibilities, we proposed the hypothesis that robust GVL would produce a significant prosurvival outcome in recipients of haplo-cord HSCT who are at high risk of relapse.

We designed a modified haplo-cord HSCT protocol with a haploidentical HSCT backbone (the Beijing protocol) plus an infusion of UCB before bone marrow grafting. We referred to this protocol as the "cord-haploidentical non-in vitro T cell-depletion allogeneic HSCT" (CHINA HSCT) protocol, or "cord-haplo HSCT" for short. This cord-haplo HSCT differs from conventional haplo-cord HSCT in the following ways: (1) the ultimate dominant chimerism is haploidentical donor-derived, (2) the technique of in vitro T cell depletion for the haploidentical donor grafts before HSCT using the anti-CD3 Miltenyi column is not used, and (3) a fixed and relatively low dose of UCB from a third-party donor is infused. Given the GVG response, we hypothesized that cord-haplo HSCT would reduce the incidence of relapse and improve the outcomes of patients with refractory AL. To that end, we retrospectively compared the characteristics and outcomes of nonremission status between patients with acute leukemia who underwent cord-haplo HSCT and those who underwent haploidentical HSCT.

PATIENTS AND METHODS

Eligibility

Patients with refractory AML or refractory acute lymphoblastic leukemia who underwent their first haploidentical allogeneic HSCT at the Department of Hematology, China Aerospace Center Hospital between May 2012 and December 2016 were included in this retrospective study. Patients with FAB M3 AML were excluded. The study was conducted in accordance with the Declaration of Helsinki and was approved by the China Aerospace Center Hospital's Institutional Review Board. All participants provided informed consent.

Definitions of Disease and Survival Status

Primary refractory disease was defined as the failure to achieve CR after 2 cycles of induction chemotherapy. Relapsed refractory acute leukemia was defined as the failure to regain CR after 2 cycles of standard salvage chemotherapy following relapse. Cytogenetic abnormalities at diagnosis were evaluated according to the 2008 World Health Organization classification [5,19,25]. Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $>.5 \times 10^9$/L, and platelet engraftment was defined as the first of 7 consecutive days with a platelet count $>20 \times 10^9$/L independent of transfusion. Acute and chronic graft-versus-host disease (GVHD) were diagnosed and graded according to consensus criteria [26,27]. Nonrelapse mortality (NRM) was defined as death without evidence of leukemia relapse. Progression-free survival (PFS) was defined as the interval between transplantation and disease progression or death from any cause, whichever occurred first. Overall survival (OS) was defined as the interval between transplantation and death from any cause.

HLA Typing and Donor Selection

HLA compatibility in haploidentical donors and recipients was confirmed by high-resolution typing of HLA-A, -B, -C, -DRB1, and -DQB1. Matched related donors were defined as siblings with identical HLA typing who had inherited the same parental haplotype as the recipient. Otherwise, donors were considered HLA-haploidentical donors. For UCB selection, the minimum HLA matching requirement was 3/6 using low-resolution HLA typing for HLA-A and -B and high-resolution typing for HLA-DRB1. The UCB units were obtained from 3 UCB banks (Beijing, Shandong, and Tianjin), all certified by the Ministry of Health. All units were clinical grade, normal in volume with depleted red blood cells, and transferred by cold-chain transportation. The cell counts and viability of each UCB were assessed immediately after thawing. Any excess remaining after infusion was discarded.

Mutual Haploidentical Donor Mismatched Antigen

To evaluate the extent of the alloreactive response to allo-HSCT, we developed a concept we termed the mutual haploidentical donor mismatched antigen (MHMA), a major HLA locus mutually harbored by the UCB and recipient but mismatched with the haploidentical donor in the haplo-versus-UCB or conventional graft-versus-host direction in the haplo-HSCT system. All the MHMAs were evaluated by a single physician and checked by a single nurse. Given that UCB HLA typing in China is based on HLA-A, -B and -DRB1, the MHMA in this study was dependent on these 3 pairs of loci. For example, HLA matchings were as follows:

Patient: HLA-A 0201, 3201; HLA-B 4001, 4403; HLA-DRB1 0701, 0901.
Donor: HLA-A 0201, 3001; HLA-B 4001, 1302; HLA-DRB1 0701, 0901.
UCB: HLA-A 02, 32; HLA-B 44, 75; HLA-DRB1 07, 09.

To estimate the number of MHMAs in the HSCT, we first identified the mutual antigens shared by UCB and recipient (ie, HLA-A 02, 32; HLA-B 44; and HLA-DRB1 07, 09). We then validated the mismatched mutual antigens in the direction of haplo-versus-UCB or conventional graft-versus-host. In the foregoing example, HLA-A 32 and HLA-B 44 were the only antigens fulfilling the requirement; thus, the number of MHMAs in this HSCT was 2.

HSCT Procedure

Details of the conditioning regimens, GVHD prophylaxis, and their withdrawal have been published previously (Figure S1) [28]. In brief, all patients received either a busulfan (Bu) -based or a total body irradiation (TBI)-based myeloablative conditioning regimen. Bu-based conditioning consisted of Bu .8 mg/kg every 6 hours i.v. on days -7 to -5, cyclophosphamide 1.8 g/m^2 i.v. on days -4 to -3, and Me-CCNU 250 mg/m^2 orally on day -4. TBI-based conditioning consisted of TBI at a dose of 200 cGy for 6 consecutive fractions on days -7 to -5, cyclophosphamide 1.8 g/m^2 i.v. on days -4 to -3, and Me-CCNU 250 mg/m^2 orally on day -4. Rabbit antithymocyte globulin (ATG)-Fresenius was infused at a total dose of 5 mg/kg on days -5 through -2. Before Bu or TBI administration, patients received a FLAG (fludarabine 30 mg/m^2 for 5 days, cytarabine 2.0 g/m^2 for 5 days, and granulocyte colony-stimulating factor 150 μg/day for 5 days) or CLAG (cladribine 5 mg/m^2 for 5 days, cytarabine 2.0 g/m^2 for 5 days, and granulocyte colony-stimulating factor 150 μg/day for 5 days) cytoreduction regimen to enhance the myeloablative effect. One of these 2 cytoreduction regimens was prescribed at the discretion of the attending physician. Cytoreduction chemotherapy was not recommended in patients with a performance score of <75.

