Biol Blood Marrow Transplant 25 (2019) 764–770

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

# Reduced-Toxicity Myeloablative Conditioning Consisting of Fludarabine/Busulfan/Low-Dose Total Body Irradiation/Granulocyte Colony-Stimulating Factor–Combined Cytarabine in Single Cord Blood Transplantation for Elderly Patients with Nonremission Myeloid Malignancies

Takaaki Konuma*, Seiko Kato, Masamichi Isobe, Mai Mizusawa, Maki Oiwa-Monna, Satoshi Takahashi, Arinobu Tojo

*Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan*

*Article history:*
Received 8 November 2018
Accepted 5 December 2018

*Key Words:*
Cord blood transplantation
Busulfan
Granulocyte colony-stimulating factor
Myeloid leukemia
Nonremission
Elderly

A B S T R A C T

The optimal intensity of a conditioning regimen might be dependent on not only age and comorbidities but also disease activity and the type of graft source. We evaluated the outcome of unrelated single cord blood transplantation (CBT) using a conditioning regimen of fludarabine 180 mg/m$^2$, i.v. busulfan 9.6 mg/kg, 4 Gy total body irradiation, granulocyte colony-stimulating factor–combined high-dose cytarabine (12 g/m$^2$) in 23 elderly patients (median, 64 years) with nonremission myeloid malignancies between 2013 and 2018 in our institution. All but 1 patient achieved neutrophil engraftment at a median of 23.5 days (range, 18 to 50). With a median follow-up of 28 months, the probabilities of overall survival (OS), disease-free survival (DFS), and cumulative incidence of relapse at 2 years were 62%, 52%, and 26%, respectively. The cumulative incidences of nonrelapse mortality at 100 days and 2 years were 9% and 22%, respectively. In the univariable analysis a higher proportion of blasts in bone marrow and in peripheral blood and a monosomal or complex karyotype were significantly associated with inferior OS and DFS. Poor cytogenetics were significantly associated with inferior DFS and increased relapse incidence. These data demonstrate that this reduced-toxicity myeloablative conditioning regimen was tolerable and effective in terms of engraftment, relapse, and survival in single CBT for elderly patients with nonremission myeloid malignancies.



## INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is the only potential curative therapy for patients with nonremission myeloid malignancies [1-3]. Cord blood transplantation (CBT) is an acceptable alternative donor source for patients without HLA-matched related or unrelated donors in need of allogeneic HCT [4-9]. Because of its rapid availability, CBT can enable patients with nonremission myeloid malignancies to perform allogeneic HCT at the optimal time. In addition, because recipient and donor ages are usually similar and the prevalence of various comorbidities increases with age, it is difficult to find suitable HLA-matched sibling donors for elderly patients. However, the use of less stringent HLA matching in CBT could increase the likelihood of identifying an appropriate donor source for elderly patients. Therefore, the use of cord blood (CB) expands the application of allogeneic HCT in elderly patients with nonremission myeloid malignancies.

The purpose of a conditioning regimen is both to eradicate the residual leukemic cells and to provide sufficient immunosuppression to facilitate donor engraftment. Therefore, the optimal intensity of conditioning regimen might be dependent of not only age and comorbidities but also disease activity and the type of graft source. Recently, reduced-toxicity myeloablative conditioning (MAC) based on fludarabine and a myeloablative dose of i.v. busulfan (FluBu4) for allogeneic bone marrow or peripheral blood stem cell transplantation (BMT/PBSCT) from related and unrelated donors has been shown to be safe and effective in elderly patients with myeloid malignancies [10,11]. However, several studies showed that FluBu4 for CBT was associated with a high rate of graft failure and relapse, although all patients received double unit grafts [12,13]. Meanwhile, we previously reported that an intensified MAC regimen consisting of the addition of granulocyte colony-stimulating factor (G-CSF)–combined high-dose cytosine

Financial disclosure: See Acknowledgments on page xxxx.

* Correspondence and reprint requests: Takaaki Konuma, MD, PhD, Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
*E-mail address:* tkonuma@ims.u-tokyo.ac.jp (T. Konuma).

https://doi.org/10.1016/j.bbmt.2018.12.004
1083-8791/

arabinoside (Ara-C) to a total body irradiation (TBI) regimen of 12 Gy and cyclophosphamide (CY) resulted in a significantly higher rate of neutrophil engraftment and better survival without a significant increase in toxicity in CBT for adult patients younger than 55 years with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) [14,15]. In an effort to improve engraftment and survival, we designed a reduced-toxicity MAC regimen consisting of fludarabine 180 mg/m$^2$, busulfan 9.6 mg/kg, 4 Gy TBI, and G-CSF–combined Ara-C for elderly patients with nonremission myeloid malignancies in single CBT.

## METHODS

### *Patients and CB Unit Selection Procedure*

This pilot study included 23 consecutive elderly patients who received single CBT for nonremission myeloid malignancies at the Institute of Medical Science, University of Tokyo between November 2013 and July 2018. In our institution CB from an unrelated donor is an alternative first-line treatment option for adult patients without a matched sibling donor, unless a patient has anti-HLA antibodies against donor-specific antigens. CB units were obtained from the Japan Cord Blood Bank Network. Preferred CB units were ≤2 locus mismatch points based on antigen level HLA-A, -B, and -DR typing and contained a minimal total nuclear cell count of approximately $1.5 \times 10^7$/kg or a minimal CD34$^+$ cell count of approximately $.5 \times 10^5$/kg before cryopreservation [16].

The institutional review board of the Institute of Medical Science, University of Tokyo approved this study. This study was conducted in accordance with the Declaration of Helsinki.

