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Feasibility of Reduced-Intensity Cord Blood Transplantation as Salvage Therapy for Graft Failure: Results of a Nationwide Survey of Adult Patients

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To evaluate whether rescue with cord blood transplantation (CBT) could improve the poor survival after graft failure (GF), we surveyed the data of 80 adult patients (median age, 51 years) who received CBT within 3 months of GF (primary 64, secondary 16), with fludarabine-based reduced-intensity regimens with or without melphalan, busulfan, cyclophosphamide, and/or 2-4 Gy total-body irradiation (TBI). A median number of 2.4×10^7/kg total nucleated cells (TNC) were infused, and among the 61 evaluable patients who survived for more than 28 days, 45 (74%) engrafted. The median follow-up of surviving patients was 325 days, and the 1-year overall survival rate was 33% despite poor performance status (2-4, 60%), carryover organ toxicities (grade 3/4, 14%), and infections (82%) prior to CBT. Day 100 transplantation-related mortality was 45%, with 60% related to infectious complications. Multivariate analysis showed that the infusion of TNC $\geq 2.5 \times 10^7$/kg and an alkylating agent–containing regimen were associated with a higher probability of engraftment, and that high risk-status at the preceding transplantation and grade 3/4 organ toxicities before CBT were associated with an increased risk of mortality. In conclusion, in an older population of patients, our data support the feasibility of CBT with a reduced-intensity conditioning regimen for GF.

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INTRODUCTION

Graft failure or rejection (GF) is a serious problem early after allogeneic stem cell transplantation (SCT) using cord blood (CB) [1-6], an HLA-mismatched donor [7], and nonmyeloablative or reduced-intensity conditioning (RIC) regimens [8-13]. The incidence of GF was low after SCT from an HLA-matched related (2%) [14] or unrelated donor (0.7%-1.7%) [15,16]. In contrast, the incidence of GF was 14%-22% for SCT from an HLA-mismatched unrelated donor [15], 8%-20% for cord blood transplantation (CBT) [17,18], and 5%-21% for SCT from an unrelated donor using RIC [12,13]. The outcome of GF becomes generally poor because of an increased risk of infectious complications, which occur during prolonged severe neutropenia with associated organ toxicities. Whereas the survival rate after GF was 8% when no rescue transplantation was performed [19], the survival rate improved to 25%-40% when a second transplantation was performed [19-22].

The treatment of GF generally depends on 2 major basic mechanisms, that is, (1) poor graft function and (2) immunologically mediated graft rejection. Although the boost infusion of $CD34^+$ stem cells, selected or unmanipulated, has been reported to be effective in the former case [23,24], in the latter case, retransplantation with immunosuppressive conditioning is required for effective reconstitution of hematopoiesis [21,25-27]. Nevertheless, transplantation-related mortality (TRM) is still high because at the second SCT, most patients have poor performance status (PS), organ toxicities, carryover infection because of prolonged cytopenia, and difficulties in finding a suitable donor on an emergency basis. An additional problem is overlapping regimen-related toxicity (RRT) because of the conditioning regimen for the second SCT.

CB is a readily available stem cell source and, with the current development of efficient banking systems, most patients can readily find a suitable CB unit [28]. Many reports have shown the feasibility of reduced-intensity cord blood transplantation (RICBT) in older patients and patients with comorbidities [29,30]. Additionally, small case series of patients who were successfully rescued with retransplantation using CB after GF have also been reported [31-36]. Hence, CBT is a potential target of clinical research for GF. Nevertheless, the inevitable risks associated with CBT, that is, slower neutrophil engraftment and resultant higher risk of GF [17,18], may become critical barriers. To investigate whether salvage therapy with RICBT is a feasible therapeutic option for adult patients suffering from GF, we conducted a nationwide survey of RICBT that was performed as salvage therapy for GF.

PATIENTS AND METHODS

Data Sources and Patient Selection

Questionnaires were sent to 131 transplant centers in Japan, and 42 centers agreed to enroll consecutive cases in this study. This study was approved by the institutional review board of the National Cancer Center. The inclusion criteria for this study were as follows: (1) patients with hematologic disorders above age 16 years who received allogeneic SCT between January 2000 and April 2006, which resulted in primary or secondary GF, and (2) those who subsequently received fludarabine-based RICBT as salvage therapy within 3 months of the diagnosis of GF. The definition of a RIC regimen was according to the previous report by Giralt [37]. Patients who had relapse or disease progression before rescue RICBT were not included.

The total number of allogeneic SCT performed during this study period in 42 centers was 5622 including related donors (n = 2556), unrelated donors (n = 1907) and cord blood donors (n = 1159). Among 240 patients who experienced GF, 146 underwent salvage SCT and 94 did not. The stem cell source was CB (n = 102) or non-CB (n = 44). Among the 102 CBT recipients, 80 patients fulfilled the criteria for this study after excluding 12 patients who received myeloablative conditioning and 10 patients who received no toxic drug as conditioning regimen (antithymocyte globulin [ATG] only, n = 5; steroid only, n = 3; total lymphoid irridiation [TLI] only, n = 1; no conditioning, n = 1).

Definitions

Neutrophil engraftment was defined as the first of 3 consecutive days after transplantation that the absolute neutrophil count (ANC) exceeded $500/mm^3$ of peripheral blood. Primary GF was defined according to a previous report [15] as (1) failure of ANC to surpass $500/mm^3$ or (2) absence of donor T cells (<5%) before relapse, disease progression, second SCT, or death. Secondary GF was defined as (1) decrease in ANC $<100/mm^3$ at 3 determinations or (2) absence of donor T cells (<5%) after the initial engraftment without recovery before relapse, disease progression, second SCT, or death. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, polymerase chain reaction (PCR) for short tandem repeats or variable numbers of tandem repeats was used to detect donor cells at

a sensitivity of 1% to 5% [38]. Whole blood, $CD3^+$ selected, or marrow cells were assessed for chimerism at the time of neutrophil engraftment depending on the decision at each transplant center. HLA matching was reported using serological typing of HLA-A and HLA-B and allele typing of HLA-DRB1 of donor-recipient pairs except for 5 patients. Standard risk was defined as all complete remission of hematologic malignancy, chronic phase of chronic myeloid leukemia, or aplastic anemia. High risk was defined as other status of hematologic malignancy and all myelodysplastic syndrome refractory anemia with excess blasts (MDS-RAEB), including nonremission atypical CML. PS was defined according to the ECOG criteria [39]. RRT was evaluated by the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0) [40]. The diagnosis and clinical grading of acute graft-versus-host disease (aGVHD) were based on the established criteria [41]. Relapse was defined as an increase of blast more than 5% in bone marrow with hematologic malignancy.

First Transplant Procedures

Patients and transplantation characteristics at the first SCT that resulted in subsequent GF are summarized in Table 1. The median age of the 80 patients was 51 years (range: 17-68). Disease risk before the first SCT was standard risk in 49 patients (61%) and high risk in 31 patients (39%). Donor source for the first SCT included unrelated CB in 74% and unrelated bone marrow (BM) in 20%. Because the Japan Marrow Donor Program does not permit the donation of granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cell (PBSC) from unrelated donors, the stem cell source from unrelated donors was BM or CB. GVHD prophylaxis varied among the transplant centers.

After the first SCT, 64 patients experienced primary GF at a median of 28 days (range: 16-56 days), and 16 patients experienced secondary GF at a median of 36 days (range: 20-156). Data for chimerism analysis were available in 65 patients (primary GF, n = 49; secondary GF, n = 16). Among them, 45 patients had <5% donor cells (primary GF, n = 40, 82%; secondary GF, n = 5, 31%), which suggested immunologically mediated graft rejection, and 20 patients had donor cells ranging from 5% to 100% (primary GF, n = 9, 18%; secondary GF, n = 11, 69%), which suggested poor graft function.

Second Rescue Transplant Procedures

Patients and transplantation characteristics at the second SCT using RICBT as salvage therapy for GF are summarized in Table 2. The median intervals between the first SCT to the second SCT and the diagnosis of GF to the second SCT were 47 days and 15 days, respectively. Forty-eight patients (60%) had poor PS at the second SCT, and 11 patients (14%) had grade 3 or 4 carryover organ toxicities. Within 3 weeks of the start of conditioning for the second SCT, 66 patients (82%) had documented infection or febrile neutropenia that required intravenous antibiotics. More than half of the patients received a graft with serologic 2- or 3-locus HLA mismatches. We also examined the effect of HLA mismatch with serologic HLA-A, B and allele DRB1 except for 5 patients whose allele typing was not performed. The median body weight of the recipients was 55 kg (range: 33-110), and the median number of total nucleated cells (TNC) was 2.4×10^7/kg recipient body weight (range: 1.03-4.3) at cryopreservation. All patients received a fludrabine-containing reduced-intensity regimen with or without 2-4 Gy TBI. As there are no

Table 1. Patients and Tranaplantation Characteristics at the First SCT

Parameters	n = 80*
Median age at first SCT (range)	51 years (17-68)
Male/female	34/46
Underlying diagnosis†	
AML	43 (54%)
MDS	10 (13%)
ALL	13 (16%)
Other	14 (18%)
Disease risk‡	
Standard risk	49 (61%)
High risk	31 (39%)
Preceding chemotherapy	
Yes	66 (83%)
No§	14 (17%)
Conditioning⊥	
Myeloablative	37 (46%)
Reduced-intensity	43 (54%)
Donor and stem cell source	
Related BM or PB	5 (6%)
Unrelated BM	16 (20%)
Unrelated CB	59 (74%)
Type of GF	
Primary	64 (80%)
Secondary	16 (20%)

SCT indicates stem cell transplantation; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; BM, bone marrow; PB, peripheral blood; CB, cord blood; GF, graft failure; RAEB, refractory anemia with excess blasts; CML, chronic myelogeneous leukemia; CY, cyclophosphamide; TBI, total-body irradiation; BU, busulfan.

*Before undergoing the SCT that resulted in GF, 6 patients had received preceding transplantation.

