Unrelated Umbilical Cord Blood Transplantation Using a TBI/FLAG Conditioning Regimen for Adults with Hematologic Malignancies

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Received October 4, 2007; accepted May 25, 2008

ABSTRACT
A combined chemotherapy regimen comprising fludarabine, cytosine arabinoside, and granulocyte colony-stimulating factor (FLAG) has been used in the treatment of relapsed or refractory leukemias. We here report 38 patients with hematologic malignancies who underwent single-unit cord blood transplantation (CBT) with a conditioning regimen comprising 12-Gy total-body irradiation (TBI) and FLAG therapy (TBI/FLAG). Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus or cyclosporin A and/or methotrexate. The median nucleated cell dose was 2.43 × 10^11/kg (range: 1.96-3.55 × 10^11/kg). Of 34 evaluable recipients, the cumulative incidence of donor engraftment was 97%. The median time to reach an absolute neutrophil count of 500/μL was 23 days (range: 18-35 days). The median time to an untransfused platelet count of 50,000/μL was 45.5 days (range: 28-208 days). Sixteen patients developed grades II-IV of acute GVHD. Fourteen patients were alive at a median follow-up of 46 months (range: 4-77 months). The estimated event-free survival at 3 years for all patients was 33.5%, with 72.7% in the standard-risk group (n = 11) and 17.7% in the high-risk group (n = 27) (P = .0075). These results showed that this novel regimen was well tolerated by patients and able to establish sustained donor cell engraftment, indicating the feasibility of TBI/FLAG as a conditioning regimen for CBT in adults with hematologic malignancies.

KEY WORDS Cord blood transplantation (CBT) • Adult • Conditioning regimen • FLAG • Total body irradiation (TBI)

INTRODUCTION
Umbilical cord blood transplantation (CBT) has increasingly been performed as an alternative to human leukocyte antigen (HLA)-matched sibling or unrelated bone marrow transplantation (BMT) [1-3]. The advantages of CBT in comparison to BMT include prompt availability of cryopreserved cells, a less stringent requirement for HLA-type matches between donors and recipients, and a low risk of inducing severe graft-versus-host disease (GVHD). The major drawbacks of CBT are slow hematopoietic recovery and a high incidence of graft failure, mainly because of a small number of progenitors being infused, which is more pronounced in adults with greater body weight [4]. Generally, the overall outcome in adult CBT needs to be improved in comparison to that in adult allogeneic BMT [2]. A standard conditioning regimen with cyclophosphamide and total-body irradiation (TBI) produces favorable results for BMT [5], but a standard conditioning regimen for CBT has not yet been firmly established.

Intensive combination chemotherapy has significantly improved the prognosis of patients with hematologic malignancy [6]. FLAG therapy using fludarabine (Flu), cytosine arabinoside (Ara-C), and
granulocyte colony-stimulating factor (G-CSF) has been shown to be effective against a variety of hematologic malignancies, including high-risk acute myeloid leukemias [7,8] and acute lymphoblastic leukemia [9]. The use of FLAG therapy for the treatment of leukemias is based on the following arguments: (1) infusion of fludarabine before Ara-C increases the accumulation of the active metabolite ara-C triphosphate in leukemic cells [10], (2) G-CSF shortens the duration of neutropenia and reduces infection rates in leukemia patients [11], and (3) G-CSF may sensitize leukemic blasts to S-phase-specific Ara-C by recruiting quiescent cells into the cell cycle and increasing Ara-C phosphorylation [12]. Thus, FLAG therapy was pharmacokinetically designed to increase antileukemic metabolites, and was intended to exert an efficient antileukemic effect in the treatment of relapsed or refractory leukemias.

Fludarabine is highly immunosuppressive and shown to be especially effective in a nonmyeloablative preparative regimen for allogeneic stem cell transplantation (SCT) [13]. Pawson et al. [14] used FLAG with or without idarubicin as a reduced-intensity conditioning (RIC) regimen for second allogeneic peripheral blood SCT in the treatment of relapsed leukemia patients. Thus, FLAG therapy may act not only as an effective antileukemic chemotherapy regimen, but also as an efficient preparative regimen for SCT.