GVHD prophylaxis consisted of mycophenolate mofetil, cyclosporine A, and a short course of methotrexate administered on days +1, +3, and +6 at a dose of 10 mg/m^2. Patients received bone marrow and peripheral blood stem cells from haploidentical donors on days 0 and 1, respectively. Notably, at least 4 hours before haploidentical bone marrow infusion on day 0, most patients received an infusion of UCB from a third-party donor at a unified cell dose of 1.0×10^7/kg total nucleated cells (TNCs). UCB infusion was omitted or withheld under the following conditions: unstable vital signs, allergic reaction, history of immune or drug-related pneumonia, and patient refusal.

After neutrophil recovery, bone marrow aspirations were repeated every 4 weeks for the first 6 months after transplantation, along with chimerism evaluation by short tandem repeat analysis, and then every 3 months thereafter until at least 1 year post-transplantation. Temporary bone marrow examination was also considered in case of suspicious disease progression. Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) viremias were assessed by a real-time PCR-based method twice weekly. Ganciclovir or foscarnet was delivered preemptively in patients who tested positive for CMV DNA viremia, whereas EBV viremia was treated with upfront rituximab, an anti-human CD20 monoclonal antibody. For patients with viremia refractory to conventional treatment, CMV- and/or EBV-specific cytotoxic lymphocyte infusions were considered.

Statistical Analysis

Data were collected from the institutional database and verified by the primary investigators and staff of the HSCT team. The data were checked for consistency and analyzed using Stata version 14 (StataCorp, College Station, TX). The last follow-up date was October 31, 2017. Descriptive analyses are reported as median and range. Continuous and categorical variables were analyzed using the Mann-Whitney test and chi-square test, respectively. Kaplan-Meier analyses were used to estimate PFS and OS, and the log-rank test was used to compare survival outcomes. The cumulative incidences of GVHD (competing risk of death in the absence of GVHD), relapse, and NRM were estimated by competing-risks analysis using the Fine-Gray method.

The influence of covariates on PFS and OS was determined using univariate and multivariate analyses via the Cox proportional hazards model. The Fine-Gray regression model was used as in a previous similar study of relapse and NRM [29]. For univariate analysis, the variables included recipient age, recipient/donor sex, diagnosis, cytogenetic risk classification at diagnosis, pre-HSCT status, interval from diagnosis to HSCT, bone marrow blast burden at HSCT, haplo-European Group for Blood and Marrow Transplantation (EBMT) score, type of conditioning regimen, and the presence of acute and chronic GVHD. Multivariate analyses of CMV and EBV reactivation, UCB infusion, mononucleated cell dose, CD34$^+$ cell dose, and CD3$^+$ cell dose were also performed. The confidence interval (CI) was reported at 95%, and a 2-sided P value $<.05$ was considered statistically significant.

RESULTS

Patient Characteristics

A total of 140 patients with primary refractory or relapsed refractory acute leukemia who underwent first haploidentical

allo-HSCT were enrolled in this study. Altogether, 97 patients receiving an additional UCB infusion were recruited into the cord-haplo group. The remaining 43 patients received conventional haplo-HSCT; 1 patient who died from infection before graft infusion was excluded. The characteristics of the enrolled patients are summarized in Table 1.

In the haplo-HSCT group, all 42 patients were exempted from UCB infusion owing mainly to ATG-related side effects, including hypoxia (n = 26; 61.9%), fever (n = 2; 4.8%), hypertension (n = 2; 4.8%), allergic reaction (n = 3; 7.1%), history of immune- or drug-related pneumonia (n = 2; 4.8%), or patient refusal (n = 7; 16.6%). However, all patients recovered from these conditions, and none of the adverse events was associated with NRM or disease relapse.

UCB Graft Characteristics and Number of MHMAs

HLA matching and MHMA information was independently reviewed by both a physician and a nurse. For UCB HLA matching, 9 units were 6/6 matched, 39 units were 5/6 matched, 43 units were 4/6 matched, and 3 units were 3/6 matched. Twelve episodes of UCB infusion-related adverse infusion events occurred within the first 24 hours after infusion in the cord-haplo cohort, including grade 1-2 hypertension (n = 45; 46.4%), grade 1-2 hypotension (n = 3; 3.1%), grade 1-2 sinus tachycardia (n = 2; 2.1%), grade 1-2 sinus bradycardia (n = 67; 69.1%), grades 1-2 dyspnea (n = 36; 37.1%), and grade 1-2 nausea and vomiting (n = 28; 28.9%) based on the Common Terminology Criteria for Adverse Events, version 4.0. No adverse infusion events of grade 3 or higher were observed. The MHMAs were categorized into 4 groups according to their numbers: N = 0 (n = 13; 13.4%), N = 1 (n = 27; 27.8%), N = 2 (n = 34; 35.1%), and N = 3 (n = 23; 23.7%).

Recovery of Hematopoiesis

The incidences and kinetics of neutrophil recovery were similar in the 2 groups. The cumulative incidence of neutrophil recovery on day +28 was 96.9% (95% CI, 90.7% to 99.0%) in the cord-haplo group and 95.2% (95% CI, 82.3% to 98.8%) in the haplo group ($P = .852$). The median time to engraftment was 16 days (interquartile range [IQR], 13 to 19 days) in the cord-haplo group and 15 days (IQR, 13 to 18 days) in the haplo group (Figure 1A).

The cumulative incidence of platelet recovery on day +90 was 87.6% (95% CI, 79.2% to 92.8%) in the cord-haplo group versus 81.0% (95% CI, 65.5% to 90.0%) in the haplo group ($P = .100$). The median time to engraftment in the 2 groups was 16 days (IQR, 12 to 24 days) and 19 days (IQR, 13 to 28 days), respectively (Figure 1B).