†AML included overt AML evolved from MDS. MDS included RAEB-I or II (n = 9) and atypical CML (n = 1). Other diagnoses included non-Hodgkin lymphoma (n = 6), aplastic anemia (n = 5), and CML (n = 3).

‡Standard risk included acute leukemia and non-Hodgkin lymphoma in any complete remission, CML in any chronic phase, and aplastic anemia. High risk included all other leukemia and non-Hodgkin lymphoma categories, and MDS-RAEB.

§Forteen patients included MDS (n = 7), AML (n = 2), or aplastic anemia (n = 5).

⊥Myeloablative conditionings included CY/TBI (n = 27), BU/CY (n = 6), and other TBI-based regimen (n = 4). Reduced-intensity conditionings included fludarabine-based (n = 37), cladribine-based (n = 2), and others (n = 4) with (n = 26) or without (n = 17) 2-4 Gy TBI.

established standard RIC regimens for CBT after GF, the different conditioning regimens were chosen at the discretion of the attending physicians. G-CSF was administered in all but 1 patient after CBT.

Statistical Analyses

The primary endpoint of this study was the engraftment rate in patients who survived for more than 28 days after salvage RICBT. The secondary endpoints were TRM, overall survival (OS), and progression-free survival (PFS) from the day of salvage RICBT. For calculation of PFS, 5 patients with aplastic anemia were excluded from the analysis. OS and PFS were estimated using the Kaplan-Meier method. The cumulative incidences of engraftment and TRM were evaluated using Gray's method, considering death without engraftment and relapse, respectively, as competing risks. The log-rank test and the generalized Wilcoxon test were used to compare the probabilities of OS, PFS, TRM, and relapse after the second transplantation over time across patient subgroups.

Factors associated with at least borderline significance ($P < .10$) in the univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. Finally, P values of $<.05$ were considered statistically significant. Clinical factors that were assessed for their association with engraftment rate, TRM, and OS included sex, patient age at the time of the first SCT (<50 years versus ≥50 years), disease risk at the first SCT (standard risk versus high risk), conditioning for the first SCT (myeloablative versus reduced-intensity), PS at the second SCT (0-1 versus 2-4), carryover organ toxicities at the second SCT (grade 0-2 versus 3-4), carryover infection at the second SCT (documented versus febrile neutropenia/none), conditioning regimens for the second SCT (containing alkylating agents versus others), including TBI at the second SCT (non-TBI versus TBI 2-4 Gy), use of MTX (yes versus no), TNC (<2.5 versus ≥2.5 × 10^7/kg), and numbers of HLA mismatches in the graft-versus-host direction (0-1 versus 2-3) and host-versus-graft direction (0-1 versus 2-3). The statistical analysis was performed with SAS ver.8 (SAS Institute, Cary, NC).

RESULTS

Neutrophil and Platelet Engraftment (Table 3)

The cumulative incidences of neutrophil engraftment and death without engraftment are shown in Figure 1A. Among 61 patients who survived for more than 28 days after the second SCT, 45 (74%) achieved neutrophil engraftment at a median of 21 days (range: 13-44) (Table 3). The other 33 patients failed to achieve engraftment because of early TRM within 28 days after RICBT (n = 17), early relapse (n = 3) at days 22-25, or primary GF (n = 13). The remaining 2 patients died of TRM within 28 days after obtaining neutrophil engraftment. Among 13 patients who experienced primary GF after second SCT, chimerism analyses were performed in 4 patients to confirm the diagnosis of GF at a median of 25 days (range: 21-28).

Table 2. Patients and Transplantation Characteristics at the Second SCT (RICBT) for GF

Parameters	n = 80
Median time interval between	
The first and second SCT	47 days (range: 27-203)
Diagnosis of GF and the second SCT	15 days (range: 4-61)
PS at the second SCT	
0-1	32 (40%)
2-4	48 (60%)
Carryover organ toxicities at the second SCT*	
Grade 0-2	69 (86%)
Grade 3-4	11 (14%)
Carryover infection at the second SCT†	
Documented	40 (50%)
Febrile neutropenia	26 (32%)
None	14 (18%)
The median TNC of CB	2.4 × 10^7/kg (range: 1.03-4.3)
Numbers of serological HLA mismatch in GVH direction	
0-1	32 (40%)
2-3	48 (60%)
HVG direction	
0-1	33 (41%)
2-3	47 (59%)
Conditioning‡	
Flu alone	20 (25%)
Flu + Mel	22 (28%)
Flu + Bu	18 (22%)
Flu + CY	17 (21%)
Flu + others	3 (4%)
with 2-4 Gy TBI	35 (44%)
without TBI	45 (56%)
GVHD prophylaxis§	
CSP alone	17 (21%)
CSP + sMTX	6 (8%)
TAC alone	40 (50%)
TAC + sMTX	8 (10%)
Others	9 (11%)

SCT indicates stem cell transplantation; RICBT, reduced-intensity cord blood transplantation; GF, graft failure; PS, performance status; TNC, total nucleated cells; CB, cord blood; HLA, human leukocyte antigen; GVH, graft-versus-host; HVG, host-versus-graft; Flu, fludarabine; Mel, melphalan; Bu, busulfan; CY, cyclophosphamide: TBI, total-body irradiation; GVHD, graft-versus-host disease; CSP, cyclosporine; sMTX, short-term methotrexate; TAC, tacrolimus.

*Grade of organ toxicities was evaluated by the CTCAE v3.0 [40]. Grade 3 toxicities included liver (n = 5), lung (n = 3), renal/bladder (n = 2), heart (n = 1), stomatitis (n = 1), and central nervus system (n = 1). Grade 4 toxicity included lung only (n = 1).

†Documented infection included bacteremia (n = 27), pneumonia (n = 5), aspergillus infection (n = 3), subcutaneous abscess (n = 2), and others (n = 3).

‡The median total doses of each conditioning regimen were as follows: Flu (138 mg/m^2), Mel (80 mg/m^2), Bu (8 mg/kg), and CY (60 mg/kg). Antithymocyte globulin was also used in 8 patients (Flu alone [n = 5], Flu + Mel [n = 1], and Flu + Bu [n = 2]). Other conditioning regimens included Flu plus thiotepa (n = 2) or etoposide (n = 1). Twelve patients received 2 Gy TBI and 23 patients received 4 Gy TBI.

§Other prophylaxis included CSP/TAC plus mycophenolate mofetil (n = 7) or prednisolone (n = 2).

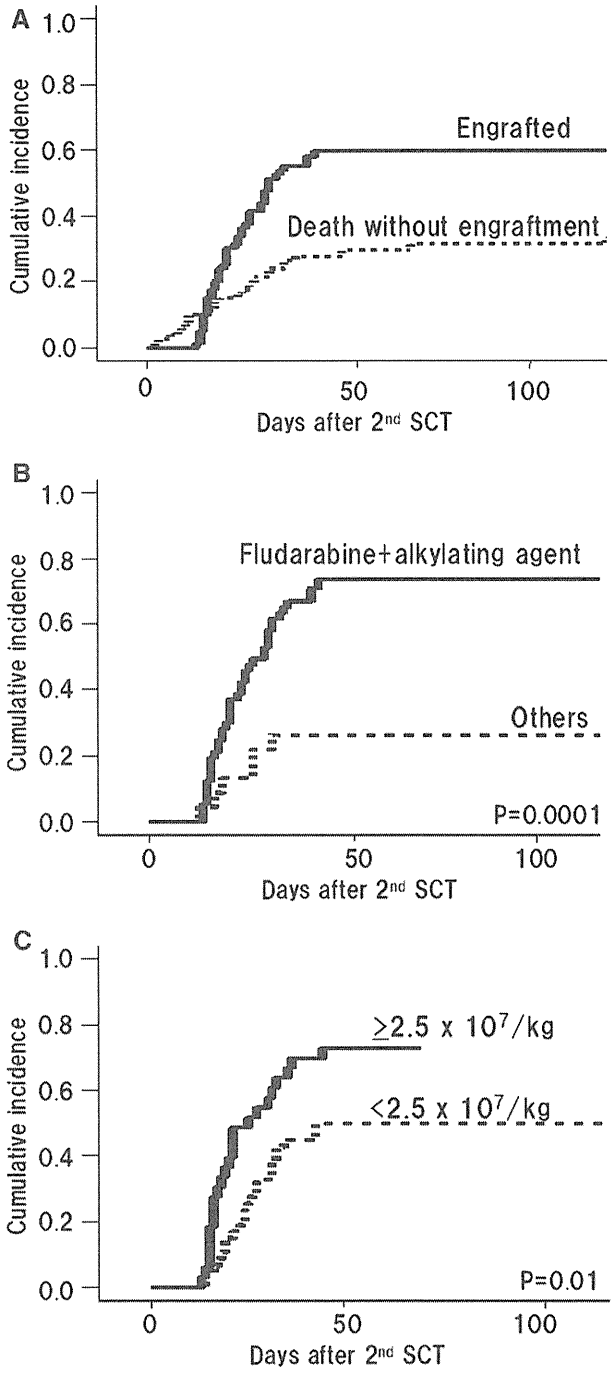

Figure 1. Cumulative incidence of neutrophil engraftment. (A) The cumulative incidences of neutrophil engraftment (solid line) and death without engraftment (dotted line) are shown. (B) The cumulative incidence of neutrophil engraftment was higher in patients who received alkylating agent–containing regimen (solid line) than in those who did not (dotted line) ($P = .0001$). (C) The cumulative incidence of neutrophil engraftment was higher in patients who received graft containing TNC $\geq 2.5 \times 10^7$/kg than in those who did not ($P = .01$).