### *Conditioning Regimen and Supportive Care*

All patients received 4 Gy TBI in 2 divided fractions on day −8, i.v. busulfan (total dose 9.6 mg/kg) on days −7 to −5, fludarabine (total dose 180 mg/m$^2$) on days −7 to −2, and Ara-C on days −4 and −2 (total dose 12 g/m$^2$, and 3 g/m$^2$ every 12 hours for 2 days) with 5 $\mu$g/kg G-CSF (lenograstim) from 12 hours before the first dose of Ara-C to the end of Ara-C dosing. All patients received cyclosporine (3 mg/kg/day, from day−1) and mycophenolate mofetil (30 mg/kg/day from days 0 to +27) as graft-versus-host disease (GVHD) prophylaxis. All patients received oral quinolones and azoles from day −14 for prophylaxis of bacterial and fungal infections. All patients also received oral trimethoprim-sulfamethoxazole from days −21 to −3 or aerosolized pentamidine before starting the conditioning regimen for prevention of pneumocystis pneumonia and oral acyclovir from day −3 for prophylaxis of herpes simplex and varicella-zoster virus reactivation. For the prevention of veno-occlusive disease continuous infusions of low-dose heparin (100 units/kg/day) and oral ursodeoxycholate acid were administered for all patients from day −9. For seizure prevention during high-dose busulfan, sodium valproate or phenytoin was orally administered for all patients from days −9 to −3.

### *Endpoints and Ddefinitions*

The study endpoints were overall survival (OS), disease-free survival (DFS), relapse, nonrelapse mortality (NRM), engraftment, and GVHD. OS was defined as the time between CBT and death or last contact. DFS was defined as the time between CBT and relapse, death, or last contact. Relapse was defined as morphologic evidence of disease. NRM was defined as death during remission. Neutrophil engraftment was defined as the first of 3 consecutive days during which the absolute neutrophil count was at least $.5 \times 10^9$/L. Platelet engraftment was achieved on the first of 3 days when the platelet count was higher than $20 \times 10^9$/L without transfusion support.

Chimerism analysis was performed using fluorescence in situ hybridization for sex-mismatched CBT or short tandem repeats for sex-matched CBT. Both acute GVHD and chronic GVHD were graded according to previously published criteria [17,18]. Regimen-related toxicity was evaluated according to the National Cancer Institute-Common Terminology Criteria for Adverse Events version 3.0 from 0 to 28 days after CBT.

The diagnoses of AML, MDS, and chronic myelomonocytic leukemia (CMML) were made according to the World Health Organization classification. Myeloid malignancies not in remission were defined as more than 5% blasts in the bone marrow (BM), or circulating blasts in peripheral blood (PB). The cytogenetic risk groups were classified according to the National Comprehensive Cancer Network criteria for AML [19] and International Prognostic Scoring System criteria for MDS and CMML [20]. Monosomal karyotype was defined as either ≥2 autosomal monosomies or 1 autosomal monosomy with other structural abnormalities [21]. Complex karyotype was defined as more than 3 clonal chromosomal abnormalities [19]. The pre-existing comorbidities were assessed according to the HCT-specific comorbidity index (HCT-CI) [22].

### *Statistical Analysis*

The probabilities of OS and DFS were estimated according to the Kaplan-Meier method, and the groups were compared using the log-rank test. The probabilities of relapse, NRM, neutrophil and platelet engraftment, and GVHD were estimated based on a cumulative incidence method to accommodate competing risks, and groups were compared using Gray's test. For relapse NRM was a competing event. In contrast, relapse was a competing event for NRM. For neutrophil and platelet engraftment death before day 28 was a competing event. For GVHD, relapse and death were competing events. Univariable analyses were performed using the following variables: age (<65 versus ≥65 years), sex (male versus female), HCT-CI score (0 to 2 versus ≥3), disease type (*de novo* AML versus AML secondary to MDS versus advanced MDS/CMML), cytogenetics (other than poor versus poor), monosomal karyotype (present versus absent), the numbers of chromosomal abnormalities (0 versus 1 to 2 versus 3 versus ≥4), complex karyotype (present versus absent), disease status at CBT (untreated versus primary induction failure/refractory relapse versus azacitidine failure), proportion of blasts in BM (<15% versus ≥15%), proportion of blasts in PB (<10% versus ≥10%), lactate dehydrogenase (no more than upper limit of normal versus more than upper limit of normal), CB total nucleated cell count (<2.5 versus ≥$2.5 \times 10^7$/kg), CB CD34$^+$ cell count (<1 versus ≥$1 \times 10^5$/kg), and HLA disparities (<2 versus ≥2). A multivariable analysis could not be performed because of the small sample size.

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphic user interface for R 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) [23]. $P < .05$ was considered to be significant.

## RESULTS

### *Characteristics of Patients and CB Units*

The characteristics of patients are listed in Table 1. The median age at CBT was 64 years (range, 57 to 69), and the median body weight was 52.2 kg (range, 38.4 to 73.8). An HCT-

**Table 1**
Patient Characteristics (N = 23)

| Characteristic | Value |
|---|---|
| Median age, yr (range) | 64 (57-69) |
| Median body weight, kg (range) | 52.2 (38.4-73.8) |
| Sex | |
| Male | 12 (52) |
| Female | 11 (47) |
| Recipients CMV serostatus | |
| Positive | 19 (82) |
| Negative | 4 (17) |
| HCT-CI score | |
| ≥3 | 5 (21) |
| Disease type | |
| *De novo* AML | 8 (34) |
| AML secondary to MDS | 6 (26) |
| MDS | 7 (30) |
| CMML | 2 (8) |
| Cytogenetics | |
| Poor | 9 (39) |
| Other than poor | 14 (61) |
| Monosomal karyotype | 5 (21) |
| Disease status at CBT | |
| Untreated | 9 (39) |
| Primary induction failure | 6 (26) |
| Refractory relapse | 2 (8) |
| Azacitidine failure | 6 (26) |
| Median BM blasts (range) | 15.7 (4.6-72.6) |
| Median PB blasts (range) | 12.5 (.3-90) |
| LDH value > ULN | 14 (60) |
| Median cryopreserved TNC, $\times 10^7$/kg (range) | 2.43 (1.73-4.20) |
| Median cryopreserved CD34$^+$ cells, $\times 10^5$/kg (range) | .95 (.48-2.08) |
| HLA disparity | |
| 0 | 2 (8) |
| 1 | 3 (13) |
| 2 | 17 (73) |
| 3 | 1 (4) |
| ABO incompatibility | |
| Match and minor mismatch | 11 (47) |
| Major and bidirectional mismatch | 12 (52) |
| Sex incompatibility | |
| Female donor to male recipient | 4 (17) |
| Others | 19 (82) |
| Median time from diagnosis to CBT | 15 (1-219) |

Values are n (%) unless otherwise defined. CMV indicates cytomegalovirus; LDH, lactate dehydrogenase; ULN, upper limit of normal; TNC, total nucleated cell.