Chimerism Analysis

On evaluation day +28, all but 2 patients in the cord-haplo group (who died from relapse) achieved both CR and full haplo donor-dominant chimerism that was maintained until relapse. All haplo group patients achieved full haplo donor chimerism and CR. Only 7 patients underwent unscheduled bone marrow evaluation around day +14. One patient showed mixed chimerism indicating haplo donor- derived chimerism of 52%, which then switched to complete haplo donor- derived chimerism on day +28.

GVHD

The 100-day cumulative incidences of grade II-IV and grade III-IV acute GVHD were 50.5% (95% CI, 40.2% to 59.9%) and 28.9% (95% CI, 20.2% to 38.1%), respectively, in the cord-haplo group and 51.2% (95% CI, 35.2% to 65.2%) and 31.7% (95% CI, 18.3% to 46.0%), respectively, in the haplo group ($P = .683$ and .662, respectively) (Figure 2A and B). The 1-year cumulative incidences of overall and extensive chronic GVHD were 33.0% (95% CI, 23.9% to 42.4%) and 18.6% (95% CI, 11.6% to 26.9%), respectively, in the cord-haplo group and 35.7% (95% CI, 21.7% to 49.9%) and 16.7% (95% CI, 7.3% to 29.3%), respectively, in the haplo group ($P = .794$ and .976, respectively) (Figure 2C and D).

Viral Reactivation

The 100-day cumulative incidence of CMV viremia was 59.8% (95% CI, 49.4% to 68.8%) in the cord-haplo group versus 47.6% (95% CI, 32.1% to 61.6%) in the haplo group ($P = .334$) (Figure 3A). Only 4 patients experienced CMV disease, including 2 in the central nervous system.

The 100-day cumulative incidence of EBV viremia was 13.4% (95% CI, 7.5% to 21.0%) in the cord-haplo group and 9.5% (95% CI, 3.0% to 20.6%) in the haplo group ($P = .521$) (Figure 3B). Two patients developed post-transplantation lymphoproliferative disease and were treated successfully.

Survival Analysis

The overall HSCT outcomes after a median follow-up period of 24 months are illustrated in Figure 4. The 2-year cumulative incidence of relapse was 25.9% (95% CI, 17.3% to 35.3%) in the cord-haplo group and 53.2% (95% CI, 36.2% to 67.5%) in the haplo group ($P = .007$) (Figure 4A). The cumulative incidence of NRM at 2 years was 38.0% (95% CI, 27.2% to 48.7%) in the cord-haplo group and 24.6% (95% CI, 12.7% to 38.5%) in the haplo group ($P = .367$) (Figure 4B). The estimated 2-year OS was 35.5% (95% CI, 24.7% to 46.5%) in the cord-haplo group versus 22.7% (95% CI, 10.8% to 37.2%) in the haplo group ($P = .049$) (Figure 4C). The estimated 2-year PFS was 35.5% (95% CI, 24.9% to 46.3%) in the cord-haplo group and 17.9% (95% CI, 7.4%–32.1%) in the haplo group ($P = .049$) (Figure 4D). In general, cord-haplo HSCT yielded a remarkable improvement in PFS and relapse rates over haplo-HSCT in this study.

Mortality

The causes of mortality are summarized in Table 2. Disease recurrence was the primary cause of death in the haplo group, accounting for 67.7%, whereas NRM caused 57.1% of all deaths in the cord-haplo group ($P = .043$). The incidence of GVHD-related deaths was similar in the 2 groups ($P = .367$). Only 3 cases of early death before engraftment were observed, all due to infectious complications.

Identifying Predictive Factors for Relapse and PFS

We performed univariate and multivariate analyses to determine the independent factors that predict relapse and PFS (Tables S1 to S3). In the cord-haplo group, a haplo-EBMT score ≥ 5 (hazard ratio [HR], .40; 95% CI, .18 to .90; $P = .026$) and chronic GVHD (HR, .30; 95% CI, .11 to .85; $P = .024$) were associated with a lower relapse rate. However, none of the examined factors was significantly associated with superior PFS.

On multivariate analysis (Table S3), cord-haplo HSCT (HR, .44; 95% CI, .21 to .92; $P = .028$), age >12 years (HR, .36; 95% CI, .15 to .89; $P = .027$), and chronic GVHD (HR, .42; 95% CI, .21 to .84; $P = .013$) were independent factors associated with a reduced risk of relapse. Furthermore, cord-haplo HSCT (HR, .58; 95% CI, .35 to .96; $P = .033$), and chronic GVHD (HR, .63; 95% CI, .40 to 1.00; $P = .052$) were predictive of improved PFS; however, the latter findings were not statistically significant.