The incidence of neutrophil engraftment was higher in patients who received alkylating agents including melphalan, busulfan, and cyclophosphamide as part of conditioning for the second SCT (73% versus 26%, $P = .0001$), as shown in Figure 1B. The engraftment rate was similar among the 3 types of conditioning regimens that included alkylating agents. The incidence of neutrophil engraftment was higher when patients received 2-4 Gy TBI (71% versus 50%, $P = .03$). The engraftment rate was higher in patients who received graft containing a higher number of TNC $\geq 2.5 \times 10^7$/kg than in those who received $<2.5 \times 10^7$/kg (73% versus 50%, $P = .01$) (Figure 1C). When 2.0×10^7/kg was used as a cutoff for TNC, the engraftment rate tended to be higher in patients who received graft that contained higher TNC (65% versus 36%, $P = .08$). The standard-risk group at the first SCT was also associated with a higher neutrophil engraftment than the high-risk group (70% versus 43%, $P = .02$). The number of $CD34^+$ cells was evaluated in 68 patients with a median of 0.6×10^5/kg (range: 0.1-4.22), and this was not associated with the neutrophil engraftment rate. In 14 patients who received MTX for GVHD prophylaxis after the second SCT, neutrophil engraftment was delayed (median 31 days; range: 14-44 days) compared to those who did not receive MTX (median 21 days; range: 13-42 days), although the ultimate engraftment rates were similar (50% versus 61%, $P = .26$). In 8 patients who received ATG for the second SCT, 3 (38%) achieved neutrophil engraftment. Anti-HLA antibody was examined before the second SCT in 28 patients. In 9 patients with positive anti-HLA antibody, only 2 (22%) achieved engraftment and 6 (67%) died within 28 days after RICBT. Among 47 patients who obtained neutrophil engraftment, with chimerism analyses available in 44 patients at a median of 30 days (range: 12-119), 42 patients (95%) achieved complete donor chimerism, and 2 continued to show mixed chimerism. Among 61 patients who survived for more than 28 days, 31 patients (51%) achieved platelet engraftment that was more than 20,000/μL, and subsequently 27 patients (44%) obtained platelet engraftment more than 50,000/μL. The median day of last platelet transfusion was 53 days (range: 15-197) after the second SCT.

RRT and aGVHD (Table 3)

Grade 3 or 4 RRT excluding febrile neutropenia was recognized in 48 patients (60%) after the second SCT, which included toxicities associated with stomatitis (n = 8), liver damage (n = 20), diarrhea (n = 11), renal and bladder (n = 10), heart (n = 8), lung (n = 21), and central nervous system (CNS) (n = 18). The details of CNS complication were limbic encephalitis including HHV-6 encephalitis (n = 8), brain hemorrhage (n = 3), cerebral aspergillosis (n = 2), and others (n = 5). TRM was 75% in 48 patients who developed grade 3 or 4 organ toxicities, and 28% in the remaining 32 patients without grade 3 or 4 organ toxicities after the second SCT. The probabilities of grades II-IV and III-IV aGVHD were 25% and 11%, respectively,

Table 3. Outcomes after the Second SCT (RICBT)

Parameters	n = 80
The engraftment rate in 61 patients surviving >28 days	45 (74%)
GF in 61 patients surviving >28 days	13 (21%)
Grade 3-4 organ toxicities*	48 (60%)
Documented infection	58 (63%)
CMV antigenemia	36 (45%)
Acute GVHD	
Grade II-IV	20 (25%)
Grade III-IV	9 (11%)
Relapse	12 (15%)
Death	51 (64%)
The median day of death after second SCT	37 days (range: 2-611)
Causes of death	
Infection	33 (65%)
Bacterial	14
Fungal	6
Viral	8
Complex or unknown	5
Relapse	6 (12%)
Acute GVHD	1 (2%)
Other†	11 (22%)

SCT indicates stem cell transplantation; RICBT, reduced-intensity cord blood transplantation; GF, graft failure; CMV, cytomegalovirus; GVHD, graft-versus-host disease.
*Grade of organ toxicities was evaluated by the CTCAE v3.0 [40].
†Other causes included cerebral hemorrhage (n = 3), multiorgan failure (n = 2), thrombotic microangiopathy (n = 2), veno-occulusive disease of the liver (n = 1), interstitial pneumonitis (n = 1), heart failure (n = 1), and secondary malignancy (n = 1).

and only 1 patient who had grade IV aGVHD died of GVHD.

TRM, Relapse, and Causes of Death (Table 3)

Fifty-one patients (64%) died at a median of 37 days (range: 2-611) after the second SCT. The cumulative incidence of TRM was 45%, 56%, and 61% at day 100, 1 year, and 2 years, respectively (Figure 2A), and infection was the most frequent cause of death. Notably, death that was directly related to bacterial infection occurred during prolonged neutropenia in the first 2 months after the second SCT. In 11 patients with grade 3 or 4 carryover organ toxicities at the second SCT, 8 (73%) died of TRM (Figure 2B). TRM was higher in patients who received an oral busulfan-based regimen (72%) than in those who received melphalan-based (50%) or cyclophosphamide-based (53%) regimens. Underlying malignancy relapsed in 12 patients (16%) at a median of 158 days (range: 22-781) after the second SCT, and 3 patients received a third SCT after relapse. Overall, 6 patients died of disease recurrence.

Survival

The median follow-up time in the surviving patients was 325 days (range: 89-1069) after the second SCT. The Kaplan-Meier curves of OS and PFS of all 80 patients are shown in Figure 3A. The estimated

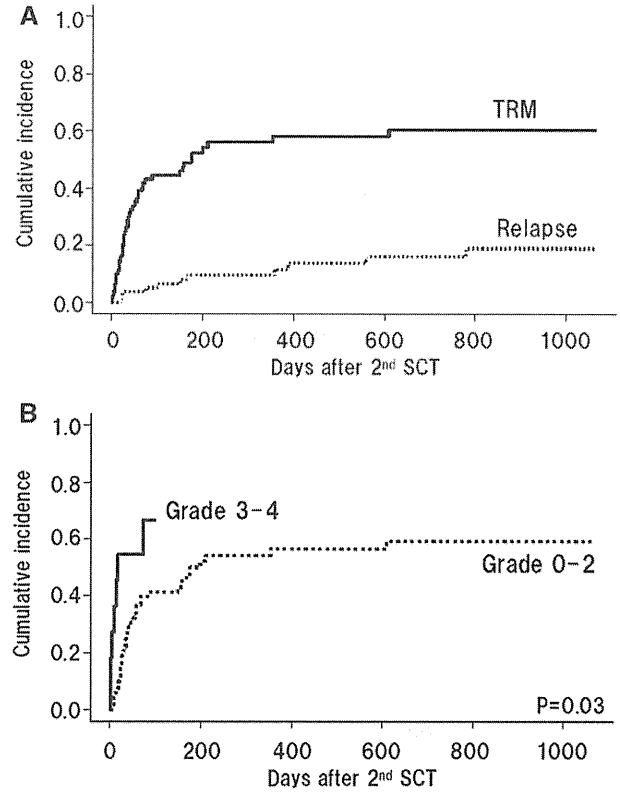

Figure 2. Cumulative incidence of transplantation-related mortality (TRM) and relapse. (A) The cumulative incidences of TRM (solid line) and relapse (dotted line) are shown. (B) The cumulative incidence of TRM was higher in patients who had grade 3 or 4 carryover organ toxicity before the second SCT (solid line) than in those who did not (dotted line) ($P = .03$).

rates of OS and PFS at 1 year after the second SCT were 33% and 29%, respectively. The OS was worse in 11 patients who had grade 3 or 4 carryover organ toxicities at the second SCT compared to the other 69 patients. OS was significantly better in patients who had standard-risk disease at the first SCT than in those who had high-risk disease (Figure 3B).

Factors Associated with Engraftment and OS

In a univariate analysis, standard risk at the first SCT, PS 0-1 at the second SCT, conditioning that included alkylating agents or 2-4 Gy TBI, and a higher dose of infused TNC ($\geq 2.5 \times 10^7$/kg) were significantly associated with a higher probability of engraftment. Carryover organ toxicities ($P = .09$) and infection at the second SCT ($P = .07$) were also included in a multivariate analysis. The type of engraftment failure after first SCT did not have an influence on outcome after the second SCT (primary versus secondary). As a result, higher TNC dose ($\geq 2.5 \times 10^7$/kg: hazard ratio [HR] = 2.14, 95% confidence interval [CI], 1.29-3.52; $P = .003$), conditioning that included alkylating agents (HR = 3.70, 95% CI, 1.51-9.09; $P = .005$), and standard risk at first SCT (HR = 2.04, 95% CI, 1.06-3.85; $P = .03$)

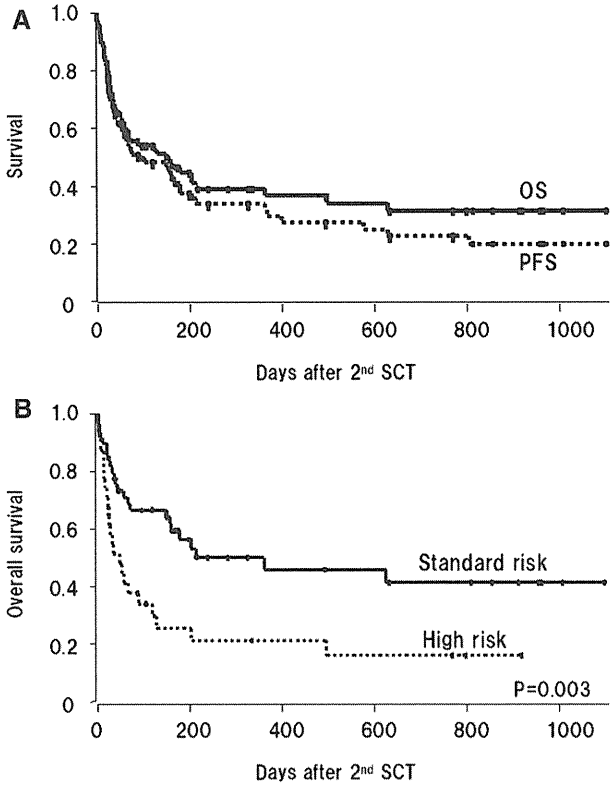

Figure 3. OS and PFS. (A) The Kaplan-Meier estimates of OS (solid line) and PFS (dotted line) are shown. (B) OS in patients who were high risk at the first SCT (dotted line) was lower than that in those who were standard risk (solid line) ($P = .003$).

remained significant in the multivariate Cox proportional hazards regression analysis (Table 4). In a multivariate Cox proportional hazards regression analysis of OS, high-risk disease at the first SCT (HR = 2.14, 95% CI, 1.20-3.81; $P = .01$) and grade 3 or 4 carryover organ toxicities at the second SCT (HR = 2.84, 95% CI, 1.33-6.06; $P = .007$) were associated with an increased risk of poor OS (Table 5).