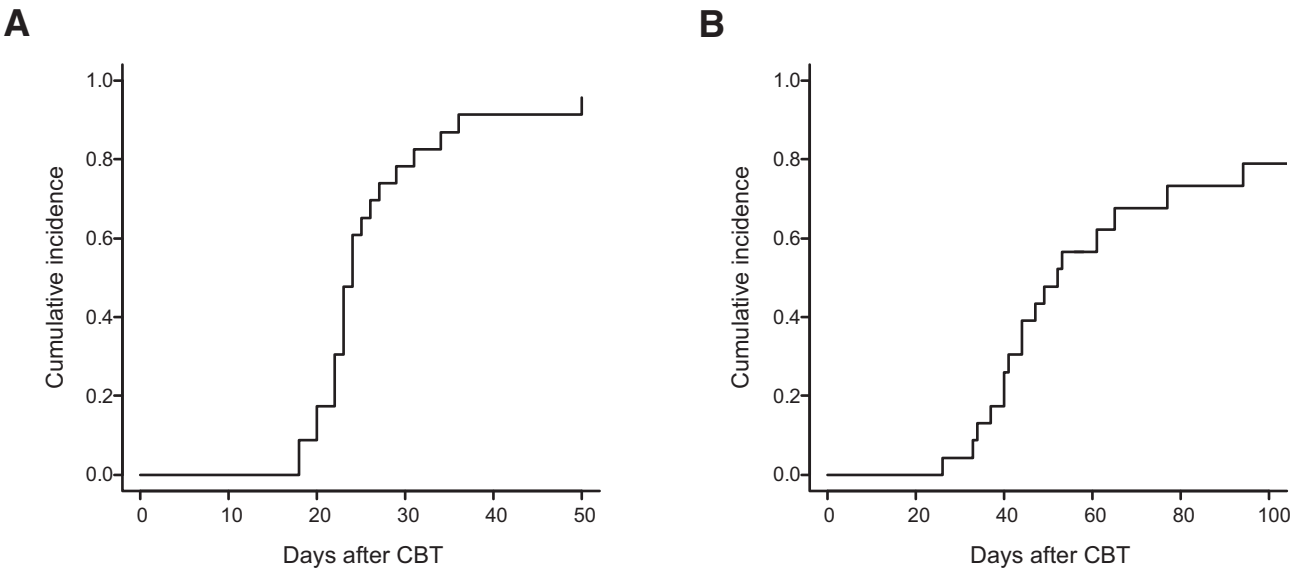


**Figure 1.** The cumulative incidences of neutrophil (A) and platelet (B) engraftment after CBT.

CI score ≥ 3 was observed in 5 patients (21%). Disease types were *de novo* AML in 8 patients, AML secondary to MDS in 6 patients, advanced MDS in 7 patients, and CMML in 2 patients. Among patients with AML, the disease status at CBT was primary induction failure in 6 patients, refractory relapse in 2 patients, and untreated status in 6 patients. Among patients with advanced MDS and CMML, the disease status at CBT was azacitidine failure in 6 patients and an untreated status in 2 patients. Among patients with MDS and AML secondary to MDS, the International Prognostic Scoring System at diagnosis was available for 9 of 13 patients: 6 were classified as intermediate-1 risk, 2 as intermediate-2 risk, and 1 as high risk. Nine patients (39%) had poor cytogenetics. The median proportions of blasts in BM and PB were 15.7% (range, 4.6% to 72.6%) and 12.5% (range, .3% to 90%) among 22 and 23 assessable patients, respectively. The median number of cryopreserved CB total nucleated cells was $2.43 \times 10^7$/kg (range, 1.73 to 4.20), and the median number of cryopreserved CB $CD34^+$ cells was $.95 \times 10^5$/kg (range, .48 to 2.08). The most common number of HLA mismatches between CB unit and recipient was found in 2 in 17 patients (73%). The median time from diagnosis to HCT was 15 months (range, 1 to 219).

**Table 2**
Regimen-Related Toxicity within 28 Days after CBT

| | Organ/System Grade | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Skin | | | | | |
| Hand-foot syndrome | 3 (13%) | 7 (30%) | 0 | 0 | 0 |
| Gastrointestinal | | | | | |
| Mucositis | 14 (60%) | 7 (30%) | 2 (8%) | 0 | 0 |
| Nausea/vomiting | 5 (21%) | 6 (26%) | 2 (8%) | 0 | 0 |
| Diarrhea | 3 (13%) | 3 (13%) | 17 (73%) | 0 | 0 |
| Hemorrhage | | | | | |
| Gastrointestinal/ urinary/pulmonary | 1 (4%) | 2 (8%) | 0 | 0 | 1 (4%) |
| Infection | | | | | |
| Febrile neutropenia | 0 | 0 | 21 (91%) | 0 | 0 |
| Liver | | | | | |
| Transaminase elevation | 6 (26%) | 0 | 1 (4%) | 0 | 0 |
| Hyperbilirubiniemia | 9 (39%) | 8 (34%) | 0 | 0 | 0 |
| Kidney | | | | | |
| Creatinine elevation | 2 (8%) | 3 (13%) | 0 | 0 | 0 |
| Neurology | | | | | |
| Seizure | — | 0 | 0 | 0 | 0 |

### *Engraftment and Chimerism*

Among the 23 patients, 1 patient died before engraftment on day 8 due to diffuse alveolar hemorrhage and 22 patients achieved neutrophil engraftment at a median of 23.5 days (range, 18 to 50). The cumulative incidence of neutrophil engraftment was 91% (95% confidence interval [CI], 63% to 98%) at day 42 after CBT (Figure 1A). Eighteen patients achieved platelet engraftment at a median of 45.5 days (range, 26 to 106). The cumulative incidence of platelet engraftment was 79% (95% CI, 51% to 92%) at day 100 after CBT (Figure 1B). Among 22 patients who achieved neutrophil engraftment, the median donor chimerism in whole BM was 98% (range, 91% to 100%) at the median time of the first BM analysis at 35 days (range, 22 to 91) after CBT.