In the present study, we developed a new conditioning regimen consisting of FLAG therapy combined with 12-Gy TBI (TBI/FLAG). We performed CBT using this regimen in 38 adult patients with hematologic malignancies in our single institution. Our results demonstrated the feasibility of this TBI/FLAG as a novel myeloablative preparative regimen for CBT.

PATIENTS AND METHODS

Eligibility

Patients were eligible if they were in a condition requiring SCT but had no 6/6 or 5/6 allele HLA-matched related donor or 6/6 HLA-matched unrelated donor available, or needed urgent SCT within 3 months. Patients receiving a transplant during the first or second complete remission of leukemia or non-Hodgkin’s lymphoma, or those who had refractory anemia of myelodysplastic syndrome (MDS) were placed in the standard-risk group. Patients in their third or subsequent remission, relapse, or partial remission with refractory leukemia and those with chronic myelogenous leukemia (CML) beyond the first chronic phase at the time of CBT were considered to be in the advanced phase of disease and were placed in the high-risk group. Patients with diseases with high-risk cytogenetics, such as acute lymphoblastic leukemia (ALL) with t(9;22) and acute myelogenous leukemia (AML) with −5, del(5q), −7, del(7) or del(11), were also included in the high-risk group [15]. This study was approved by the institutional review board of Hyogo College of Medicine. All patients provided written informed consent.

CB grafts

Appropriate cord blood (CB) was identified through the Japan Cord Blood Bank Network (JCBBN), which maintains information on the holdings of 11 local CB banks in Japan [16]. In the first 19 patients, CB grafts were selected on the basis of serologic matching at 4-6 of 6 HLA loci (class I HLA-A and -B, and class II HLA-DR alleles) as determined by a standard complement-dependent microlymphocytotoxicity test [17]. In the subsequent 19 patients, high-resolution DNA typing of class II DRB1 alleles was used for selection of class II alleles according to the availability of the high-resolution class II data. CB grafts selected had a cryopreserved cell dose of at least 2 × 10⁷ nucleated cells (NC) per kilogram of recipient body weight (NC/kg). Confirmatory high-resolution DNA typing of class I HLA-A and -B and class II DRB1 alleles was also performed [18-20]. All CB used were single units and were not depleted of T lymphocytes.

Preparative Regimen

The TBI/FLAG regimen comprised TBI (12 Gy), Flu (150 mg/m²), Ara-C (10 g/m²), and G-CSF. TBI was administered daily at 3 Gy for 4 days (day −10 to day −7). Flu, Ara-C, and G-CSF were administered daily for 5 days (day −6 to −2). Flu (30 mg/m²) was administered intravenously over 2 hours. Four hours after the completion of Flu infusion, Ara-C (2 g/m²) was administered intravenously over 2 hours. The TBI/FLAG regimen was performed irrespective of prior Ara-C treatment. G-CSF (300 μg/m²) was administered subcutaneously. In the first 24 consecutive patients, G-CSF was administered to all patients, but in the subsequent 14 patients, the G-CSF administration was omitted in patients with lymphoid leukemias and lymphomas (n = 7), because efficacy of G-CSF on lymphoid malignancy is not firmly established.

GVHD Prophylaxis and Treatment

GVHD prophylaxis was tacrolimus (n = 11) or cyclosporin A (CsA) (n = 1) alone during the years 2000 to 2002. Tacrolimus plus short-term methotrexate (MTX) (n = 9) or CsA plus short-term MTX (n = 17) was used since August 2002. Administration of tacrolimus (0.02 mg/kg/day) or CsA (3 mg/kg/day) in a continuous infusion was started on day −1 and continued until the patient became tolerant to oral administration. Short-term MTX was administered at 10 mg/m² on day 1 and 7 mg/m² on days 3 and 6 [21]. After neutrophil engraftment, and in the absence of acute
GVHD (aGVHD), tacrolimus or CyA was tapered 10% per week starting at approximately day 35. Acute GVHD was clinically diagnosed using the criteria of Glucksberg et al. [22]. Grade II to IV aGVHD was treated with methylprednisolone at 1-2 mg/kg/day. Patients who survived for >100 days were analyzed for chronic GVHD (cGVHD).

