学位論文

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

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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).

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).

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).

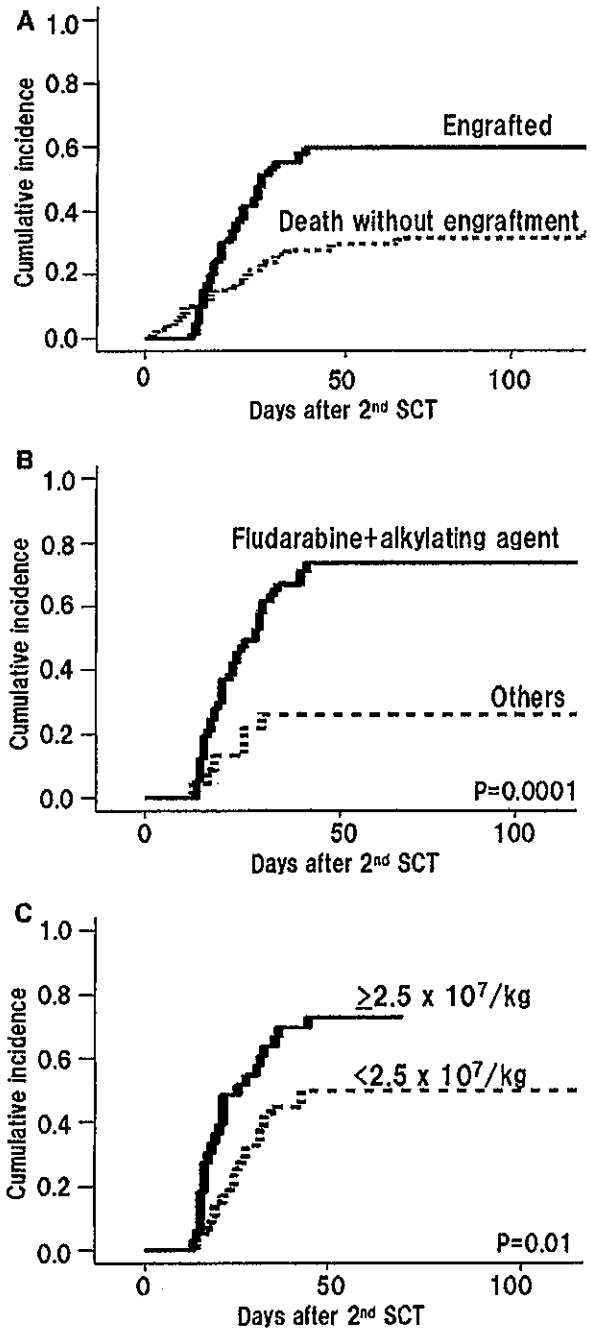

Figure 1. Cumulative incidence of neutrophil engraftment. (A) The cumulative incidences of neutrophil engraftment (solid line) and death without engraftment (dotted line) are shown. (B) The cumulative incidence of neutrophil engraftment was higher in patients who received alkylating agent–containing 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

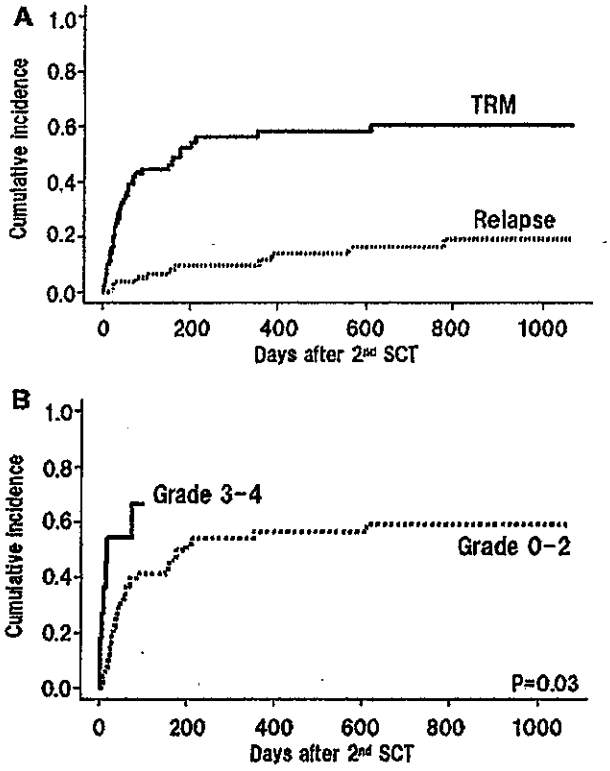

Figure 2. Cumulative incidence of transplantation-related mortality (TRM) and relapse. (A) The cumulative incidences of TRM (solid line) and relapse (dotted line) are shown. (B) The cumulative incidence of TRM was higher in patients who had grade 3 or 4 carryover organ toxicity before the second SCT (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; $P = .03$)

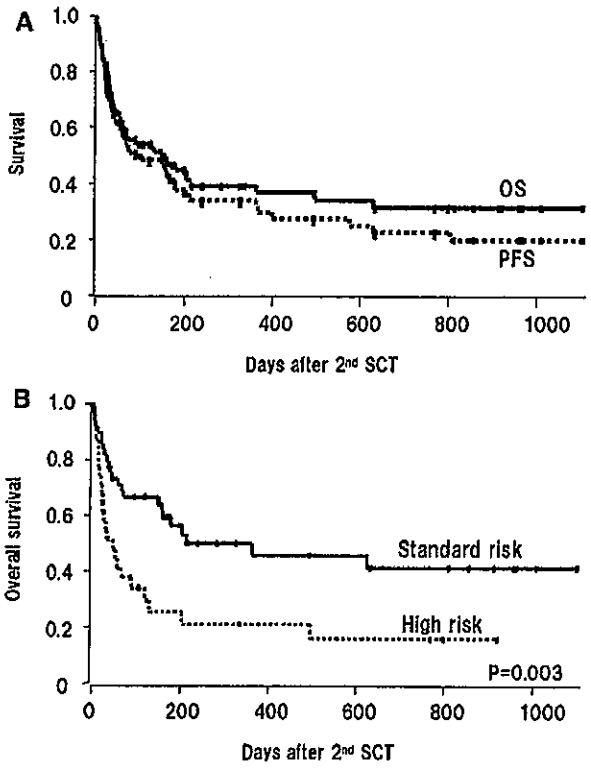

Figure 3. OS and PFS. (A) The Kaplan-Meier estimates of OS (solid line) and PFS (dotted line) are shown. (B) OS in patients who were high risk at the first SCT (dotted line) was lower than that in those who were standard risk (solid line) ($P = .003$).

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 P	Multivariate Hazard Ratio (95% CI)	Multivariate 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
≥2.5 × 10^7/kg	73		2.14 (1.29-3.52)	
<2.5 × 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 P	Multivariate Hazard Ratio (95% CI)	Multivariate 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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