DISCUSSION

Based on data obtained from this large cohort of patients, we showed that neutrophil engraftment can be achieved in >70% of adult patients who received RICBT as salvage therapy for GF. Although our cohort was composed of rather older patients, the engraftment rate was comparable to that reported in primary CBT [17,18,29,34]. Considering the poor PS and carryover infection and organ toxicities, salvage therapy with RICBT is a feasible option that gave a 1-year OS of 33%. Nevertheless, this procedure is still associated with a high rate of TRM (45% at day 100), 60% of which was related to infectious complications, and we performed analyses to identify the risk factors for engraftment and survival.

Guardiola et al. [22] reported in 82 patients with various hematological diseases who underwent second allogeneic SCT that the neutrophil engraftment rate and 3-year OS were 70% and 30%, respectively. They showed that a longer intertransplant interval of ≥80 days was associated with a higher neutrophil engraftment rate and survival in a multivariate analysis. McCann et al. [19] also reported that a longer interval of ≥60 days was associated with a higher engraftment rate and OS in 41 patients with aplastic anemia. In our study, we did not find any association between interval and neutrophil engraftment or OS, and this discrepancy may be because of differences in the cohorts of patients evaluated. In the report by Guardiola et al. [22], the proportions of patients who experienced secondary GF and who received transplant from an HLA-matched sibling donor were much higher than in our study (66% versus 20%, 78% versus 6%, respectively). Grandage et al. [25] reported successful engraftment in 12 patients who underwent a second SCT from the same unrelated donor after GF. In the current study, however, it was not possible to perform a second SCT using an unrelated BM donor because most patients had poor PS, organ toxicities, or infections with prolonged cytopenia (ANC $<100/mm^3$).

Our data confirmed that a higher number of infused CB cells (TNC $\geq 2.5 \times 10^7$/kg) was associated with a higher probability of neutrophil engraftment after the second RICBT ($P = .01$), which was consistent with previous reports [4,42]. Because the median body weight of patients in this study was 55 kg, CB units containing $>2.0 \times 10^7$/kg were available in >80% of patients. A double cord blood unit strategy might be favorable as previous reported, because a higher cell dose was associated with better survival [43]. Although in a previous study by Wagner et al. [44], the total number of $CD34^+$ cells was reported to be a major determinant of neutrophil recovery after CBT, our present findings did not confirm this point. Another discrepancy with previous reports [44] is that HLA disparity between the donor and recipient was not related to the engraftment rate in our study. We also examined the effect of HLA mismatch with serological HLA-A, B and allele DRB1 except for 5 patients whose allele typing was not performed. However, the results remained unchanged, and there was no impact on engraftment and OS.

The need for an intensive immunosuppressive conditioning regimen before the second SCT for GF depends on the mechanism of GF, and we found that a fludarabine-based regimen that included alkylating agents was associated with a higher neutrophil engraftment rate. Whereas the use of cytotoxic drugs is not mandatory before stem cell boost for patients who have poor graft function [23,24], intensive immunosuppressive conditioning is essential to suppress residual host T and natural killer cells to

Table 4. Univariate and Multivariate Analysis of Factors Predicting Engraftment after the Second SCT

Covariates	Proportion (%)*	Univariate	Multivariate	
		P	Hazard Ratio (95% CI)	P
Disease risk at the first SCT†		.02		.03
Standard risk	70		2.04 (1.06-3.85)	
High risk	43		1.00	
Type of graft failure		.57		—
Primary	58		—	
Secondary	56		—	
Interval between the first SCT and second SCT		.87		—
<50 days	60		—	
≥50 days	59		—	
PS		.01		—
0-1	81		—	
2-4	46		—	
Carryover organ toxicities at the second SCT‡		.09		—
Grade 0-2	65		—	
Grade 3-4	27		—	
Carryover infection at the second SCT		.07		—
Febrile neutropenia/none	69		—	
Documented infection	51		—	
Conditioning§		.0001		.005
Alkylating agent–containing	73		3.70 (1.51-9.09)	
Other	26		1.00	
TBI		.03		—
2-4 Gy TBI	71		—	
No TBI	50		—	
TNC of the CB		.01		.003
$\geq 2.5 \times 10^7$/kg	73		2.14 (1.29-3.52)	
$< 2.5 \times 10^7$/kg	50		1.00	

SCT indicates stem cell transplantation; PS, performance status; TBI, total-body irradiation; TNC, total nucleated cells; CB, cord blood; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; CML, chronic myelogeneous leukemia; MDS, myelodysplatic syndrome; RAEB, refractory anemia with excess blasts; CI, confidence interval.

Other covariates examined included sex, patient age, conditioning of the first SCT, use of methotrexate as GVHD prophylaxis, and numbers of selorogic HLA mismatch in graft-versus-host and host-versus-graft directions.

*Proportions of patients who achieved neutrophil engraftment.

†Standard risk included acute leukemia and non-Hodgkin lymphoma in any complete remission, CML in any chronic phase, and aplastic anemia. High risk included all other types of leukemia and non-Hodgkin lymphoma categories, and MDS-RAEB.

‡Grade of organ toxicities was evaluated by the CTCAE v3.0 [40].

§Alkylating agents included melphalan, busulfan, and cyclophosphamide. Other conditioning included fludarabine alone and a combination of fludarabine plus thiotepa or etoposide.

overcome immunologic rejection [21,26,45]. As previously reported in patients with aplastic anemia [19,46], the addition of 2-4 Gy TBI to the RIC regimen increased the probability of engraftment in a univariate analysis, although it did not have a significant effect in a multivariate analysis. In our preliminary data, 6 of the 10 patients who received second CBT without cytotoxic conditioning regimen (ie, ATG only, steroid only, etc.) experienced GF again after second SCT. Whereas the addition of alkylating agent and low-dose TBI to the conditioning regimen for the second RICBT enhanced neutrophil engraftment, it did not affect the overall outcomes in our study. To determine the best conditioning regimen for salvage RICBT after GF, further studies to evaluate regimens including fludarabine plus melphalan or cyclophosphamide with or without 2-4 Gy TBI will be required.

In our study, the TRM early after the second RICBT was extremely high (45% at day 100), mainly because of infectious complications, which was consistent with previous reports on CBT [5,17,29,30,47]. This is probably because of a prolonged period of severe neutropenia before and after the second RICBT in patients complicated with GF, which incubated carryover infections. To reduce the incidence of infection-related TRM, frequent monitoring and extensive treatment including granulocyte transfusion to support the intertransplant period may be needed [48]. Alternatively, the earlier application of RICBT while patients are still in better condition without infection may be preferred to reduce TRM.

When patients require a second SCT for GF, the selection of the donor source is critical. Based on the feasibility of second RICBT in our study, we suggest that CB carries the highest priority for selection because of its ready availability. Although the possibility of a second SCT or boost of stem cells from the same related donor of the first SCT has been reported [19,22], 75% of our patients had undergone CBT at the first transplant, which reflects the difficulty of finding a suitable donor. Another possibility is a second SCT from a haploidentical related donor [49,50]. The more rapid neutrophil engraftment after SCT using PBSC

Table 5. Univariate and Multivariate Analysis of Overall Survival after the Second SCT

Covariates	Proportion at 1 Year (%)	Univariate	Multivariate	
		P	Hazard Ratio (95% CI)	*P*
Disease risk at the first SCT*		.03		.01
Standard risk	50		1.00	
High risk	26		2.14 (1.20-3.81)	
Type of graft failure		.87		—
Primary	36		—	
Secondary	39		—	
Interval between the first SCT and second SCT		.38		—
<50 days	40		—	
≥50 days	31		—	
PS		.2		—
0-1	39		—	
2-4	35		—	
Carryover organ toxicities at the second SCT†		.001		.007
Grade 0-2	41		1.00	
Grade 3-4	0		2.84 (1.33-6.06)	
Carryover infection at the second SCT		.14		—
Febrile neutropenia/none	46		—	
Documented infection	27		—	
Conditioning‡		.69		—
Alkylating agent–containing	35		—	
Other	40		—	
TBI		.56		—
2-4 Gy TBI	37		—	
No TBI	37		—	
TNC of the CB		.77		—
≥2.5 × 10^7/kg	41		—	
<2.5 × 10^7/kg	33		—	

SCT indicates stem cell transplantation; PS, performance status; TBI, total-body irradiation; TNC, total nucleate cells; CB, cord blood; GVHD, graft-versus-host disease; HLA. human leukocyte antigen; CML, chronic myelogeneous leukemia; MDS, myelodysplatic syndrome; RAEB, refractory anemia with excess blasts; CI, confidence interval.

Other covariates examined included sex, patient age, conditioning of the first SCT, use of methotrexate as GVHD prophylaxis, and numbers of serological HLA mismatch in graft-versus-host and host-versus-graft directions.

*Standard risk included acute leukemia and non-Hodgkin lymphoma in any complete remission, CML in any chronic phase, and aplastic anemia. High risk included all other types of leukemia and non-Hodgkin lymphoma categories, and MDS-RAEB.

†Grade of organ toxicities was evaluated by the CTCAE v3.0. [40].