### *Regimen-Related Toxicity and GVHD*

Regimen-related toxicities within 28 days after CBT are shown in Table 2. Grade 1 or 2 toxicities, including hand-foot syndrome (43%), mucositis (90%), nausea/vomiting (47%), diarrhea (26%), hemorrhage (12%), transaminase elevation (26%), hyperbilirubinemia (73%), and creatinine elevation (21%), frequently occurred in most patients. The most common grade 3 toxicities included febrile neutropenia in 21 patients (91%). The other grade 3 toxicities were diarrhea in 17 patients (73%), mucositis in 2 patients (8%), nausea/vomiting in 2 patients (8%), and transaminase elevation in 1 patient (4%). One patient died of diffuse alveolar hemorrhage on day 8 after CBT. Eleven patients (47%) experienced bloodstream infection.

The cumulative incidence of grades II to IV acute GVHD and grades III to IV acute GVHD was 87% (95% CI, 61% to 96%) and 22% (95% CI, 8% to 40%) at 100 days. Chronic GVHD developed in 11 of 20 assessable patients. According to the National Institutes of Health consensus criteria for chronic GVHD, 6 patients had mild type and 5 patients had moderate type.

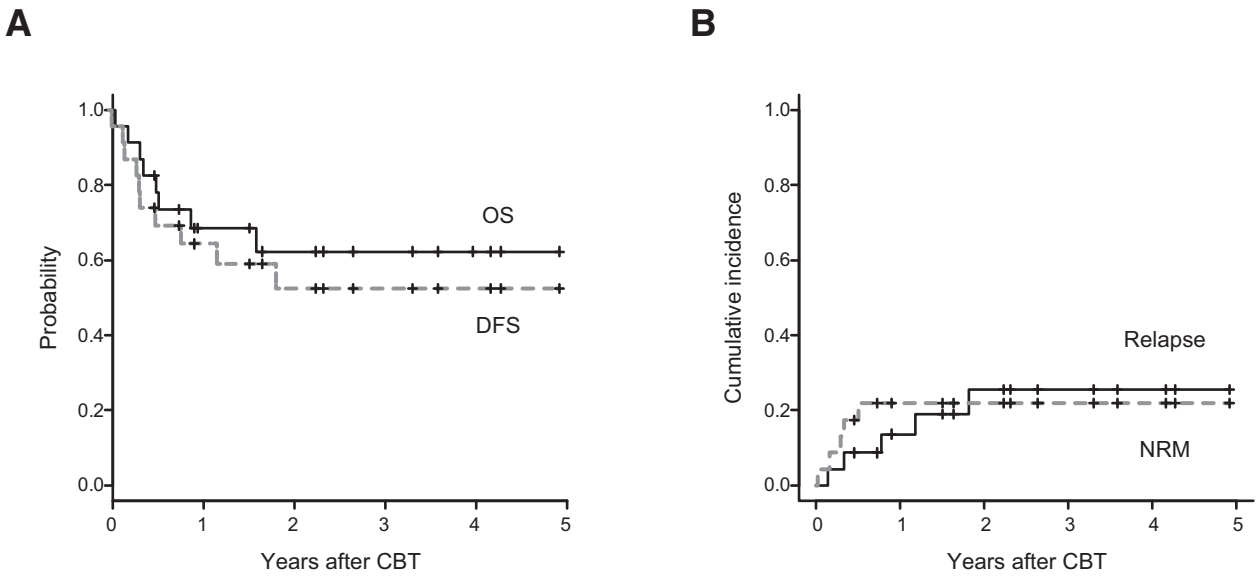


**Figure 2.** The probabilities of OS and DFS (A) and the cumulative incidences of relapse and NRM (B) after CBT.

***Survival, Relapse, NRM, and Cause of Death***

With a median follow-up of 28 months (range, 6 to 60) for survivors, the probabilities of OS and DFS at 2 years were 62% (95% CI, 38% to 79%) and 52% (95% CI, 29% to 72%), respectively (Figure 2A). The cumulative incidence of relapse at 2 years was 26% (95% CI, 9% to 47%). The cumulative incidences of NRM at 100 days and 2 years were 9% (95% CI, 1% to 25%) and 22% (95% CI, 8% to 41%), respectively (Figure 2B).

In the univariable analysis, higher proportions of blasts in BM and in PB and a monosomal or complex karyotype were significantly associated with inferior OS and DFS (Table 3, Figure 3, Supplementary Figures 1, and 3). Poor cytogenetics were significantly associated with inferior DFS and increased relapse incidence (Table 3, Figure 3, Supplementary Figure 2). The numbers of chromosomal abnormalities were significantly associated with inferior OS and DFS and increased relapse incidence (Supplementary Figure 3). In contrast, disease type, disease status at CBT, and lactate dehydrogenase value did not affect the survival and relapse incidence (Table 3, Figures 3, Supplementary Figures 1 and 2).

At the last follow-up, 8 patients had died. The causes of death were relapse in 3 patients, pneumonia in 2 patients, diffuse alveolar hemorrhage in 1 patient, organ failure in 1 patient, and acute GVHD in 1 patient.