Table 1
Patient Characteristics

Characteristics	Cord-Haplo Group (n = 97)	Haplo Group (n = 42)	*P* Value
Age, yr, median (range)	26 (3-59)	26 (2-56)	.185
Sex, male/female, n (%)	64/33 (66.0/34.0)	25/17 (59.5/40.5)	.466
Diagnosis, n (%)			.747
AML	69 (71.1)	31 (73.8)	
Acute lymphoblastic leukemia	28 (28.9)	11 (26.2)	
Cytogenetic risk, n (%)			.194
Favorable	23 (23.7)	10 (23.8)	
Moderate	27 (27.8)	6 (14.3)	
Poor	47 (48.5)	26 (61.9)	
Bone marrow blasts at HSCT, %, n (%)			.117
5-20	25 (25.8)	5 (11.9)	
21-50	21 (21.6)	6 (14.3)	
51-80	19 (19.6)	13 (31.0)	
81-99	32 (33.0)	18 (42.9)	
CNS involvement/normal, n (%)	14/83 (14.4/85.6)	4/38 (9.5/90.5)	.585
EMD involvement/normal, n (%)	24/73 (24.7/75.3)	7/35 (16.7/83.3)	.294
Time between diagnosis and HSCT, n (%)			.243
≤12 mo	45 (46.4)	15 (35.7)	
>12 mo	52 (53.6)	27 (64.3)	
Response before HSCT, n (%)			.211
Primary refractory	36 (37.1)	11 (26.2)	
Relapsed refractory	61 (62.9)	31 (73.8)	
Haplo-EBMT score, n (%)			.333
2	1 (1.0)	0 (0)	
3	4 (4.1)	1 (2.4)	
4	22 (22.7)	5 (11.9)	
5	32 (33.0)	18 (42.9)	
6	25 (25.8)	16 (38.1)	
7	12 (12.4)	2 (4.8)	
8	1 (1.0)	0 (0)	
Conditioning regimen, n (%)			.426
TBI-based	65 (67.0)	31 (73.8)	
Bu-based	32 (33.0)	11 (26.2)	
Cytoreduction chemotherapy, n (%)			
Used	67 (69.1)	27 (64.3)	.580
Not used	30 (30.9)	15 (35.7)	
Donors-recipients, n (%)			.937
Paternal	41 (42.3)	18 (42.9)	
Maternal	12 (12.4)	4 (9.5)	
Child	13 (13.4)	6 (14.3)	
Sibling	27 (27.8)	11 (26.2)	
Second-degree	4 (4.1)	3 (7.1)	
HLA matching, n (%)			.519
3/6	76 (78.4)	36 (85.7)	
4/6	14 (14.4)	3 (7.1)	
5/6	6 (6.2)	2 (4.8)	
6/6	1 (1.0)	1 (2.4)	
MNCs, 10^8/kg, median (range)	9.12 (7.44-24.40)	9.20 (8.21-10.86)	.050
CD34$^+$ cells, 10^6/kg. median (range)	3.27 (.80-8.04)	3.49 (.88-8.25)	.806
CD3$^+$ cells, 10^8/kg, median (range)	1.71 (.61-9.91)	2.27 (.64-11.32)	.196

CNS indicates central nervous system; EMD, extramedullary disease; MNC, mononucleated cell.

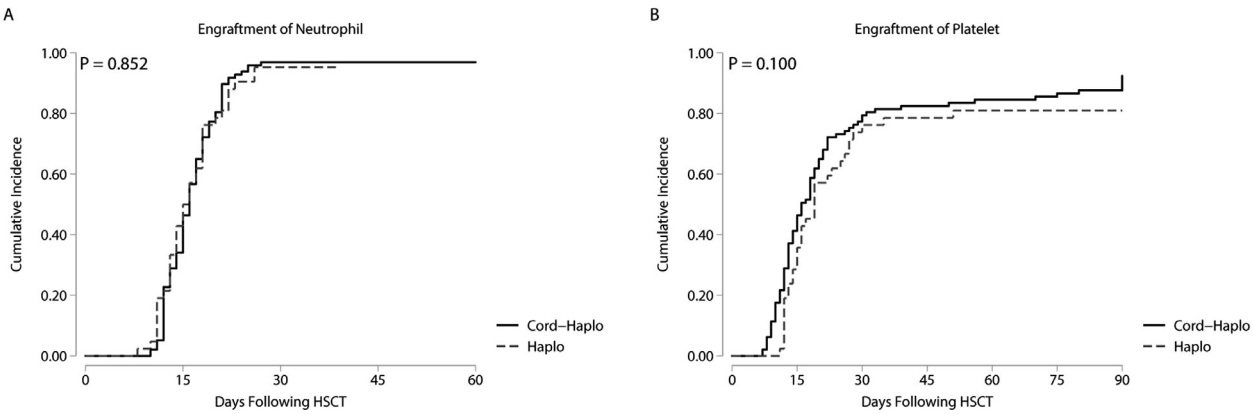

Figure 1. Neutrophil and platelet engraftment. (A) Neutrophils. (B) Platelets.