Supportive Care
Each patient was isolated in a laminar air-flow room. Ciprofloxacin at 400 mg/day and fluconazole at 300 mg/day were administered from day −14 until neutrophil recovery. G-CSF at 300 μg/m² was again administered to all patients from day 5 until neutrophil recovery. Acyclovir was administered at 750 mg/day for 5 weeks after transplantation to prevent herpes simplex virus infection. Ganciclovir 10 mg/kg was administered in 2 divided doses from day −10 to day −3 as prophylaxis for cytomegalovirus (CMV) infection. Detection of CMV antigenemia was performed using an immunoperoxidase-conjugated antibody, HRP-C7, which binds to an immediate-early antigen of CMV, pp65 antigen. After grafting, ganciclovir administration was re instituted in patients demonstrating positive CMV antigenemia.

Donor Chimerism Analysis
Donor chimerism was analyzed using marrow and/or blood samples. Chimerism was determined by quantitative PCR analysis of informative short tandem repeat regions in the recipients and donors (STR-PCR) [21,23]. DNA was extracted from marrow or blood cells using a SepaGene isolation kit (Sankyo Pure Chemical, Tokyo, Japan), and amplified with fluorescent PCR primers (AmpFlSTR profiler PCR amplification kit; Applied Biosystems, San Jose, CA). The fluorescent PCR products were separated by capillary electrophoresis using a 310 Genetic Analyzer (Applied Biosystems). GeneScan software and GeneMapper software (Applied Biosystems) were used to calculate the percentage of donor and recipient DNA.

Engraftment
Engraftment was considered to have occurred when whole blood cell counts of absolute neutrophil counts of >500/μL were obtained for 3 consecutive days after transplantation, accompanied by the detection of donor chimerism. Graft failure was considered to have occurred when peripheral and marrow hypoplasia were noted after transplantation, and donor markers could not be detected by using cytogenetic and/or molecular techniques.

Regimen-Related Toxicity (RRT) and Transplantation-Related Mortality (TRM)
RRT, the nonhematologic toxicities directly caused by a given preparative regimen by day 28, were analyzed using Bearman’s criteria [24]. TRM was defined as death without primary disease progression.

Statistical Analysis
The probability of event-free survival (EFS) was estimated using the Kaplan-Meier method with Mantel-Cox log rank test. In this analysis, graft failure, relapse, disease progression, and death were defined as events. We used Cox proportional hazards models to determine which independent patient-, disease- and transplant-related variables predict EFS. We first fitted univariable models, then all variables with P < .10 were included in a multivariable model. Hazard ratios were estimated with 95% confidence intervals. Categoric variables were compared using the χ² test. Values of P < .05 were considered to be significant. All statistical analyses were carried out with StatView version 5.0 software (SAS Institute, Cary, NC).

RESULTS
Patient Characteristics
Thirty-eight patients underwent CBT with a TBI/FLAG conditioning regimen between December 2000 and February 2007 at our institution. The median age of the patients was 38.5 years (range: 16-52 years) and the median weight was 58 kg (range: 39-81 kg). Details of patients’ characteristics are listed in Table 1. Eleven patients (29%) who were in first or second remission were placed in the standard-risk group. The remaining 27 patients (71%), who were placed in the high-risk group, include 14 in relapse or partial remission with