## DISCUSSION

The optimal intensity of a conditioning regimen might be dependent of the type of graft source. Arai et al. [24] showed that the addition of high-dose Ara-C to a TBI/CY regimen was significantly associated with reduced relapse and improved survival after CBT for AML, but this effect was not observed after BMT/PBSCT from related and unrelated donors [25]. Moreover, Nakasone et al. [26] reported the TBI regimens were significantly associated with a higher rate of neutrophil engraftment after CBT regardless of conditioning intensity but not for matched or mismatched BMT. Indeed, the preferred conditioning regimens used by adult CBT centers in the United States are intensified regimens, such as addition of fludarabine or thiotepa to the TBI/CY regimen for younger patients [27]. By contrast, although the use of a reduced-intensity conditioning expanded the application of allogeneic HCT in elderly patients even in the setting of CBT, the outcome of reduced-intensity conditioning CBT was dismal with a higher relapse incidence and NRM [28-30]. Several studies demonstrated that FluBu4 has a similar efficacy with a lower toxicity than BuCy [11], suggesting that elderly patients may benefit from FluBu4 after BMT/PBSCT from related and unrelated donors. However, FluBu4 has been associated with an increased risk of graft failure after CBT [12,13]. Based on these data a higher intensity of conditioning regimen might be appropriate for CBT. In fact, engraftment rate and early toxicity have been reported to be improved by the addition of melphalan to FluBu4 [31], addition of thiotepa and antithymocyte globulin to FluBu3 [32], or addition of 2-Gy TBI and clofarabine to FluBu4 [13] in single or double CBT for adults. Thus, to avoid the toxicity of full-dose TBI in elderly patients, we added FluBu3 with 4 Gy TBI to G-CSF–combined high-dose Ara-C to sustain engraftment and to reduce relapse with accepted toxicity. Our data showed that neutrophil engraftment with donor-dominant chimerism was achieved in all assessable patients, and the incidences of OS and relapse at 2 years were 62% and 26%, respectively. These findings suggested that our reduced-toxicity myeloablative conditioning regimen improved engraftment rate and maintained antitumor effect after single CBT for elderly patients with nonremission myeloid malignancies.

Based on the concept that the concomitant use of G-CSF and Ara-C enhanced the antitumor activity by inducing dormant leukemia cells to enter the cell cycle, as shown in basic research [33,34] and clinical studies [35,36], we used the addition of G-CSF–combined Ara-C to a TBI/CY regimen for adult patients younger than 55 years in CBT [4,8,14,15,37]. Our previous studies demonstrated that the G-CSF–combined Ara-C and TBI/CY regimen was effective for relatively young adult patients with nonremission myeloid malignancies [37]. Interestingly, our retrospective studies showed that the G-CSF–combined Ara-C regimen was significantly associated with a higher rate of neutrophil engraftment and better survival without increased NRM in CBT for AML and MDS [14,15]. Indeed, the addition to G-CSF–combined high-dose Ara-C to FluBu3 and a low-dose TBI regimen in the present study had a relatively low early NRM (9% at 100 days), although grade 3 diarrhea and febrile neutropenia were frequently observed even in elderly patients. These data

**Table 3**
Univariable Analysis of OS, DFS, and Relapse

| | | Number | 2-Year OS (95% CI) | *P* | 2 -Year DFS (95% CI) | *P* | 2 -Year Relapse (95% CI) | *P* |
|---|---|---|---|---|---|---|---|---|
| Age | <65 yr | 12 | 63 (27-85) | .849 | 44 (14-70) | .716 | 31 (6-62) | .824 |
| | ≥65 yr | 11 | 61 (27-84) | | 64 (30-85) | | 18 (2.5-46) | |
| Sex | Male | 12 | 46 (16-72) | .066 | 37 (10-64) | .075 | 21 (2.1-52) | .632 |
| | Female | 11 | 78 (35-94) | | 72 (35-90) | | 28 (5.9-57) | |
| HCT-CI | 0-2 | 18 | 64 (35-82) | .858 | 51 (25-72) | .95 | 27 (7.4-51) | .975 |
| | ≥ 3 | 5 | 60 (13-88) | | 60 (13-88) | | 20 (.4-62) | |
| Disease type | *De novo* AML | 8 | 45 (11-75) | .419 | 47 (12-76) | .697 | 13 (.5-45) | .183 |
| | AML secondary to MDS | 6 | 56 (7-88) | | 50 (11-80) | | 50 (7.7-83) | |
| | MDS/CMML | 9 | 78 (37-94) | | 62 (21-86) | | 16 (1-53) | |
| Cytogenetics | Other than poor | 14 | 76 (42-92) | .102 | 77 (44-92) | **.008** | 9 (1-33) | **.028** |
| | Poor | 9 | 42 (11-71) | | 15 (1-47) | | 52 (10-83) | |
| Monosomal karyotype | Absent | 18 | 75 (45-90) | **.027** | 61 (32-81) | **.036** | 22 (5-.48) | .357 |
| | Present | 5 | 20 (1-58) | | 20 (1-58) | | 40 (3-79) | |
| Disease status at CBT | Untreated | 9 | 65 (25-87) | .435 | 53 (18-80) | .733 | 24 (3-58) | .854 |
| | PIF/refractory relapse | 8 | 45 (11-75) | | 47 (12-76) | | 28 (3-64) | |
| | Azacitidine failure | 6 | 83 (27-98) | | 63 (14-89) | | 21 (1-65) | |
| BM blasts | <15% | 10 | 90 (47-99) | **.022** | 75 (30-93) | **.024** | 15 (1-51) | .193 |
| | ≥15% | 12 | 35 (10-63) | | 29 (7-56) | | 36 (9-65) | |
| PB blasts | <10% | 10 | 90 (47-99) | **.031** | 75 (30-93) | **.039** | 15 (1-51) | .215 |
| | ≥10% | 13 | 39 (11-66) | | 35 (11-61) | | 33 (9-60) | |
| LDH value | ≤ ULN | 9 | 78 (37-94) | .336 | 78 (37-94) | .136 | 11 (1-41) | .366 |
| | > ULN | 14 | 52 (22-76) | | 36 (11-62) | | 35 (8-64) | |
| Cryopreserved TNC | $<2.5 \times 10^7$/kg | 12 | 56 (24-79) | .614 | 46 (16-72) | .613 | 29 (6-59) | .798 |
| | $\geq 2.5 \times 10^7$/kg | 11 | 71 (34-90) | | 61 (27-84) | | 19 (3-48) | |
| Cryopreserved $CD34^+$ cells | $<1 \times 10^5$/kg | 14 | 45 (18-70) | .060 | 38 (13-63) | .095 | 26 (5-54) | .952 |
| | $\geq 1 \times 10^5$/kg | 9 | 89 (43-98) | | 78 (37-94) | | 22 (3-53) | |
| HLA disparity | <2 | 5 | 75 (13-96) | .362 | 50 (6-85) | .599 | 50 (3-88) | .365 |
| | ≥2 | 18 | 60 (34-79) | | 55 (29-74) | | 17 (4-38) | |

*P* values in bold are statistically significant (<.05). PIF indicates primary induction failure.