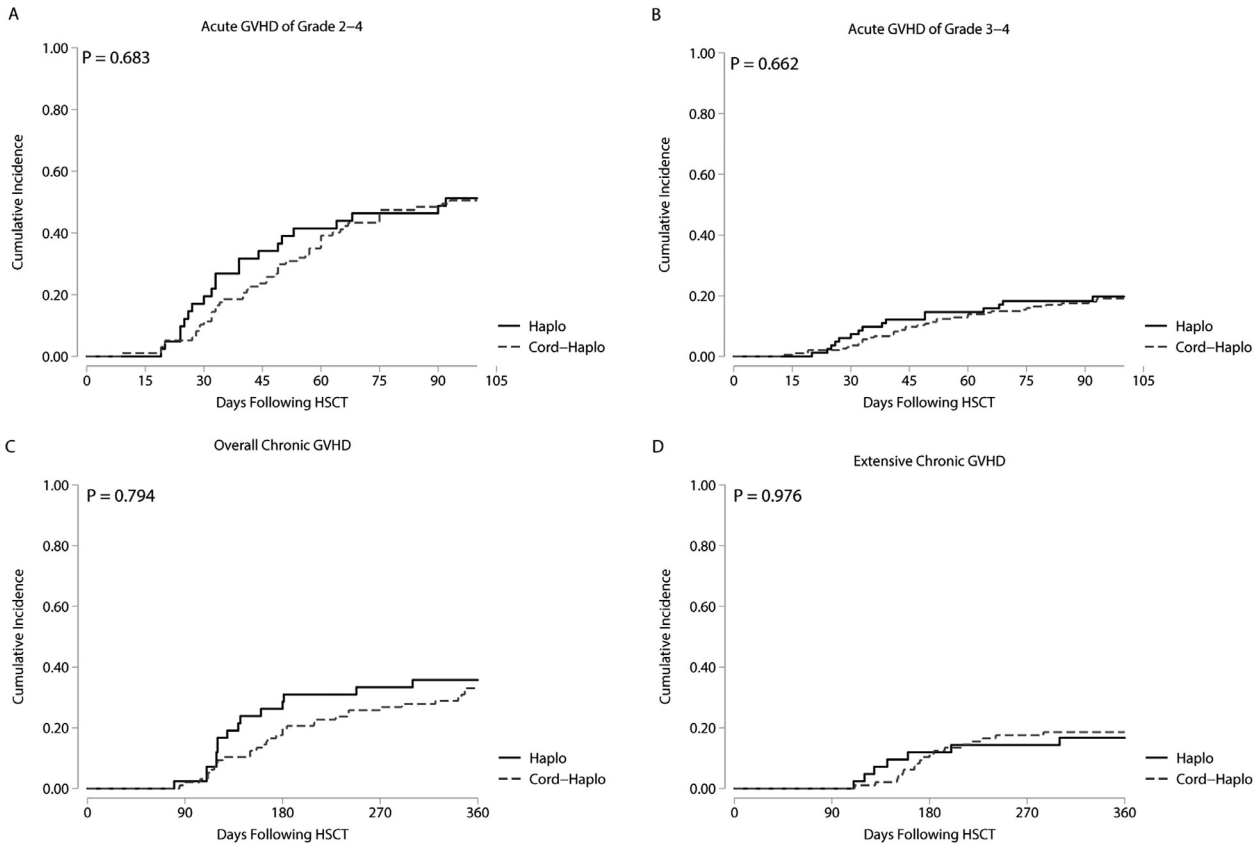

Figure 2. Cumulative incidence of GVHD. (A) Grade II-IV acute GVHD. (B) Grade III-IV acute GVHD. (C) Overall chronic GVHD. (D) Extensive chronic GVHD.

MHMA Influences HSCT Outcomes

The number of MHMAs influenced both relapse and NRM in patients of the cord-haplo group. The NRM gradually increased as the number of MHMAs increased, while the relapse rates decreased (Table 3). Of note, relapse rates were comparable in the haplo group and the subgroup of cord-haplo patients with no MHMAs (HR, 1.01; 95% CI .41 to 2.45; $P = .985$). The subgroup of patients with 1 MHMA had the most favorable PFS rate (41.8%).

DISCUSSION

In this study, we treated patients with refractory acute leukemias by cord-haplo HSCT (also known as the CHINA HSCT protocol), which involves the usual haplo-HSCT procedure plus a low-dose UCB infusion. We assessed the characteristics and therapeutic effects of this treatment and found a significantly enhanced GVL effect and reduced relapse, along with a GVG response, in the cord-haplo group, consistent with the findings of previous dUCBT studies [20,21].

We postulate that the superior disease control with cord-haplo HSCT is attributable to an enhanced GVL effect, a phenomenon discovered by Wagner et al [21] in a 2009 study of dUCBT. The authors speculated that the increased alloreactivity may be induced by the graft-graft interaction between the 2 UCB units, which may be responsible for the apparent reduced risk of relapse following dUCBT. Consistent with that study, cord-haplo HSCT in our patients with refractory acute leukemias manifested a GVG response, resulting in

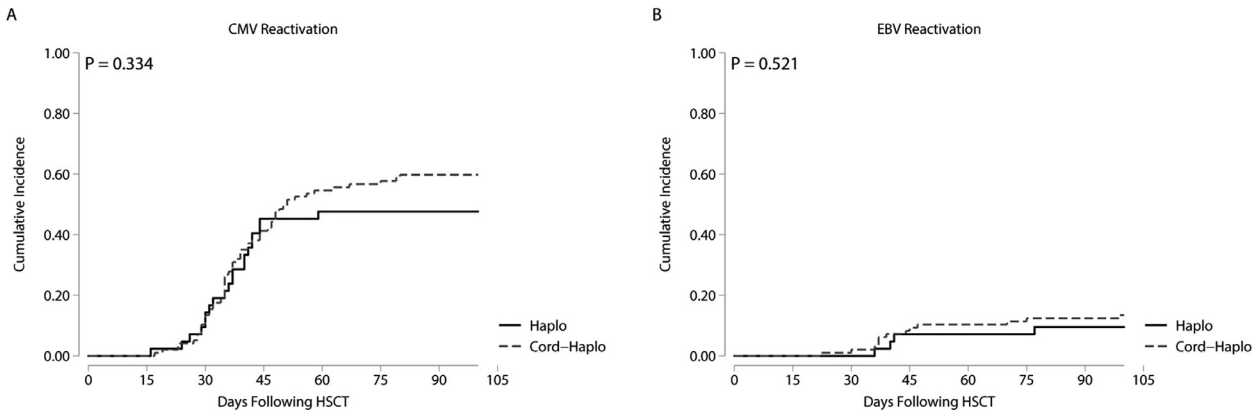

Figure 3. Cumulative incidence of CMV (A) and EBV (B) reactivation within 100 days following transplantation.