‡Alkylating agents included melphalan, busulfan, and cyclophosphamide. Other conditioning included fludarabine alone and a conbination of fludarabine plus thiotepa or etoposide.

from a haploidentical donor may decrease the risk of infectious complications in patients suffering from GF. However, compared to CBT, the feasibility of this procedure has not yet been established and the incidence of acute GVHD increases. In addition, collection of autologous stem cells prior to CBT might be an option to salvage a fraction of patients who experienced GF as previously reported [51]. Nevertheless, further studies are warranted to determine which types of transplant, CBT or SCT from a haploidentical related donor, can achieve better outcomes for patients suffering from GF.

This study has several inherent limitations. First, the patients and transplantation characteristics including the conditioning regimen, GVHD prophylaxis, and supportive care varied among the different centers. Second, the timing of and general conditions at the second RICBT differed among patients. Third, there may be unrecognized biases because only successful cases may have been collected. Finally, the duration of follow-up for patients in this study was too short to draw any definite conclusions. Nevertheless, the large cohort of 80 patients who received RICBT as salvage therapy for GF in the current study allowed us to make several clinically relevant observations.

In conclusion, we suggest that salvage therapy with a second RICBT is a feasible therapeutic option for patients who are suffering from GF. To achieve stable neutrophil engraftment after the second RICBT, conditioning with fludarabine plus alkylating agents and the infusion of CB containing ≥2.5 × 10^7/kg cells are preferable. A high TRM early after RICBT emphasizes the need for the earlier application of RICBT while patients still have better PS and have not yet acquired infection and organ toxicity. Prospective trials are needed to determine the ultimate utility of rescue RICBT using a fludarabine-based regimen including alkylating agents for patients suffering from GF.

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AUTHORSHIP STATEMENT

F. Waki and T. Fukuda played a major role in designing and performing the research, verifying the integrity of and analyzing the data, and writing the manuscript. Y. Kanda played a major role in the statistical analyses and in developing the concept of the research. K. Masuoka, T. Yamashita, A. Wake, and S. Takahashi designed the research and contributed vital data to generate the final database. Y. Takaue and S. Taniguchi designed the research and contributed to writing or interpreting relevant parts of the manuscript. All other coauthors contributed vital data to generate the final database and interpreted relevant parts of the manuscript.

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APPENDIX

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ORIGINAL ARTICLE

Allogeneic cord blood transplantation for adult acute lymphoblastic leukemia: retrospective survey involving 256 patients in Japan

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We investigated the efficacy of cord blood transplantation (CBT) for adult acute lymphoblastic leukemia (ALL) by reviewing medical records of 256 patients reported to the Japan Cord Blood Bank Network between June 1997 and August 2006. Cumulative incidence of neutrophil engraftment at day 100 was 78%. Infused CD34-positive cell dose ($>1 \times 10^5$ cells/kg) was associated with successful neutrophil engraftment. Cumulative incidence of grade II-IV acute graft-versus-host disease (GVHD) at day 100 was 37%. A 2-year disease-free and overall survival (OS) rates were 36% and 42%, respectively. Multivariate analysis showed that age (51 or older vs younger than 50) (hazard ratio 1.9, 95% confidence interval (CI), 1.3-2.8, $P=0.001$), disease status (non-remission vs remission) (hazard ratio 2.2, 95% CI, 1.5-3.2, $P<0.0001$), grade III-IV acute GVHD (hazard ratio 2.0, 95% CI, 1.2-3.2, $P=0.006$) and absence of chronic GVHD (hazard ratio 2.4, 95% CI, 1.1-5.1, $P=0.02$) were negatively associated with OS. CBT is effective for some patients with advanced ALL. It is worth considering for further evaluation.

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Keywords: cord blood transplantation; adult acute lymphoblastic leukemia; graft-versus-host disease; graft-versus-leukemia effect; engraftment

INTRODUCTION

Adult patients with acute lymphoblastic leukemia (ALL) achieved complete remission with induction chemotherapy in the high rate of 85-90%, although most of them relapse and finally die of disease progression. The indication and timing of allogeneic hematopoietic cell transplantation continue to be debated for adult ALL.[1-5]

Umbilical cord blood is a promising alternative for allogeneic transplantation. It has a great advantage over bone marrow and peripheral blood because of its immediate availability and lack of invasive interventions to donors. The value of cord blood transplantation (CBT) has been intensively evaluated in previous studies.[6-9]

Graft-versus-leukemia effects are associated with graft-versus-host disease (GVHD) in allogeneic transplantation for hematological malignancies.[10] Some clinical studies suggested the presence of graft-versus-leukemia effects after CBT in pediatric and adult patients,[7,11-14] most of them were small sized. At present, limited information is available on the graft-versus-leukemia effects after CBT for adult ALL.

We conducted a retrospective nation-wide study to investigate the usefulness of CBT for adult ALL.

PATIENTS AND METHODS

Data collection

The recipient's clinical data were provided by the Japan Cord Blood Bank Network (JCBBN). All 11 CB banks in Japan are affiliated to JCBBN. The data management committee of JCBBN collects all the recipients' clinical information at day 100, then 1-5 years after CBT. The numbers of nucleated and CD34-positive cells were provided by the CB banks. The numbers of these cells were measured before cryopreservation. The information on the number of CD3-positive cells in the cord blood was not available.

Between March 1998 and June 2006, 424 adult patients with ALL received CBT and were registered to JCBBN. All recipients received a single cord blood unit. We excluded 77 patients with a history of any types of allogeneic transplantation before CBT. We also excluded adult T-cell leukemia/lymphoma because of its different disease entity. Finally, a total of 256 patients met the criteria. Some of them were reported previously in other studies.[15-18]

Approval for this study was obtained from the JCBBN institutional review board. CB units were provided with written informed consent in accordance with the Declaration of Helsinki Principles, which approved by the institutional review board of each participating institution.

Definitions and endpoints

Day of neutrophil engraftment was defined as the first of 3 consecutive days on which absolute neutrophil count was >500 cells/μl. Graft failure was diagnosed when neutrophil recovery was not achieved within 60 days of transplantation. GVHD was graded according to the criteria published previously.[19] Relapse was defined as presence of ALL cells based on morphological evaluation of the bone marrow or other sites. Patients who had never achieved complete remission after CBT were considered to have

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progression on day 0. Treatment-related mortality (TRM) was defined as deaths without progression of ALL. Reduced-intensity regimens were defined as reported previously.[20,21]

Statistical analysis

The available data were as of October 2006. The data sets were fixed in March 2008, and we analyzed them between April 2008 and October 2010. The probabilities of overall survival (OS) and disease-free survival (DFS) were estimated by the Kaplan-Meier method. Cumulative incidence curves were used in a competing-risk setting to calculate the probability of engraftment, acute and chronic GVHD, relapse and TRM. For neutrophil and platelet engraftment, death before neutrophil and platelet recovery within 60 days of transplant was the competing event; for GVHD, disease relapse and engraftment failure without GVHD, and deaths within 60 days of transplant without GVHD were the competing events; for relapse, death without relapse was the competing event; and for TRM, death with disease relapse was the competing event.[22]

Associations between potential prognostic factors and outcomes were evaluated using the Cox's proportional hazard regression models. The following variables were considered as covariates: age, body weight, human leukocyte antigen mismatch, blood-type mismatch, sex mismatch, infused nucleated cell dose and CD34-positive cell dose, status of underlying disease at transplantation, chromosomal abnormality, preparative regimens and GVHD prophylaxis. Occurrence of acute GVHD was added to the models as a time-dependent covariate. SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

RESULTS

Patients' characteristics

Patients' characteristics are shown in Table 1. The median follow-up of the surviving patients was 20.5 months (range, 1.1-86.4). Ph-positive ALL comprised 39% of the population analyzed. No patients received tyrosine kinase inhibitors for maintenance following CBT.

Engraftment

Of the 256 patients, 190 (74%) achieved primary neutrophil engraftment at a median of day 24 (range 11-51) and 44 (17%) died without engraftment. Median time to platelet recovery (>20 000/μl) was day 46 (range 20-179).

Of the remaining 22, who survived without neutrophil engraftment, 14 received second transplantation as stem-cell rescue. The other eight survived without neutrophil engraftment. Their disease status at last follow-up was remission after autologous recovery ($n=4$) and non-remission ($n=4$). The diagnosis of primary and secondary graft failure was established in 22 and 10 patients, respectively.

Cumulative incidence of neutrophil engraftment at day 100 was 78% (95% confidence interval (CI), 73-84%). The cumulative incidence of platelet recovery to 20 000/μl at day 100 was 64% (95% CI, 58-70%). The prognostic factors for neutrophil and platelet engraftment were shown in Table 2.

GVHD

The cumulative incidence of grade II-IV acute GVHD at day 100 is 37% (95% CI, 30-43%). No prognostic factors were identified in multivariate analysis for grade II-IV acute GVHD, whereas the number of infused nucleated cells, conditioning regimens, GVHD prophylaxis and the number of human leukocyte antigen disparities were examined.

Chronic GVHD was diagnosed in 41 of the 180 evaluable patients, who survived longer than 100 days. The cumulative incidence of chronic GVHD at 2 years after CBT was 24% (95% CI, 21-28%). Of the 41 patients, 27 and 14 developed limited and extensive diseases, respectively. The presence of grade III-IV acute GVHD and the number of CD34-positive cells were the prognostic factors of chronic GVHD (Table 2).

Table 1. Patient characteristics

Variables	n
Patients	256
Male/female	120/136
Median age, years (range)	40 (16-74)
Median body weight, kg (range)	54 (23.5-81.5)
Median duration from diagnosis to transplantation, months (range)	7 (2-127)
Disease linage	
B cell/T cell/other/unknown	145/29/42/39
Chromosomal abnormality	
t(9;22)	100
t(4;11)	8
Complex karyotype	36
Other abnormality	21
Normal	53
Not avairable	38
Disease status at transplant	
First remission	125
Second remission	44
Third remission	8
Primary refractory	20
Relapse	58
Unknown	1
Preparative regimens	
Myeloablative	
TBI (10-14 Gy) containing regimens	184
Non-TBI regimens	6
Reduced intensity regimens	
TBI (2-8 Gy) containing regimens	50
Non-TBI regimens	16
GVHD prophylaxis	
CyA+MTX	115
CyA+PSL	5
CyA only	38
FK+MTX	45
FK only	42
Others	6
Not avairable/unspecified	5
Infused cord blood	
Median number of nucleated cells, 10^7/kg (range)	2.50 (1.51-5.00)
Median number of CD34-positive cells, 10^5/kg (range)	0.78 (0.08-5.80)
Number of HLA-A, B and DRB1 mismatches	
GVHD direction 0/1/2/3	27/82/143/2
Rejection direction 0/1/2/3	27/86/140/1
ABO compatibility	
Match	81
Major/minor mismatch	111/62

Abbreviations: CyA, cyclosporin; FK, tacrolimus; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; MTX, methotrexate; PSL, predonisolone; TBI, total-body irradiation.