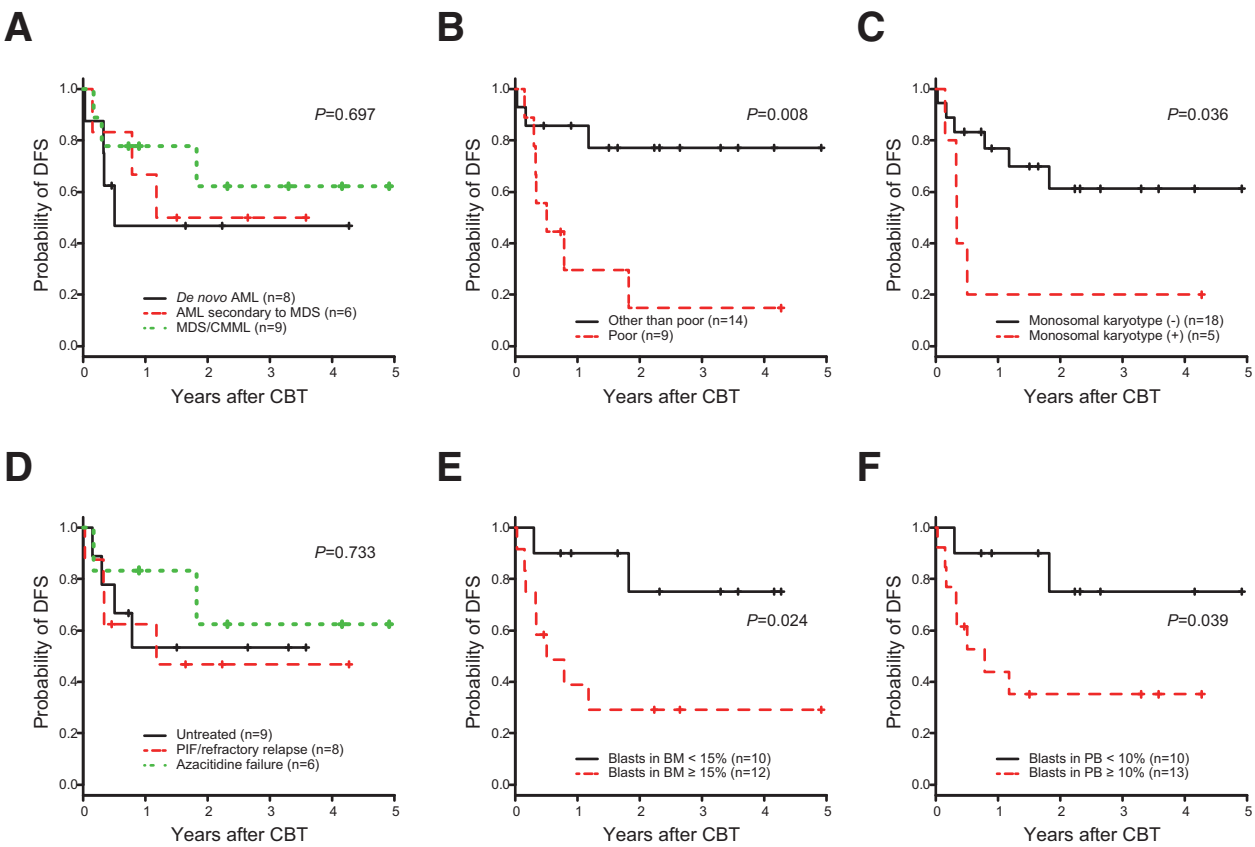


**Figure 3.** The probability of DFS according to the disease type (A), cytogenetic risk (B), monosomal karyotype (C), disease status at CBT (D), the proportion of blasts in BM (E), and the proportion of blast in PB (F).

suggested that the addition of G-CSF–combined Ara-C to FluBu3 and 4 Gy TBI was well tolerated with an antitumor effect in elderly patients with nonremission myeloid malignancies.

In our study approximately 40% of patients received CBT without prior treatment. The efficacy of pretransplant induction chemotherapy has not been demonstrated in advanced MDS and AML secondary to MDS [38-40], although the disease burden at HCT has been shown to be associated with relapse after HCT [41,42]. In fact, the efficacy of induction chemotherapy has been limited in patients with poor cytogenetic risk, and the use of CB as a graft source enables patients to undergo HCT as an upfront treatment. Our recent report showed that upfront HCT had a significantly reduced incidence of leukemia-related mortality among patients with poor cytogenetic risk and those who received MAC and CBT compared with those who received induction chemotherapy followed by HCT for AML with multilineage dysplasia in a propensity score matched analysis on the Japanese registration data [43]. These data indicated that upfront CBT should be considered for patients with AML with multilineage dysplasia, AML secondary to MDS, or those with poor cytogenetics.

