that this observation serves as an important guide for considering the indications for allo-HCT in AML in CR1 by incorporating the evaluation of QOL. Adjustment for QOL with the use of utility values provided by patients who live with the disease should add valuable clues for weighing the value of a postremission strategy for each person. In addition, an investigation that applies a prospectively collected database for a multiethnic population should help to further show the roles of allo-HCT and chemotherapy in AML in CR1.

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Authorship

Contribution: S.K. designed the study, prepared the data file, performed the analysis, interpreted data, and wrote the manuscript; T. Yamaguchi was primarily responsible for the study design, data analysis, and interpretation of the data; S.M., N.U., H.K., K.U., T. Yamashita, M.W., K.Y., S.Y., Y. Nawa, J. Taguchi, J. Takeuchi, J. Tomiyama, and Y. Nakamura obtained the patients' data and interpreted data; I.M. reviewed the cytogenetic reports and interpreted data; Y.K. helped to design the study and to interpret data; Y.T. interpreted data and helped to write the paper; and T.F. was primarily responsible for the entire paper as an accurate and verifiable report.

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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 $\times 10^7$/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).

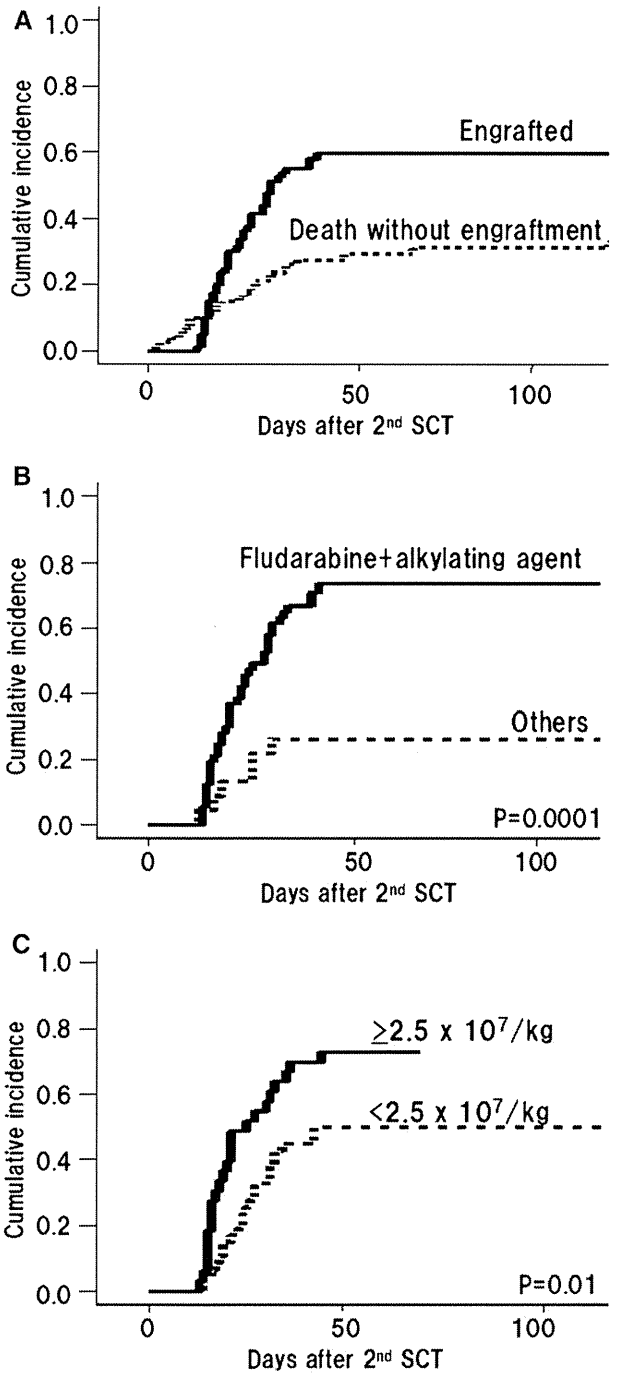

Figure 1. Cumulative incidence of neutrophil engraftment. (A) The cumulative incidences of neutrophil engraftment (solid line) and death without engraftment (dotted line) are shown. (B) The cumulative incidence of neutrophil engraftment was higher in patients who received alkylating agent–containing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