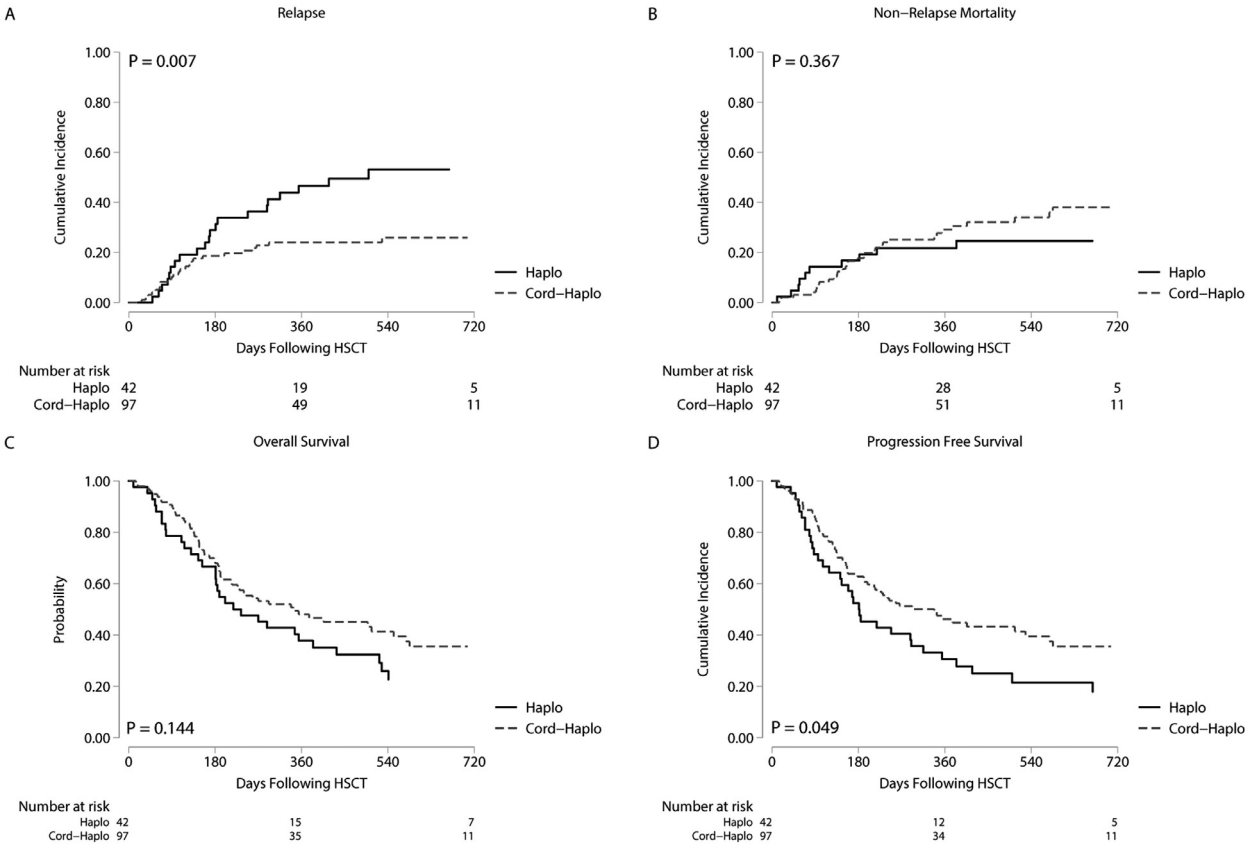

Figure 4. Two-year major outcomes of transplantation. (A) Cumulative incidence of relapse. (B) Cumulative incidence of NRM. (C) Probability of OS. (D) Probability of PFS.

significantly reduced relapse rates. Notably, previous analyses seldom focused on HLA matching between the "loser" unit and the recipient [30]. Overall, the MHMA algorithm should be helpful in predicting whether higher-degree HLA mismatching improves outcomes in terms of relapse and NRM rates [18,20].

In our cohort, the number of MHMAs was associated with relapse rate, and we postulate that haplo donor-derived, UCB-specific T cells may be responsible for the apparent reduced risk of relapse following cord-haplo HSCT. Previous studies have shown that rejected graft-specific $CD4^+$ and $CD8^+$ T cells derived from the "winner" UCB can recognize leukemic cells in dUCBT studies [7,8,12,23]. In addition, mismatch of HLA-DPB1 between the "winner" UCB and the recipient likely would reduce the risk of relapse in different patterns of HSCT [22,31,32]. The mechanism and effector cell population(s) remain unclear, however, requiring further laboratory research. However, patients in the subgroup with no MHMAs had a similar risk of disease recurrence as those who underwent conventional haplo-HSCT. This not only demonstrates an association between the GVL effect and MHMA number, but also alludes to a possible role of other mechanisms in inducing GVL when using our cord-haplo protocol [33-38].

Table 2
Causes of Death

Cause of Death	Cord-Haplo Group (n = 56/97), n (%)	Haplo Group (n = 31/42), n (%)	*P* Value
Relapse	24 (42.9)	21 (67.7)	.043
NRM	32 (57.1)	10 (32.3)	
DAH	2 (3.6)	1 (3.2)	1.000
GVHD	15 (26.8)	3 (9.7)	.367
Infection	14 (25.0)	4 (12.9)	.765
TMA	1 (1.8)	1 (3.2)	.474
Other/unknown	0 (.0)	1 (3.2)	.273
Total	56 (100)	31 (99.9)	

DAH indicates diffuse alveolar hemorrhage; TMA, thrombotic microangiopathy.

The GVG response did not increase the risk of GVHD in our patients who underwent cord-haplo HSCT. This finding differs from that in a previous study that found higher incidences of both acute and chronic GVHD in patients who underwent dUCBT compared with those who underwent single UCBT [21]. However, previous studies also have shown a significantly lower incidence of GVHD with dUCBT than with single UCBT [39], and also with haplo-cord HSCT than with haplo-HSCT and post-transplantation cyclophosphamide [16]. Moreover, another study using a similar protocol treating myelodysplasia syndrome found a lower incidence of GVHD than seen with HSCT from identical sibling donors (particularly the acute form) [40]. Similar to the foregoing conclusions, we believe that a disturbance in the balance between the protective role of regulatory T cells in UCB and the detrimental effect of the GVG response might be responsible for our observations. Furthermore, given that our cohort of patients had refractory acute leukemia, it remains to be further investigated whether advanced disease stage affects the risk of GVHD when using our protocol or those of other aforementioned platforms.

Although stable UCB donor chimerism predominance has been successfully achieved with conventional haplo-cord HSCT, we chose haploidentical donors for ultimate chimerism predominance, because post-transplantation donor lymphocyte infusion when using UCB is problematic. Thus,