Previous studies demonstrated that relapse incidence after CBT is not inferior to that after BMT/PBSCT from unrelated donors, although the incidence of severe GVHD is significantly lower after CBT than BMT/PBSCT from unrelated donors [5,6,8], indicating that the graft-versus-leukemia (GVL) effect might not be compromised after CBT. By contrast, the GVL effect might be enhanced because most CBTs have HLA mismatches. Indeed, several studies showed that a lower relapse incidence was associated with a specific HLA locus mismatch [44-46] and an increased number of HLA mismatches [47,48]. Moreover, Kanda et al. [49] demonstrated that development of mild acute and chronic GVHD reduced the relapse incidence after single CBT. Interestingly, a recent study from the Fred Hutchinson Cancer Research Center demonstrated that among patients with acute leukemia or MDS, relapse incidence was significantly lower after CBT than for HLA-matched or -mismatched unrelated donors in patients with minimal residual disease at the time of HCT, but this effect was not observed in patients without minimal residual disease [7]. Those data clearly indicate that in patients with a similar bulk of disease at transplant, CBT would reduce relapse incidence and be preferable to other hematopoietic cell sources. More recently, an encouraging report from China showed that the relapse incidence was significantly lower after CBT than for BMT/PBSCT from matched sibling donors for AML [50], suggesting again that the GVL effects after CBT might be stronger than those after matched related BMT/PBSCT. Our study showed the relapse incidence at 2 years after CBT was 26% even in patients with nonremission myeloid malignancies, which was better than previous reports of allogeneic BMT/PBSCT for nonremission patients [41,51]. However, whether a major cause of antitumor activity after CBT for patients with nonremission myeloid malignancies was due to these GVL effect is unclear because of a limited number of patients in the current study. Further studies are required to clarify the role of a meaningful GVL effect after CBT for patients with nonremission myeloid malignancies.

In summary, although this study was a single-institute retrospective analysis that included only a relatively small number of patients and the information of molecular mutation profiles was lacking, our data concluded that this reduced-toxicity myeloablative conditioning regimen based on 4 Gy TBI, G-CSF–combined Ara-C, and FluBu3 was tolerable and effective in terms of engraftment, relapse, and survival in CBT for elderly patients with nonremission myeloid malignancies.

## ACKNOWLEDGMENTS

The authors thank all physicians and staff at the hospital and the cord blood bank in Japan for their help in this study.

*Financial disclosure:* This work was supported in part by grants-in-aid from the Japanese Foundation for Promotion of Cancer Research, and from the Japanese Foundation for Multidisciplinary Treatment of Cancer.

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

## SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.12.004.

## REFERENCES

1. Duval M, Klein JP, He W, et al. Hematopoietic stem-cell transplantation for acute leukemia in relapse or primary induction failure. *J Clin Oncol*. 2010;28:3730–3738.
2. Nagler A, Savani BN, Labopin M, et al. Outcomes after use of two standard ablative regimens in patients with refractory acute myeloid leukaemia: a retrospective, multicentre, registry analysis. *Lancet Haematol*. 2015;2:e384–e392.
3. 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.
4. Takahashi S, Ooi J, Tomonari A, et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood*. 2007;109:1322–1330.
5. Eapen M, Rocha V, Sanz G, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. *Lancet Oncol*. 2010;11:653–660.
6. Terakura S, Atsuta Y, Tsukada N, et al. Comparison of outcomes of 8/8 and 7/8 allele-matched unrelated bone marrow transplantation and single-unit cord blood transplantation in adults with acute leukemia. *Biol Blood Marrow Transplant*. 2016;22:330–338.
7. Milano F, Gooley T, Wood B, et al. Cord-blood transplantation in patients with minimal residual disease. *N Engl J Med*. 2016;375:944–953.
8. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood*. 2004;104:3813–3820.
9. Konuma T, Tsukada N, Kanda J, et al. Comparison of transplant outcomes from matched sibling bone marrow or peripheral blood stem cell and unrelated cord blood in patients 50 years or older. *Am J Hematol*. 2016;91:E284–E292.
10. Alatrash G, de Lima M, Hamerschlak N, et al. Myeloablative reduced-toxicity i.v. busulfan-fludarabine and allogeneic hematopoietic stem cell transplant for patients with acute myeloid leukemia or myelodysplastic syndrome in the sixth through eighth decades of life. *Biol Blood Marrow Transplant*. 2011;17:1490–1496.
11. Ben-Barouch S, Cohen O, Vidal L, Avivi I, Ram R. Busulfan fludarabine vs busulfan cyclophosphamide as a preparative regimen before allogeneic hematopoietic cell transplantation: systematic review and meta-analysis. *Bone Marrow Transplant*. 2016;51:232–240.
12. Horwitz ME, Morris A, Gasparetto C, et al. Myeloablative intravenous busulfan/fludarabine conditioning does not facilitate reliable engraftment of dual umbilical cord blood grafts in adult recipients. *Biol Blood Marrow Transplant*. 2008;14:591–594.
13. Mehta RS, Di Stasi A, Andersson BS, et al. The development of a myeloablative, reduced-toxicity, conditioning regimen for cord blood transplantation. *Clin Lymph Myeloma Leuk*. 2014;14:e1–e5.
14. Konuma T, Ooi J, Uchida N, et al. Granulocyte colony-stimulating factor combined regimen in cord blood transplantation for acute myeloid leukemia: a nationwide retrospective analysis in Japan. *Haematologica*. 2014;99:e264–e268.
15. Konuma T, Takahashi S, Uchida N, et al. Effect of granulocyte colony-stimulating factor-combined conditioning in cord blood transplantation for myelodysplastic syndrome and secondary acute myeloid leukemia: a retrospective study in Japan. *Biol Blood Marrow Transplant*. 2015;21:1632–1640.
16. Konuma T, Kato S, Oiwa-Monna M, et al. Cryopreserved CD34+ cell dose, but not total nucleated cell dose, influences hematopoietic recovery and extensive chronic graft-versus-host disease after single-unit cord blood transplantation in adult patients. *Biol Blood Marrow Transplant*. 2017;23:1142–1150.
17. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995;15:825–828.
18. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-