Complications other than GVHD

Infections were documented in 129 patients (50%). They included bacterial ($n=85$), fungal ($n=16$) and viral infection ($n=52$). Cytomegalovirus antigenemia was detected in 111 patients (43%).

Non-infectious complications other than GVHD occurred in 41 patients (16%); interstitial pneumonitis ($n=13$), acute respiratory distress syndrome ($n=12$), hepatic veno-occlusive disease

Table 2. Prognostic factors following CBT in multivariate analysis

	Factors		*Hazard ratio*	*P-value*	*95% Confidence interval*
Neutrophil engraftment	Number of CD34-positive cells	$<1\times10^5$/kg vs $\geqslant1\times10^5$/kg	1.58	<0.0001	1.27-1.95
Platelet engraftment ($\geqslant$20 000/mm^3)	Number of CD34-positive cells	$<1\times10^5$/kg vs $\geqslant1\times10^5$/kg	1.92	<0.0001	1.33-2.26
	Disease status	non-remission vs remission	1.88	0.004	1.23-2.88
	ABO misatch	Major/minor mismatch vs match	1.34	0.003	1.10-1.64
	Number of nucreated cells	per 1×10^7/kg	0.63	0.005	0.46-0.87
	GVHD prophylaxis	With MTX vs no MTX	0.62	0.02	0.42-0.92
Relapse	Disease status	non-remission vs remission	2.28	0.0012	1.38-3.76
	Number of nucreated cells	per 1×10^7/kg	1.84	0.002	1.25-2.71
Acute GVHD	*	*			
Chronic GVHD	Acute GVHD	3-4 vs 0-2	1.92	0.02	1.12-3.28
	Number of CD34-positive cells	$<1\times10^5$/kg vs $\geqslant1\times10^5$/kg	1.51	0.04	1.02-2.23
Overall survival	Disease status	non-remission vs remission	2.2	< 0.0001	1.53-3.15
	Age	51 or older vs younger than 50	1.89	0.0009	1.30-2.75
	Acute GVHD	3-4 vs 0-2	1.98	0.006	1.21-3.24
	Chronic GVHD	Absent vs present	2.39	0.02	1.13-5.08
Disease-free survival	Disease status	non-remission vs remission	2.51	< 0.0001	1.78-3.56
	Acute GVHD	3-4 vs 0-2	1.65	0.04	1.02-2.66

Abbreviations: CBT, cord blood transplantation; GVHD, graft-versus-host disease. *Indicates no prognostic factors were identified in multivariate analysis.

($n=13$), thrombotic microangiopathy ($n=20$) and hemorrhage from any sites ($n=18$).

A total of 89 patients (35%) died of TRM at a median day of 46 days (range, 4-466). The cumulative incidence of TRM at 2 years after CBT was 32% (95% CI, 26-38%). Causes of death included infection ($n=36$), acute GVHD ($n=11$), veno-occlusive disease ($n=10$), graft failure ($n=6$), idiopathic pneumonia syndrome ($n=7$), thrombotic microangiopathy ($n=6$), multiple organ failure ($n=6$), hemorrhage ($n=5$) and infarction ($n=2$).

Response to CBT

Out of 78 patients, 24 who were transplanted in non-remission achieved durable remission lasting 60 days or longer and 19 survived without relapse longer than 1 year.

Relapse

The cumulative incidence of relapse at 2 years was 43% (95% CI, 38-47%). Disease status and the number of nucleated cells at cryopreservation were the prognostic factors in multivariate analysis (Table 2).

DFS and OS

Probabilities of 2-year DFS and OS were 36% (95% CI, 33-39%) and 42% (95% CI, 39-45%), respectively. In the 177 patients transplanted in remission, DFS and OS were 45% (95% CI, 41-49%) and 51% (95% CI, 46-55%), respectively. In those who were transplanted in non-remission, DFS and OS were 16% (95% CI, 12-21%) and 23% (95% CI, 17-28%), respectively (Figure 1). In patients with Ph-positive ALL, probabilities of 2-year DFS and OS were 32% (95% CI, 27-37%) and 43% (95% CI, 37-48%), respectively.

Multivariate analysis showed that age (51 or older vs younger than 50), disease status (non-remission vs remission), absence of chronic GVHD and grade III-IV acute GVHD were negatively associated with OS (Table 2). Disease status and grade III-IV acute GVHD were negatively associated with DFS (Table 2).

OS was shown according to the presence of acute and chronic GVHD among the 180 patients who survived longer than 100 days (Figure 2). Patients with grade III-IV acute GVHD had poor prognosis compared with those with grade 0-I and grade II acute GVHD. The patients who developed chronic GVHD was expected higher survival rates than patients of absence chronic GVHD.

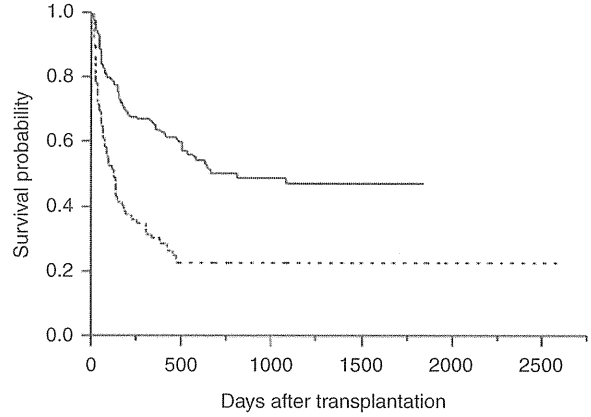

Figure 1. OS according of disease status at CBT. Probability of 2-year OS rates of patients in remission and non-remission were 51% (95% CI, 46-55) and 23% (95% CI, 17-28), respectively ($P<0.001$). Solid line indicates patients in remission ($n=177$). Broken line indicates patients in non-remission ($n=78$).

DISCUSSION

The present study showed that 24 of the 78 patients transplanted in primary refractory or relapse achieved durable remission. Considering that combination chemotherapy is usually ineffective at prolong survival in adult patients with advanced or Ph-positive ALL,[23,24] the long-term DFS after CBT suggests that durable allogeneic immune reactions continue to suppress leukemic progression after CBT. These findings support the hypothesis that graft-versus-leukemia effects exist after CBT for adult ALL.

Cumulative incidence of grade II-IV acute GVHD at day 100 is 37%. These findings were comparable with previous studies on CBT (26-60%).[6,25] However, it should be noted that grade III-IV acute GVHD negatively impacted OS, and that there was no significant differences between patients with grade 0-I acute GVHD and those with grade II acute GVHD. These findings suggest that control of acute GVHD is important in CBT for ALL.

Previous studies showed that presence of chronic GVHD protected relapse following CBT for adult ALL,[9,14] whereas it had

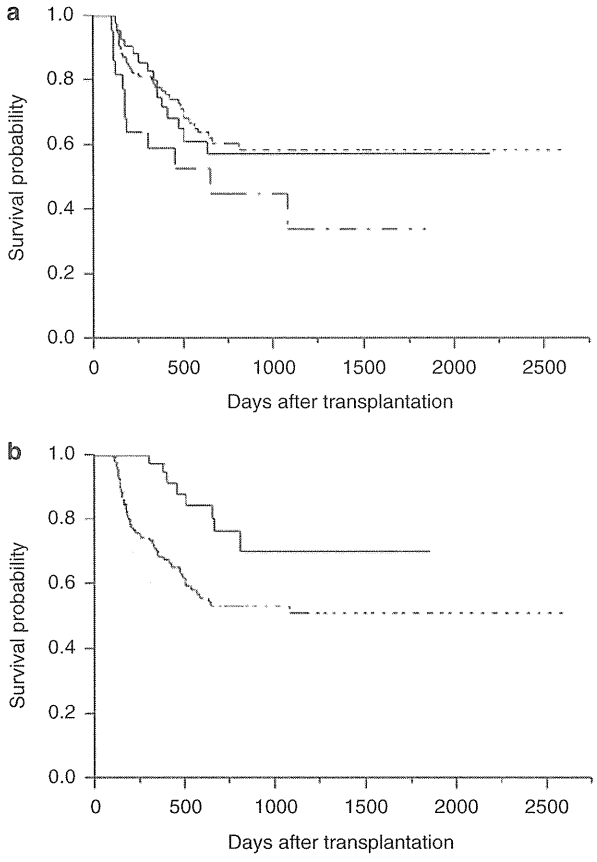

Figure 2. (**a**) Influence of acute GVHD on OS. Solid line indicates patients with acute GVHD less than grade II ($n=99$). Broken line indicates patients with grade II acute GVHD ($n=48$). Wavy line indicates patients with grades III-IV acute GVHD ($n=33$). (**b**) Influence of chronic GVHD on OS. Solid line indicates patients with chronic GVHD ($n=41$). Broken line indicates patients without chronic GVHD ($n=139$).

no significant impact on OS and DFS. Chronic GVHD was not identified as a prognostic factor for relapse in either analysis for patients transplanted in remission or that for patients with active disease at time of transplant. These findings were in contrast to our study, in which chronic GVHD was associated with better OS. Notably, chronic GVHD was limited-type in two-thirds of the patients in this study, and extensive-type chronic GVHD was dominant in the previous study.[14] The mild features of chronic GVHD among Japanese patients following CBT probably lead to the favorable impact of chronic GVHD on OS.[15]

This study provided detailed information on the prognostic factors of CBT for adult ALL patients. The number of infused CD34-positive cells was associated with engraftment. Concerning OS, patients' age, disease status and development of acute and chronic GVHD were significant risk factors. These findings were comparable to previous reports.[7,25]

Clinical decision making as to when to proceed with CBT in patients with ALL is difficult. Disease status is an important prognostic factor in CBT. As some patients with second or further remission and those with induction failure achieved durable remission after CBT, they are candidates for CBT. However, it is controversial whether CBT should be offered to patients in first remission, as CBT is associated with a high mortality.