Table 3
Comparison of Outcomes in the Cord-Haplo and Haplo Groups

MHMA	Relapse			NRM			PFS		
	% (95% CI)	HR (95% CI)	*P*	% (95% CI)	HR (95% CI)	*P*	% (95% CI)	HR (95% CI)	*P*
Haplo	53.2 (36.2-67.5)	1		24.6 (12.7-38.5)	1		17.9 (7.4-32.1)	1	
Cord-Haplo									
0	46.2 (19.2-69.6)	1.01 (.41-2.45)	.985	23.1 (3.2-53.4)	.61 (.13-2.73)	.515	30.8 (6.5-60.2)	.75 (.34-1.62)	.461
1	28.3 (12.1-47.1)	.46 (.20-1.08)	.075	29.9 (12.6-49.6)	1.00 (.38-2.63)	.996	41.8 (20.7-61.6)	.53 (.29-.98)	.041
2	20.7 (9.1-35.6)	.38 (.16-.92)	.031	43.6 (26.0-59.9)	2.02 (.86-4.73)	.104	35.7 (19.5-52.2)	.83 (.47-1.41)	.467
3	17.9 (5.6-35.8)	.29 (.10-.86)	.025	45.0 (22.6-65.1)	1.62 (.67-3.92)	.288	35.2 (15.8-55.5)	.58 (.31-1.09)	.090

guaranteeing the stability and reliability of this predominance is crucial, especially in those recipients with a high risk of post-HSCT relapse. Non-in vitro T cell depletion for haploidentical grafts and infusion of TNCs at low doses are the key factors for haplo donor chimerism dominance in cord-haplo HSCT. Several factors were identified as correlated with the "winner" graft, including the number and viability of $CD34^+$ cells, HLA matching, and $CD3^+$ cells [14,17,41]. Based on these data, we hypothesized that the number of infused TNCs would predict the "winner" graft. Moreover, as described previously [11], in conventional haplo-cord HSCT, physicians deliver the haplo donor-derived grafts with a restricted number of $CD3^+$ cells with T cell depletion in vitro to promote the UCB engraftment. We thus reasoned that the restricted number of T cells in haplo donor grafts was attributable to the reliability of UCB predominance. However, in view of the limited studies on the correlations between predominance and HLA typing, further investigations are warranted.

Our study has several limitations. First, as a retrospective analysis, it may carry an inherent selection bias. Second, the enrollment of subjects who withheld UCB infusion owing to adverse events into the control group might have interfered with the reliability of the results. Although 80% of the adverse events were ATG-related (ie, hypoxia, fever, hypertension, and allergic reaction), which are believed to have relatively mild effects on outcomes, administering ATG earlier than day 0 should be considered, to avoid the resultant adverse reactions and blunting of the GVL effect. Finally, subgroup analysis stratified by other factors, such as donor lymphocyte infusion and chronic GVHD rather than MHMA, was not feasible given the small sample size of each group. Thus, our main conclusions require validation in further independent and more extensive studies.

In conclusion, our modified cord-haplo HSCT protocol relies on a GVG response that has shown an enhanced GVL effect and reduced relapse rates. Our findings may encourage induction of the GVL effect by considering internal HLA matching between the 2 grafts. Moreover, MHMAs illustrate a potential mechanism for enhancing the GVL effect by triggered a GVG response.

ACKNOWLEDGMENTS

The authors thank all the patients and their families for participating in the study. The authors are grateful to the nurses and staff from the Transplantation Unit in China Aerospace Central Hospital for their dedication and contributions to making this work possible, as well as staff at the Beijing, Shandong and Tianjin UCB banks for their technical assistance.

Financial disclosure: This work was supported by the China Capital Characteristic Clinic Project (Grant Z171100001017103).

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: L.Y. analyzed data and wrote the manuscript; W.W. and J.G., managed patients and contributed data collection; Z.W. and W.Z. selected the donors, analyzed data, and contributed to manuscript preparation; H.C., X.F., and Y.Y. enrolled patients, oversaw the study, and contributed to data analysis and manuscript preparation; T.Z provided scientific and technical input in the preparation of cellular products and laboratory work; and J.W. designed and supervised the study.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.bbmt.2018.09.002.

REFERENCES

1. Gyurkocza B, Lazarus HM, Giralt S. Allogeneic hematopoietic cell transplantation in patients with AML not achieving remission: potentially curative therapy. *Bone Marrow Transplant*. 2017;52:1083–1090.
2. Schmid C, Schleuning M, Schwerdtfeger R, et al. Long-term survival in refractory acute myeloid leukemia after sequential treatment with chemotherapy and reduced-intensity conditioning for allogeneic stem cell transplantation. *Blood*. 2006;108:1092–1099.
3. Yan CH, Wang Y, Wang JZ, et al. Minimal residual disease- and graft-vs.-host disease-guided multiple consolidation chemotherapy and donor lymphocyte infusion prevent second acute leukemia relapse after allotransplant. *J Hematol Oncol*. 2016;9:87.
4. Baron F, Nagler A. Novel strategies for improving hematopoietic reconstruction after allogeneic hematopoietic stem cell transplantation or intensive chemotherapy. *Expert Opin Biol Ther*. 2017;17:163–174.
5. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *J Clin Oncol*. 2003;21:4642–4649.
6. Yoo SH, Koh Y, Kim DY, et al. Salvage therapy for acute chemorefractory leukemia by allogeneic stem cell transplantation: the Korean experience. *Ann Hematol*. 2017;96:605–615.
7. Gutman JA, Turtle CJ, Manley TJ, et al. Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8+ T-cell response against the nonengrafted unit. *Blood*. 2010;115:757–765.
8. Lamers CH, Wijers R, van Bergen CA, et al. CD4+ T-cell alloreactivity toward mismatched HLA class II alleles early after double umbilical cord blood transplantation. *Blood*. 2016;128:2165–2174.
9. Michel G, Galambrun C, Sirvent A, et al. Single- vs double-unit cord blood transplantation for children and young adults with acute leukemia or myelodysplastic syndrome. *Blood*. 2016;127:3450–3457.
10. van Besien K, Hari P, Zhang MJ, et al. Reduced intensity haplo plus single cord transplant compared to double cord transplant: improved engraftment and graft-versus-host disease-free, relapse-free survival. *Haematologica*. 2016;101:634–643.
11. van Besien K, Childs R. Haploidentical cord transplantation—the best of both worlds. *Semin Hematol*. 2016;53:257–266.
12. Cornelissen JJ, Kalin B, Lamers CHJ. Graft predominance after double umbilical cord blood transplantation: a review. *Stem Cell Investig*. 2017;4:47.
13. Rettman P, Willem C, Volteau C, et al. Impact of graft-versus-graft natural killer cell alloreactivity on single unit dominance after double umbilical cord blood transplantation. *Transplantation*. 2017;101:2092–2101.
14. Kwon M, Bautista G, Balsalobre P, et al. Haplo-cord transplantation using CD34+ cells from a third-party donor to speed engraftment in high-risk patients with hematologic disorders. *Biol Blood Marrow Transplant*. 2014;20:2015–2022.
15. Lindemans CA, Te Boome LC, Admiraal R, et al. Sufficient immunosuppression with thymoglobulin is essential for a successful haplo-myeloid bridge