Table 1. Patient Characteristics
<table>
<thead>
<tr>
<th>Number of patients</th>
<th>38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>20/18</td>
</tr>
<tr>
<td>Age (year); median (range)</td>
<td>38.5 (16-52)</td>
</tr>
<tr>
<td>Disease</td>
<td>Standard-Risk</td>
</tr>
<tr>
<td>CR1/CR2 RA, CP</td>
<td>&gt; CR3/&gt;AP HRC</td>
</tr>
<tr>
<td>AML</td>
<td>5</td>
</tr>
<tr>
<td>ALL</td>
<td>4</td>
</tr>
<tr>
<td>MLL</td>
<td>2</td>
</tr>
<tr>
<td>NHL</td>
<td>0</td>
</tr>
<tr>
<td>ATL</td>
<td>0</td>
</tr>
<tr>
<td>MDS</td>
<td>0</td>
</tr>
<tr>
<td>CML</td>
<td>0</td>
</tr>
<tr>
<td>CLL</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
</tr>
</tbody>
</table>

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MLL, mixed-lineage leukemia; NHL, non-Hodgkin lymphoma; ATL, adult T cell leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; CR1, CR2, CR3 first, second, and third complete remission; RA, refractory anemia; CP, chronic phase of CML; Rel, relapse; Ref, refractory disease; AP, accelerated phase of CML; HRC, high-risk cytogenetics.
refractory disease, 13 in their third or subsequent remission or with high-risk cytogenetics (Table 1).

**Graft Characteristics**

The median number of nucleated cells infused was $2.43 \times 10^{7}/kg$ body weight (range: 1.96-3.55 $\times 10^{7}/kg$) and that of CD34+ cells was $0.87 \times 10^{5}/kg$ body weight (range: 0.24-3.98 $\times 10^{5}/kg$) (Table 2). CB grafts were primarily selected on the basis of serologic matching at HLA-A, -B, and -DR alleles (n = 19) or serologic matching at HLA-A and -B and high-resolution DNA typing of DRB1 alleles (n = 19). Only 1 graft (2%) in primary selection was 3 HLA mismatches. However, confirmatory high-resolution DNA typing of both class I and class II alleles revealed that 12 (31%) of the CB grafts had 3 or 4 mismatched antigens (Table 2).

**Recovery of Peripheral Blood Cell Counts and Engraftment**

Four of 38 patients were not evaluated for donor engraftment because of early death from sepsis (n = 2) (day 7, day 21) or bleeding (n = 2) (days 17, 22). Of 34 evaluable recipients, the cumulative incidence of primary donor engraftment was 97% (33 patients) as 1 patient experienced graft rejection with autologous marrow recovery. The median time for neutrophil recovery (>500/µL) was 23 days (range: 18-35 days; n = 33) (Figure 1A). All of these patients accompanied by donor chimerism by 86% to 100% using STR-PCR analysis of bone marrow cells at approximately day 21. After neutrophil recovery, 6 patients did not achieve subsequent reticulocyte recovery; 5 patients died between day 25 and day 100, and 1 patient experienced relapse. The median time for reticulocyte recovery (>1%) was 29 days (range: 25-57 days; n = 27) (Figure 1B). Thereafter, 1 patient, who experienced relapse, failed to achieve platelet recovery. The median time for platelet recovery (>50,000/µL) was 45.5 days (range: 28-208 days; n = 26) (Figure 1C).

**Early Organ Toxicity**

Early organ toxicity caused by the TBI/FLAG preparative regimen by day 28 was graded by the regimen-related toxicity (RRT) grading system [24]. Toxicities because of infection, bleeding, GVHD, and drugs administered posttransplant were excluded from this.

<table>
<thead>
<tr>
<th>Table 2. Graft Characteristics and GVHD Prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cord blood</strong></td>
</tr>
<tr>
<td><strong>Total cells</strong> ($\times 10^7$/kg) 2.43 (1.96-3.55)</td>
</tr>
<tr>
<td><strong>CD34+ cells</strong> ($\times 10^5$/kg) 0.87 (0.24-3.98)</td>
</tr>
<tr>
<td><strong>HLA mismatch</strong></td>
</tr>
<tr>
<td><strong>Primary</strong></td>
</tr>
<tr>
<td>0/6</td>
</tr>
<tr>
<td>1/6</td>
</tr>
<tr>
<td>2/6</td>
</tr>
<tr>
<td>3/6</td>
</tr>
<tr>
<td>4/6</td>
</tr>
<tr>
<td><strong>GVHD prophylaxis</strong></td>
</tr>
<tr>
<td>CsA/CsA + sMTX</td>
</tr>
<tr>
<td>Tacrolimus/tacrolimus + sMTX</td>
</tr>
</tbody>
</table>