TRM is a significant concern in CBT. The present study demonstrated that most TRM occurred early after transplantation, as consistent with the previous studies.[9,17] Early infection before engraftment is a significant complication in CBT, and the long duration of neutropenia might have a major role in its pathogenesis. As the higher number of infused CD34-positive cells was a significant predictive factor for engraftment in this study, selection of cord blood units with a higher number of CD34-positive cells may be reasonably expected to reduce the risk of graft failure. Use of double CBT was another option. Some researchers reported that infused cell doses and increased alloreactivity induced by interaction between the two CB units may be responsible for reduced risk of relapse.[11]

Our study indicates that CBT is worth considering options for adult ALL further intense evaluation. As a result of the advances in supportive care with better management of infectious complications, and the accumulation of knowledge of better cord blood selection, the outcomes of CBT were significantly improved over time.[8] In addition, reduced-intensity regimens are used widely for CBT, and some small-sized clinical trials suggested that reduced-intensity transplantation potentially results in modest TRM, limited risk of relapse and promising survival in adult patients with ALL.[26] Use of reduced-intensity regimen might offer chance of cure to elderly patients with ALL. Further investigations are warranted to investigate its role in the therapeutic options for adult patients with ALL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contribution

TM designed the research and wrote the manuscript. TY analyzed the data. MK, KY, EK, STaniguchi, STakahashi, MO and HS performed the research. HA, MT, HK, SKai and TI-N managed the data. KK and SKato took the leadership of authors.

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ORIGINAL ARTICLE

Unrelated cord blood transplantation after myeloablative conditioning in adults with advanced myelodysplastic syndromes

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We analyzed the disease-specific outcomes of adult patients with advanced myelodysplastic syndrome (MDS) treated with cord blood transplantation (CBT) after myeloablative conditioning. Between August 1998 and June 2009, 33 adult patients with advanced MDS were treated with unrelated CBT. The diagnoses at transplantation included refractory anemia with excess blasts ($n=7$) and MDS-related secondary AML (sAML) ($n=26$). All patients received four fractionated 12 Gy TBI and chemotherapy as myeloablative conditioning. The median age was 42 years, the median weight was 55 kg and the median number of cryopreserved nucleated cells was 2.51×10^7 cells per kg. The cumulative incidence of neutrophil recovery at day 50 was 91%. Neutrophil recovery was significantly faster in sAML patients ($P=0.04$). The cumulative incidence of plt recovery at day 200 was 88%. Plt recovery was significantly faster in CMV seronegative patients ($P<0.001$). The cumulative incidence of grade II–IV acute GVHD (aGVHD) and extensive-type chronic GVHD was 67 and 34%, respectively. Degree of HLA mismatch had a significant impact on the incidence of grade II–IV aGVHD ($P=0.021$). TRM and relapse at 5-years was 14 and 16%, respectively. The probability of EFS at 5 years was 70%. No factor was associated with TRM, relapse and EFS. These results suggest that adult advanced MDS patients without suitable related or unrelated BM donors should be considered as candidates for CBT.

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Introduction

The prognosis of advanced myelodysplastic syndrome (MDS) is poor. Although some patients with advanced MDS achieve remission with standard intensive chemotherapy, the duration is usually limited.[1] Therefore, Allo-SCT is considered as the only curative therapy for MDS patients. Alternative donor sources other than HLA-identical siblings have been used as allogeneic stem cell sources.[2–6] Recently, umbilical cord blood from unrelated donors has been used as an alternative stem cell source for adult patients.[7–15] However, reports of disease-specific outcomes for adult patients with advanced MDS after cord blood transplantation (CBT) are still limited. We have previously reported the results of a group of 19 adult patients with advanced MDS who received unrelated CBT.[16,17] Here, we have updated the results of unrelated CBT after myeloablative conditioning for 33 adult patients with advanced MDS. The main purpose of this retrospective single-center study was to confirm the safety and efficacy of unrelated CBT for adult advanced MDS patients after a myeloablative conditioning regimen, as well as to identify pretransplant factors related to the transplant outcomes on long-term follow-up.

Patients and methods

Patients

This was a retrospective single-center analysis. Between August 1998 and June 2009, 33 adult patients with advanced MDS were treated with unrelated CBT at The Institute of Medical Science, University of Tokyo. The diagnosis of MDS was made for all patients according to the World Health Organization classification. The diagnosis at transplantation included refractory anemia with excess blasts (1/2) ($n=7$) and MDS-related secondary AML (sAML) ($n=26$). MDS-related sAML was defined as AML that developed during the follow-up period of MDS. The cytogenetic subgroups according to a transplantation-specific cytogenetics grouping for MDS[18] were adverse risk (abnormalities of chromosome 7 and complex karyotype) in 10 patients and standard risk (all others) in 23 patients. Written informed consent for treatment was obtained from all patients.

Cord blood unit selection
The HLA-A and HLA-B Ags were identified by serological typing. HLA-DRB1 alleles were determined by high-resolution molecular typing using PCR sequence-specific primers. Patients without a suitable, closely HLA-matched family donor were eligible for CBT if a matched unrelated BM donor was unavailable as a first treatment option. If there was insufficient time for an unrelated BM donor search because of disease status or because preliminary search indicated a low likelihood of obtaining a matched unrelated BM donor, we attempted to locate cord blood grafts. Preferred cord blood units matched three or more of six HLA loci and contained a minimal cell count of 1.5×10^7 nucleated cells per kg before freezing. All but one cord blood units were obtained from cord blood banks in the Japan Cord Blood Bank Network.

Conditioning
All patients received four fractionated 12 Gy TBIs on days −9, −8 or on days −8 and −7, recombinant human G-CSF combined Ara-C and CY. Ara-C was administered i.v. over 2 h at a dose of 3 g/m^2 every 12 h on days −6 and −5 or on days −5 and −4 (total dose, 12 g/m^2). G-CSF was administered by continuous infusion at a dose of 5 μg/kg per day. Infusion of G-CSF was started 12 h before the first dose of Ara-C and stopped at the completion of the last dose. CY was administered i.v. over 2 h at a dose of 60 mg/kg once daily on days −4 and −3 or on days −3 and −2 (total dose, 120 mg/kg). At 2 or 3 days after the completion of conditioning, patients received a CBT.

GVHD prophylaxis
All patients received standard CYA and MTX as GVHD prophylaxis. CYA was given i.v. every day starting on day −1 at a dose of 3 mg/kg per day. MTX (15 mg/m^2 i.v.) was given on day 1, and 10 mg/m^2 of MTX was administered on days 3 and 6. Once oral intake could be tolerated, patients were administered oral CYA at a dose of 1:2, in two divided doses per day, on the basis of the last i.v. dose. In the absence of GVHD, CYA was tapered beginning between weeks 6 and 9 until it could be discontinued in the absence of chronic GVHD (cGVHD) between 6 and 12 months after transplantation. CYA was reduced when serum creatinine levels rose 1.5 times above baseline or when other serious agent-associated toxicities occurred. Physicians could freely modify the CYA dose for patients experiencing severe acute GVHD (aGVHD) or risk of disease relapse. Corticosteroid-based treatment was considered when grade II or higher severe aGVHD occurred (0.5–2 mg/kg).

Supportive care
All patients received G-CSF by i.v. infusion starting on day 1 until durable granulocyte recovery was achieved. The supportive care regimen, including prophylaxis for infection, was the same as previously reported.[13]

End points and definitions
Myeloid engraftment was defined as the first of the 3 consecutive days during which the ANC was at least 0.5×10^9 cells per l. Plt recovery time was achieved on the first of 3 days when the plt count was higher than 5×10^9 cells per l without transfusion support. The chimerism status after CBT was determined either by FISH with a Y chromosome probe for sex-mismatched CBT or by quantitative PCR analysis for microsatellite DNA markers. Primary engraftment failure was defined as the absence of donor-derived myeloid cells on the day of death, the day of relapse and on day 60 in patients surviving after CBT. A second allogeneic transplantation or autologous hematopoietic reconstitution before donor-derived myeloid recovery was considered as primary engraftment failure. Both aGVHD and cGVHD were graded according to the previously published criteria.[19,20] TRM was defined as death from any cause except relapse. Relapse was defined by morphological evidence of disease in peripheral blood, BM or extramedullary sites. EFS was defined as the time from CBT to graft failure, relapse, death or the last observation.

Statistical analysis
Cumulative incidences were estimated for hematopoietic recovery, GVHD, TRM and relapse to take competing risks into account. The probability of EFS was estimated from the time of CBT according to the Kaplan–Meier method. Variables considered in univariate analysis were body weight, age, recipient sex, degree of HLA matching, recipient CMV serology, diagnosis (MDS-related sAML or others), cytogenetic subgroups, year of transplant, total nucleated cell dose and CD34-positive cell dose. Variables with a value of $P<0.1$ for each end point were tested in multivariate analysis. End points were calculated at the last contact, the date of the last follow-up being 1 December 2009.

Results

Characteristics of patients and cord blood units
The characteristics of 33 patients and cord blood units are shown in Table 1. Among the patients, the median age was 42 years (range, 19–52 years), the median weight was 55 kg (range, 41–75 kg), the median number of cryopreserved nucleated cells was 2.51×10^7 cells per kg (range, 1.71–4.60×10^7 cells per kg), and the median number of cryopreserved CD34-positive cells was 0.91×10^5 cells per kg (range, 0.27–2.14×10^5 cells per kg). All patients received a single and HLA-mismatched cord blood unit.