in haploidentical-cord blood transplantation. *Biol Blood Marrow Transplant*. 2015;21:1839–1845.
16. Kwon M, Bautista G, Balsalobre P, et al. Haplo-Cord transplantation compared to haploidentical transplantation with post-transplant cyclophosphamide in patients with AML. *Bone Marrow Transplant*. 2017;52:1138–1143.
17. van Besien K, Koshy N, Gergis U, et al. Haplo-cord transplant: HLA-matching determines graft dominance. *Leuk Lymphoma*. 2017;58:1512–1514.
18. Tozatto-Maio K, Giannotti F, Labopin M, et al. Cord blood unit dominance analysis and effect of the winning unit on outcomes after double unit umbilical cord blood transplantation in adults with acute leukaemia: a retrospective study on behalf of Eurocord, the Cord Blood Committee of Cellular Therapy, Immunobiology Working Party and the Acute Leukaemia Working Party of the European Group for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2018;24:1657–1663.
19. Eapen M, Kurtzberg J, Zhang MJ, et al. Umbilical cord blood transplantation in children with acute leukemia: impact of conditioning on transplantation outcomes. *Biol Blood Marrow Transplant*. 2017;23:1714–1721.
20. 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;22:487–492.
21. Verneris MR, Brunstein CG, Barker J, et al. Relapse risk after umbilical cord blood transplantation: enhanced graft-versus-leukemia effect in recipients of 2 units. *Blood*. 2009;114:4293–4299.
22. Yabe T, Azuma F, Kashiwase K, et al. HLA-DPB1 mismatch induces a graft-versus-leukemia effect without severe acute GVHD after single-unit umbilical cord blood transplantation. *Leukemia*. 2018;32:168–175.
23. Milano F, Heimfeld S, Gooley T, Jinneman J, Nicoud I, Delaney C. Correlation of infused CD3+CD8+ cells with single-donor dominance after double-unit cord blood transplantation. *Biol Blood Marrow Transplant*. 2013;19:156–160.
24. Kurtzberg J, Prasad VK, Carter SL, et al. Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies. *Blood*. 2008;112:4318–4327.
25. Hong M, He G. 2016 revision to the WHO Classification of acute myeloid leukemia. *J Transl Int Med*. 2017;5:69–71.
26. Aisa Y, Mori T, Kato J, et al. Validation of NIH consensus criteria for diagnosis and severity-grading of chronic graft-versus-host disease. *Int J Hematol*. 2013;97:263–271.
27. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825–828.
28. Wang J, Yuan L, Cheng H, et al. Salvaged allogeneic hematopoietic stem cell transplantation for pediatric chemotherapy-refractory acute leukemia. *Oncotarget*. 2017;9:3143–3159.
29. Zhang X, Zhang MJ, Fine J. A proportional hazards regression model for the subdistribution with right-censored and left-truncated competing risks data. *Stat Med*. 2011;30:1933–1951.
30. Brunstein C, Zhang MJ, Barker J, et al. The effect of inter-unit HLA matching in double umbilical cord blood transplantation for acute leukemia. *Haematologica*. 2017;102:941–947.
31. Moyer AM, Hashmi SK, Kroning CM, et al. Clinical outcomes of HLA-DPB1 mismatches in 10/10 HLA-matched unrelated donor-recipient pairs undergoing allogeneic stem cell transplant. *Eur J Haematol*. 2017;99:275–282.
32. Fleischhauer K, Shaw BE. HLA-DP in unrelated hematopoietic cell transplantation revisited: challenges and opportunities. *Blood*. 2017;130:1089–1096.
33. van Rood JJ, Scaradavou A, Stevens CE. Indirect evidence that maternal microchimerism in cord blood mediates a graft-versus-leukemia effect in cord blood transplantation. *Proc Natl Acad Sci U S A*. 2012;109:2509–2514.
34. Willemze R, Rodrigues CA, Labopin M, et al. KIR-ligand incompatibility in the graft-versus-host direction improves outcomes after umbilical cord blood transplantation for acute leukemia. *Leukemia*. 2009;23:492–500.
35. van Rood JJ, Stevens CE, Smits J, Carrier C, Carpenter C, Scaradavou A. Reexposure of cord blood to noninherited maternal HLA antigens improves transplant outcome in hematological malignancies. *Proc Natl Acad Sci U S A*. 2009;106:19952–19957.
36. Kloosterboer FM, van Luxemburg-Heijs SA, Willemze R, Falkenburg JH. Umbilical cord blood-naive T cells but not adult blood-naive T cells require HLA class II on antigen-presenting cells for allo-immune activation. *Hum Immunol*. 2004;65:328–339.
37. Zhao XY, Chang YJ, Xu LP, et al. HLA and KIR genotyping correlates with relapse after T cell-replete haploidentical transplantation in chronic myeloid leukaemia patients. *Br J Cancer*. 2014;111:1080–1088.
38. Mancusi A, Ruggeri L, Urbani E, et al. Haploidentical hematopoietic transplantation from KIR ligand-mismatched donors with activating KIRs reduces nonrelapse mortality. *Blood*. 2015;125:3173–3182.
39. Sanz J, Kwon M, Bautista G, et al. Single umbilical cord blood with or without $CD34^+$ cells from a third-party donor in adults with leukemia. *Blood Adv*. 2017;1:1047–1055.
40. Ke P, Bao XB, Hu XH, et al. Myeloablative conditioning regimens with combined of haploidentical and cord blood transplantation for myelodysplastic syndrome patients. *Bone Marrow Transplant*. 2018;53:162–168.
41. Choe HK, van Besien K. Against the odds: haplo-cord grafts protect from GvHD and relapse. *Bone Marrow Transplant*. 2017;52:1590–1591.