GVHD indicates graft-versus-host disease; CsA, cyclosporin A; sMTX, short-term methotrexate.

*Primary HLA mismatches were detected on the basis of serological HLA-A, -B, and -DR alleles (n = 19) or serologic HLA-A and -B and high-resolution DRB1 alleles (n = 19).

†Confirmatory HLA mismatches were detected on the basis of high-resolution HLA -A, -B, and -DRB1 alleles.
Grade I stomatitis was observed in 13 patients, grade I hepatic toxicity in 7 patients, and Grade I gastrointestinal toxicity (diarrhea) in 11 patients. No patient developed cardiac toxicity (electrocardiograph abnormality), pulmonary toxicity (dyspnea), renal toxicity (increase in creatinine), or bladder toxicity (haematuria).

Infection

Five patients developed sepsis, 6 pneumonia, 1 human herpesvirus-6 (HHV-6) encephalitis and 1 interstitial pneumonitis. Reactivation of cytomegalovirus was documented in 16 patients and gancyclovir was administered. One of them developed fatal interstitial pneumonitis because of CMV. No obvious fungemia and invasive aspergillosis were observed.

GVHD

Twenty-eight patients who attained engraftment and survived >40 days were evaluated for aGVHD. The cumulative incidence of grade II-IV aGVHD was 57% (16/28), with grades II, III, and IV occurring in 7, 8, and 1 patients, respectively. Appearance of aGVHD varied depending on GVHD prophylaxis used. The incidence of grade II-IV aGVHD was 88% (n = 8) when single agent (tacrolimus or CsA alone) was used in 2000 to 2002, and it was significant reduced (45%, n = 20) when short-term MTX was used in combination with tacrolimus or CsA after August 2002 (P = .04 by the χ² test). Chronic GVHD developed in 11 (41%) of 27 evaluable patients who survived >100 days. Of the 11 patients, 8 patients developed limited cGVHD and 3 extended cGVHD.

Relapse

Overall, 11 patients (28.9%) relapsed after CBT. Cumulative incidence of relapse is shown in Figure 2. Of these patients, 10 were in the high-risk group (3 with AML, 1 with MDS, 3 with ALL, 2 with non-Hodgkin lymphoma), and 1 was in the standard risk group (1 with ALL).

Causes of Death

TRM within 100 days was 23.6% (9 of 38 patients). The main cause of death was bleeding (pulmonary and cerebral) in 2 cases, sepsis in 2, multiple organ failure in 2, herpes simplex virus-6 encephalitis in 1, and pneumonia in 2. One patient died of relapse within 100 days. The cause of death after 100 days was relapse in 10 cases, sepsis in 1, pneumonia in 1, interstitial pneumonitis in 1, and cGVHD in 1. TRM at day 365, which excluded primary disease progression, was 34.2% (13 out of 38 patients).

Survival and Prognostic Factors

Fourteen out of 38 patients were alive at a median follow-up of 46 months (range: 4-77 months). Three-year EFS was 33.5% (Figure 3). Using Cox proportional hazards models, sex, weight, HLA match (primary and confirmatory), cell dose, GVHD prophylaxis, and the presence of grade II-IV GVHD had no apparent effect on EFS (Table 3). In contrast, age and disease status at transplantation had significant impacts on EFS in both univariable and multivariable analysis (Table 3). Kaplan-Meier estimates indicated that patients who were 42 years old or younger (n = 26) showed significantly better survival (39.8%) than those who were older than 42 years (n = 12) (19.4%) (P = .0422) (Figure 4). Regarding the disease status at transplantation, EFS was 72.7% in the standard risk group (n = 11) and 17.7% in the high-risk group (n = 27) (P = .0075) (Figure 5). As the number of patients included in this study is small, the results shown above should be interpreted with caution.