Hematopoietic recovery
A total of 30 patients had myeloid reconstitution, and the median time to achieve more than 0.5×10^9 cells per l ANC was 22 days (range, 18–35 days). Three patients experienced primary engraftment failure. Of the three patients, one relapsed at day 53 and two died at days 24 and 28 without myeloid engraftment. The cumulative incidence of neutrophil recovery at day 50 was 91% (95% confidence interval

Table 1 Characteristics of patients and cord blood units

Characteristics	
Patients, *n*	33
Male/female, *n*	22/11
Median age, years (range)	42 (19–52)
Median wt, kg (range)	55 (41–75)
Median no. of cryopreserved nucleated cells ($\times 10^7$ cells per kg (range))	2.51 (1.71–4.60)
Median no. of cryopreserved CD34-positive cells ($\times 10^5$cells per kg (range))	0.91 (0.21–2.14)
Median time from diagnosis to transplantation, months (range)	9 (2–223)
Recipient CMV status, positive/negative, *n*	27/6
Diagnosis	
RAEB, *n*	7
MDS-related secondary AML, *n*	26
Cytogenetic subgroups	
Standard, *n*	23
Adverse, *n*	10
Conditioning regimen	
TBI + Ara-C/G-CSF + CY, *n*	33
GVHD prophylaxis	
CyA + MTX, *n*	33
No. of HLA-A, -B and -DRB1 mismatches	
1, *n*	5
2, *n*	15
3, *n*	11
4, *n*	2
Year of transplant	
1998–2003, *n*	15
2004–2009, *n*	18

Abbreviations: RAEB = refractory anemia with excess blasts; MDS = myelodysplastic syndrome; G-CSF = recombinant human G-CSF.

Table 2 Multivariate analysis of factors associated with hematopoietic recovery and acute GVHD

Outcome/variables	*Hazard ratio (95% CI)*	P-*values*
Neutrophil recovery		
Diagnosis		
sAML	2.48 (1.04–5.92)	0.04
RAEB	1	
Plt recovery		
Recipient CMV status		
Positive	0.21(0.09–0.48)	0.00021
Negative	1	
Acute GVHD (grade II–IV)		
No. of HLA mismatches		
3 or 4	3.04 (1.19–7.78)	0.021
1 or 2	1	

Abbreviations: CI = confidence interval; sAML = myelodysplastic syndrome-related secondary acute myelogenous leukemia; RAEB = refractory anemia with excess blasts.

(CI), 79.7–100%). In multivariate analysis, neutrophil recovery was significantly faster for sAML patients ($P = 0.04$) (Table 2). A self-sustained plt count of more than 50×10^9 cells per l was achieved in 29 patients at a median time of 51 days (range, 30–179 days). The cumulative incidence of plt recovery at day 200 was 88% (95% CI, 75.1–100%). In multivariate analysis, plt recovery was significantly faster for CMV seronegative patients ($P < 0.001$) (Table 2).

aGVHD and cGVHD

Acute GVHD occurred in 28 of 30 evaluable patients. The grading of aGVHD was grade I in 12 patients, grade II in 10, grade III in 4 and grade IV in 2. The cumulative incidence of grade II–IV and grade III and IV aGVHD at day 100 after CBT was 67% (95% CI, 34.0–90.8%) and 41% (95% CI, 5.5–75.5%), respectively. In multivariate analysis, degree of HLA mismatch had a significant impact on the incidence of grade II–IV aGVHD ($P = 0.021$) (Table 2). cGVHD occurred in 25 of 28 evaluable patients. Among 25 cGVHD patients, 11 patients were of the extensive type. The cumulative incidence of overall and extensive-type cGVHD was 76% (95% CI, 60.4–91.2%) and 34% (95% CI, 17.4–51.2%), respectively. No factor was associated with the incidence of overall and extensive-type cGVHD.

Relapse

The cumulative incidence of relapse at 5 years was 16% (95% CI, 2.7–29.1%). No factor was associated with the incidence of relapse.

TRM and causes of death

The cumulative incidence of TRM at day 100 and at 5 years was 6% (95% CI, 0–14.4%) and 14% (95% CI, 0.8–27.0%), respectively. No factor was associated with TRM. Eight patients died. Of the eight patients, four died of relapse. In the remaining four patients, the causes of death were treatment related (organ failure ($n = 1$), infection ($n = 1$), cGVHD with or without infection ($n = 2$)).

EFS

Of 33 patients, 24 are alive and event-free after CBT. Median follow-up of event-free survivors was 59 months (range, 5–137 months). The probability of EFS at 5 years was 70% (95% CI, 55.3–88.9%) (Figure 1). No factor was associated with EFS.

Discussion

Several studies have suggested the promising results of unrelated CBT after myeloablative conditioning for adult patients.[7–15] Recently, reports of disease-specific outcomes for adult patients with acute leukemia after CBT have been published;[21–24] however, there have been no reports detailing the long-term follow-up results of disease-specific outcomes of adult advanced MDS patients treated with CBT after myeloablative conditioning. Although we have previously reported the results of 19 adult patients with advanced MDS who received CBT after myeloablative conditioning,[16,17] the follow-up duration after CBT was

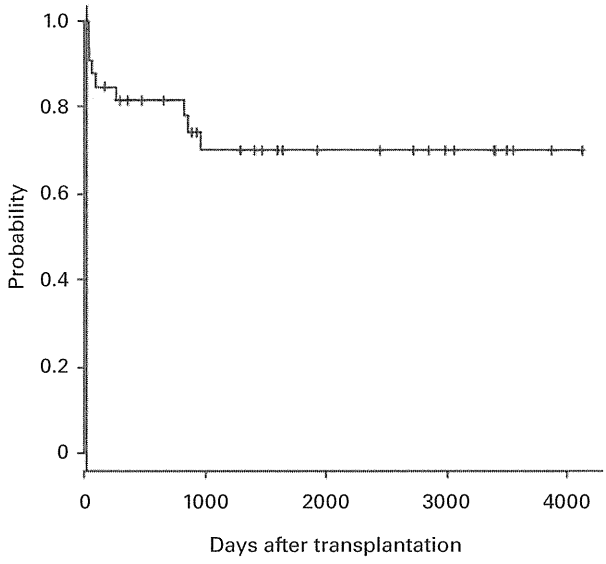

Figure 1 Probability of EFS after cord blood transplantation.

short and risk factors associated with transplant outcomes were not analyzed. In addition, our recent report of the results of 77 adult patients with AML who received CBT included 20 patients with sAML;[21] however, the details of sAML-specific outcomes were not fully analyzed. Therefore, we updated the results of unrelated CBT after myeloablative conditioning for 33 adult patients with advanced MDS and identified pretransplant factors related to the transplant outcomes on long-term follow-up.

In this study, the cumulative incidence of neutrophil recovery at day 50 was 91% and the median time to neutrophil recovery was 22 days. Of 33 patients, only 3 patients experienced primary engraftment failure. The diagnosis of sAML was identified as a significant factor affecting faster neutrophil recovery. One reason may be that two of the three patients who experienced primary engraftment failure had refractory anemia with excess blasts (2 of 7 refractory anemia with excess blasts patients experienced primary engraftment failure). The cumulative incidence of plt recovery at day 200 was 88%, and plt recovery was significantly faster for CMV seronegative patients. This finding is consistent with our previous report.[25] The cumulative incidence of grade II–IV aGVHD, grade III and IV aGVHD, overall and extensive-type cGVHD was 67, 41, 76 and 34%, respectively. As described before,[13] because immunosuppressants for CBT recipients tended to be discontinued faster in our institution, the incidence of aGVHD and cGVHD was relatively higher than other reports of adult CBT. However, the cumulative incidence of TRM at day 100 and at 5 years was very low (6 and 14%, respectively), and aGVHD and cGVHD were not related to TRM. In multivariate analysis, degree of HLA mismatch had a significant impact on the incidence of grade II–IV aGVHD. Recently, Arcese *et al.*[14] reported the updated results of a large series of adult patients with different hematopoietic malignancies transplanted in 63 centers of the Eurocord group. In total, 171 adult patients received CBT after myeloablative conditioning. They analyzed outcomes and risk factors after CBT. The cumulative incidence of grade II–IV aGVHD was 32%, and no factor was found to significantly influence the development or severity of aGVHD in multivariate analysis. Our previous studies[21,22] and those of others,[7,24] as well as that of Arcese *et al.*[14] reported that HLA matching had no effect on aGVHD after CBT in adults; however, the degree of HLA mismatch had a significant impact on aGVHD in this study. The reasons for this finding remain unclear because of the limited number of patients. The cumulative incidence of relapse at 5 years was 16% and the probability of EFS at 5 years was 70%. No factor was associated with relapse and EFS. Recently, a transplantation-specific cytogenetics grouping scheme for patients with MDS has been reported.[18] Under this scheme, abnormalities of chromosome 7 and complex karyotype are considered to be adverse risks, whereas all others are considered standard risk. Although, we performed univariate and multivariate analyses according to this transplantation-specific grouping, cytogenetics had no impact on the incidence of relapse and EFS. In this analysis, unrelated CBT after myeloablative conditioning can cure ~70% of the adult patients with advanced MDS. As previously described,[13,15,21] a lower TRM rate and the use of G-CSF-combined preparative regimen,[21] which was capable of reducing the post transplant relapse rate in refractory myeloid malignancies, may be associated with encouraging outcomes in this study. In addition, Japanese patients might have some advantages in the setting of HLA-mismatched transplantation because of HLA or non-HLA immune genetics.

In summary, we updated the results of unrelated CBT for adult advanced MDS patients. These results suggest that unrelated CBT after myeloablative conditioning could be safely and effectively used for adult patients with advanced MDS without suitable related or unrelated BM donors.

Conflict of interest

The authors declare no conflict of interest.

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