versus-host disease. I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2015;21:389–401.
19. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology, acute myeloid leukemia (version 2.2016) Available at: https://www.nccn.org/professionals/physician_gls/pdf/aml.pdf. Accessed 20 January 2017.
20. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood*. 1997; 89:2079–2088.
21. Breems DA, Van Putten WL, De Greef GE, et al. Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. *J Clin Oncol*. 2008;26:4791–4797.
22. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood*. 2005;106:2912–2919.
23. Kanda Y. Investigation of the freely available easy-to-use software "EZR" for medical statistics. *Bone Marrow Transplant*. 2013;48:452–458.
24. Arai Y, Takeda J, Aoki K, et al. Efficiency of high-dose cytarabine added to CY/TBI in cord blood transplantation for myeloid malignancy. *Blood*. 2015;126:415–422.
25. Arai Y, Aoki K, Takeda J, et al. Clinical significance of high-dose cytarabine added to cyclophosphamide/total-body irradiation in bone marrow or peripheral blood stem cell transplantation for myeloid malignancy. *J Hematol Oncol*. 2015;8:102.
26. Nakasone H, Fuji S, Yakushijin K, et al. Impact of total body irradiation on successful neutrophil engraftment in unrelated bone marrow or cord blood transplantation. *Am J Hematol*. 2017;92:171–178.
27. Barker JN, Kurtzberg J, Ballen K, et al. Optimal practices in unrelated donor cord blood transplantation for hematologic malignancies. *Biol Blood Marrow Transplant*. 2017;23:882–896.
28. Cutler C, Ballen K. Reduced-intensity conditioning and umbilical cord blood transplantation in adults. *Bone Marrow Transplant*. 2009; 44:667–671.
29. Oran B, Wagner JE, DeFor TE, Weisdorf DJ, Brunstein CG. Effect of conditioning regimen intensity on acute myeloid leukemia outcomes after umbilical cord blood transplantation. *Biol Blood Marrow Transplant*. 2011;17:1327–1334.
30. Baron F, Ruggeri A, Beohou E, et al. RIC versus MAC UCBT in adults with AML: a report from Eurocord, the ALWP and the CTIWP of the EBMT. *Oncotarget*. 2016;7:43027–43038.
31. Yamamoto H, Uchida N, Yuasa M, et al. A novel reduced-toxicity myeloablative conditioning regimen using full-dose busulfan, fludarabine, and melphalan for single cord blood transplantation provides durable engraftment and remission in nonremission myeloid malignancies. *Biol Blood Marrow Transplant*. 2016;22:1844–1850.
32. Sanz J, Boluda JC, Martín C, et al. Single-unit umbilical cord blood transplantation from unrelated donors in patients with hematological malignancy using busulfan, thiotepa, fludarabine and ATG as myeloablative conditioning regimen. *Bone Marrow Transplant*. 2012;47:1287–1293.
33. Beekman R, Touw IP. G-CSF and its receptor in myeloid malignancy. *Blood*. 2010;115:5131–5136.
34. Saito Y, Uchida N, Tanaka S, et al. Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. *Nat Biotechnol*. 2010;28:275–280.
35. Löwenberg B, van Putten W, Theobald M, et al. Effect of priming with granulocyte colony-stimulating factor on the outcome of chemotherapy for acute myeloid leukemia. *N Engl J Med*. 2003;349:743–752.
36. Pabst T, Vellenga E, van Putten W, et al. Favorable effect of priming with granulocyte colony-stimulating factor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. *Blood*. 2012;119:5367–5373.
37. Konuma T, Kato S, Ooi J, et al. Single-unit cord blood transplantation after granulocyte colony-stimulating factor-combined myeloablative conditioning for myeloid malignancies not in remission. *Biol Blood Marrow Transplant*. 2014;20:396–401.
38. Anderson JE, Gooley TA, Schoch G, et al. Stem cell transplantation for secondary acute myeloid leukemia: evaluation of transplantation as initial therapy or following induction chemotherapy. *Blood*. 1997; 89:2578–2585.
39. Nakai K, Kanda Y, Fukuhara S, et al. Value of chemotherapy before allogeneic hematopoietic stem cell transplantation from an HLA-identical sibling donor for myelodysplastic syndrome. *Leukemia*. 2005;19:396–401.
40. Scott BL, Storer B, Loken MR, et al. Pretransplantation induction chemotherapy and posttransplantation relapse in patients with advanced myelodysplastic syndrome. *Biol Blood Marrow Transplant*. 2005;11:65–73.
41. Craddock C, Labopin M, Pillai S, et al. Factors predicting outcome after unrelated donor stem cell transplantation in primary refractory acute myeloid leukaemia. *Leukemia*. 2011;25:808–813.
42. Ogawa H, Ikegame K, Daimon T, et al. Impact of pretransplant leukemic blast% in bone marrow and peripheral blood on transplantation outcomes of patients with acute myeloid leukemia undergoing allogeneic stem cell transplantation in non-CR. *Bone Marrow Transplant*. 2018;53:478–482.
43. Konuma T, Harada K, Yamasaki S, et al. Upfront allogeneic HCT vs remission induction chemotherapy followed by allogeneic HCT for acute myeloid leukemia with multilineage dysplasia: a propensity score matched analysis. *Am J Hematol*. 2019;94:103–110.
44. Atsuta Y, Kanda J, Takanashi M, et al. Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia. *Haematologica*. 2013;98:814–822.
45. Konuma T, Kato S, Ishii H, et al. HLA-DRB1 mismatch is associated with a decreased relapse in adult acute myeloid leukemia after single-unit myeloablative cord blood transplantation. *Ann Hematol*. 2015;94:1233–1235.
46. 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.
47. Cunha R, Loiseau P, Ruggeri A, et al. Impact of HLA mismatch direction on outcomes after umbilical cord blood transplantation for hematological malignant disorders: a retrospective Eurocord-EBMT analysis. *Bone Marrow Transplant*. 2014;49:24–29.
48. Sanz J, Jaramillo FJ, Planelles D, et al. Impact on outcomes of human leukocyte antigen matching by allele-level typing in adults with acute myeloid leukemia undergoing umbilical cord blood transplantation. *Biol Blood Marrow Transplant*. 2014;20:106–110.
49. Kanda J, Morishima Y, Terakura S, et al. Impact of graft-versus-host disease on outcomes after unrelated cord blood transplantation. *Leukemia*. 2017;31:663–668.
50. Zheng CC, Zhu XY, Tang BL, et al. Clinical separation of cGvHD and GvL and better GvHD-free/relapse-free survival (GRFS) after unrelated cord blood transplantation for AML. *Bone Marrow Transplant*. 2017;52:88–94.
51. Magenau J, Westervelt P, Khaled S, et al. A multicenter trial of myeloablative clofarabine and busulfan conditioning for relapsed or primary induction failure AML not in remission at the time of allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2017;52:59–65.