DISCUSSION

Intensified chemotherapy can be effective in the treatment of chemotherapy-sensitive malignant
The curative effect of allogeneic SCT is derived partly from the antileukemic effect of myeloablative therapy and partly from a graft-versus-leukemia effect of donor immune cells on the residual leukemia. Transplantation using CB cells as alternative to the bone marrow cells or peripheral blood cells has increasingly been performed for the treatment of hematologic malignancies [1-3]. However, a standard preparative conditioning regimen has not been firmly established. We here report the results of CBT using a new myeloablative regimen, TBI/FLAG.

In the present study, we used 12-Gy TBI (in 4 fractions) and FLAG comprising 10 g/m² Ara-C, 150 mg/m² Flu, and G-CSF. High-dose Ara-C has been found to be effective in the treatment of myeloid and lymphoid leukemia patients with poor prognoses [25,26]. A conditioning regimen using TBI and high cumulative doses of Ara-C (24 or 36 g/m²) achieves a lower relapse rate [27]. However, a significant proportion of allogeneic or autologous BMT patients who received high cumulative doses of Ara-C (36 g/m²) has been reported to die early as a result of toxicity [28]. The incidence of pulmonary complications, including interstitial pneumonia and obvious infection, and the risk of pulmonary toxicity, increases with age. These results suggest that the use of high cumulative doses of Ara-C (36 g/m²) for conditioning should be avoided. Tomonari et al. [29] carried out a preliminary trial in which 5 patients who received CBT were conditioned with 24 g/m² Ara-C, 90 mg/m² Flu, and 12-Gy TBI. All patients showed favorable prognosis. Furthermore, Takahashi et al. [30] reported that a conditioning regimen comprising 12-Gy TBI, Ara-C (12 g/m²), cyclophosphamide (120 mg/kg), and G-CSF produced very good outcomes. Thus, appropriate doses of Ara-C may be effective as part of a preparative regimen. In the TBI/FLAG regimen used in this study, the total dose of Ara-C was limited to 10 g/m², and its activity was pharmacokinetically augmented by concomitant use of fludarabine [10]. This preparative regimen was found to be associated with minimal early RRT within 28 days and without any enhancement of later pulmonary or other life-threatening toxicities.

GVHD indicates graft-versus-host disease; CsA, cyclosporin A; MTX, methotrexate.

*Primary HLA matches were detected on the basis of serological HLA-A, -B, and -DR alleles (n = 19) or serologic HLA-A and -B and high-resolution DRB1 alleles (n = 19).

†Confirmatory HLA matches were detected on the basis of high-resolution HLA-A, -B, and -DRB1 alleles.

Table 3. Risk Factors for Event-Free Survival (EFS) after CBT

<table>
<thead>
<tr>
<th>Factors</th>
<th>Hazard Ratio</th>
<th>95% Confidential Interval</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.881</td>
<td>0.394-1.966</td>
<td>.7568</td>
</tr>
<tr>
<td>Age &gt;42</td>
<td>2.325</td>
<td>1.005-5.493</td>
<td>.0481</td>
</tr>
<tr>
<td>Weight</td>
<td>1.016</td>
<td>0.969-1.064</td>
<td>.5168</td>
</tr>
<tr>
<td>Disease status:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high risk</td>
<td>4.604</td>
<td>1.356-15.634</td>
<td>.0143</td>
</tr>
<tr>
<td>Cell dose: total cells</td>
<td>0.696</td>
<td>0.239-2.064</td>
<td>.5140</td>
</tr>
<tr>
<td>HLA match</td>
<td>0.885</td>
<td>0.450-1.708</td>
<td>.7152</td>
</tr>
<tr>
<td>(primary)*</td>
<td>1.022</td>
<td>0.666-1.567</td>
<td>.9218</td>
</tr>
<tr>
<td>HLA match (confirmatory)†</td>
<td>0.701</td>
<td>0.301-1.585</td>
<td>.3939</td>
</tr>
</tbody>
</table>

CSA vs tacrolimus: 0.701 0.301-1.585 0.3939

MTX 1 vs 2: 0.688 0.215-2.200 0.5282

Acute GVHD

Age >42 2.828 1.140-7.091 0.0250

Disease status: 5.245 1.505-18.281 0.0093

Figure 4. EFS in relation to age. Kaplan-Meier estimates of EFS in patients 42 years old or younger (n = 26) and older than 42 (n = 12) (P = .0422).

Figure 5. EFS in relation to disease status. Kaplan-Meier estimates of EFS in standard-risk patients (n = 11) and high-risk patients (n = 27) (P = .0075).
II-IV aGVHD was high (88%, n = 8), but it was significantly reduced to 45% (n = 20) when short-term MTX was used together with tacrolimus or CsA after August 2002. However, the combined use of MTX for GVHD prophylaxis did not result in the improvement of the survival rate in our analysis (Table 3). Rather, the disease status had the strongest impact on the survival rate (Table 3). Our patients' response to steroid therapy was generally good for those with grade II and III aGVHD (data not shown).

Rocha et al. [33] reported the results of CBT on 98 patients in multicenter analysis, which showed 36% of 2-year survival. Laughlin et al. [2] also reported multicentric analysis of CBT including 150 patients that showed 26% of 3-year survival. In single-institution studies of adult CBT, Long et al. [34] reported 3-year survival of 19% of 57 patients, whereas Takahashi et al. [30] showed 2-year survival of 74% of 113 patients. In our single-institution study using a single-conditioning regimen, 3-year EFS was 33.5% (Figure 3). Cell dose of CB graft is known to be one of the critical factors that affect EFS in CBT [31], but we did not find this to be the case in our analysis (Table 3). This is probably because we used CB with relatively large number of cells, with the median cell number of 2.43 × 10^7 cells/kg. We found that patients older than 42 showed poor EFS (Figure 4), and this FLAG/TBI conditioned CBT is favorable to those who are 42 or younger. Regarding disease status and survival, 3-year EFS was 72.7% in the standard risk group (n = 11) and 17.7% in the high-risk group (n = 27) (P = .0075) (Figure 5). The EFS of 17.7% in the high-risk group in our study is comparable to previously reported rates of 15% to 20% [2]. The results of the present study are encouraging because standard-risk patients had 72.7% survival, which is comparable to that seen in standard-risk patients receiving allogeneic BMT or peripheral blood stem cell transplantation from HLA-matched donors [35]. This may indicate that CBT has almost the same efficacy as BMT in standard-risk patients. Although the finding must be confirmed in a larger scale study, our study suggests that CBT following conditioning with the TBI/FLAG regimen may be a reasonable option for adults with hematologic malignancies.

The results presented above show that CBT with a TBI/FLAG preparative regimen was well tolerated without significant RRT, and offered sustained donor cell engraftment. Patients who are 42 years old or younger and in standard risk may obtain a favorable outcome in this TBI/FLAG regimen. Further studies are needed to optimize this procedure to establish an effective treatment modality for hematologic malignancies.

ACKNOWLEDGMENTS

We thank the medical, nursing, and laboratory staff of the participating departments for their contributions to this study. We are also grateful to Dr. Toshimitsu Hamasaki (Department of Bio-medical statistics, Osaka University Graduate School of Medicine) for the assistance of statistical analysis, and to Ms. Shoko Yagi, Ms. Ikuyo Kasumoto and Ms. Hiromi Takeda for their excellent technical assistance. This study was supported by a grant from the Research on Human Genome, Tissue Engineering, Food Biotechnology of the Ministry of Health and Welfare of Japan, and a research grant from the High-Tech Research Center Program of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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