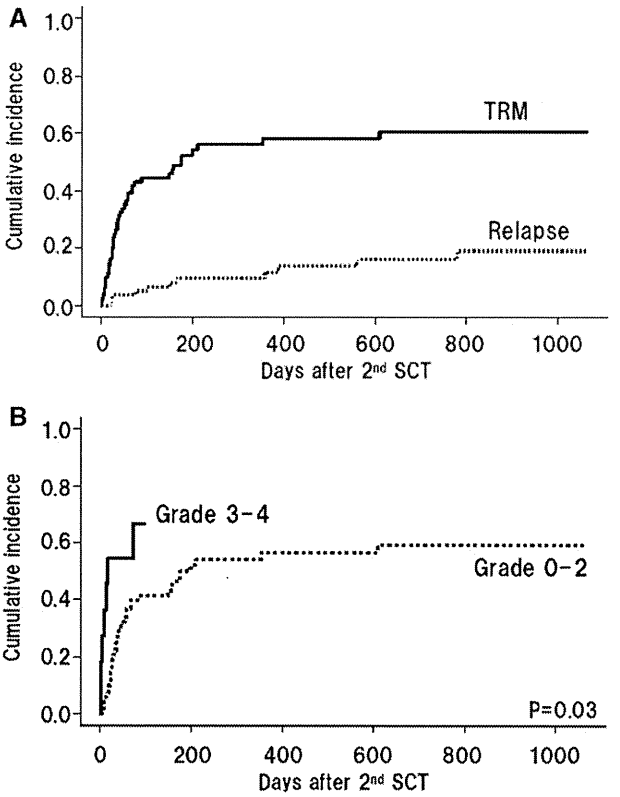

Figure 2. Cumulative incidence of transplantation-related mortality (TRM) and relapse. (A) The cumulative incidences of TRM (solid line) and relapse (dotted line) are shown. (B) The cumulative incidence of TRM was higher in patients who had grade 3 or 4 carryover organ toxicity before the second SCT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

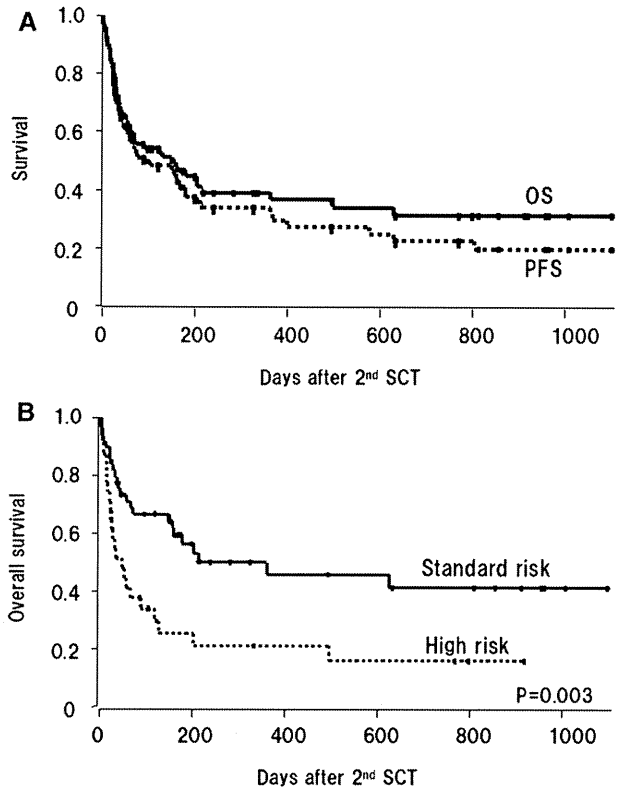

Figure 3. OS and PFS. (A) The Kaplan-Meier estimates of OS (solid line) and PFS (dotted line) are shown. (B) OS in patients who were high risk at the first SCT (dotted line) was lower than that in those who were standard risk (solid line) ($P = .003$).

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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Incidence, risk factors, and clinical outcomes of cataracts following hematopoietic stem cell transplantation

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To evaluate the characteristics of cataracts following hematopoietic stem cell transplantation (HSCT), 561 patients without any ocular complications before HSCT were reviewed. Ocular complications occurred in 196 patients (34.9%). Cataract was diagnosed in 45 of 561 patients. The 10-year estimated cataract incidence was 14%. The median onset time was 498 days after transplant (range, 38–4,535 days). The onset of cataracts was later than that of other ocular complications. Phacoemulsification was performed in 13 patients (29%) at a median of 1,028 days after HSCT (range, 375–4,549 days). In 6 of 13 patients (46%) who underwent cataract surgery, visual acuities gradually deteriorated because of the development of posterior capsule opacification. YAG (yttrium-aluminum-garnet) laser posterior capsulotomy successfully recovered their sight without any sequelae. A multivariate analysis revealed that age, acute and chronic graft versus host disease (GVHD), and systemic infection were significant risk factors for cataract, whereas neither steroid nor total body irradiation (TBI)-containing regimens affected the development of cataract. Our results showed that the clinical outcomes of cataract after HSCT are favorable and comparable to those in the general population. Hyperfractionated TBI with eye shielding may be effective for the prevention of cataract.

Numerous advances in allogeneic hematopoietic stem cell transplantation (HSCT) techniques have improved recipients' long-term survival. Currently, patients with acute leukemia or chronic myeloid leukemia who survive without recurrent malignancy for 2 years after allogeneic HSCT have an 89% probability of surviving for at least 5 years, and recently, the quality of life (QOL) of such long-term survivors has been investigated [1]. Several reports revealed that a significantly large population of patients developed ocular complications [2,3], such as cataract, dry eye, retinal bleeding, or intraocular infection, and some of these complications affected patients' QOL. Although cataract formation is one of the major late complications following HSCT, its risk factors are still controversial. Previous reports showed that total body irradiation (TBI) increased the risk of cataract, and single dose TBI was more cataractogeneic than hyperfractionated TBI [4]. In the largest series evaluating a cohort of 1,064 patients, risk factors for the development of cataract were older age, higher dose rate (>0.04 Gy/min), allogeneic HSCT, and steroid administration [5].

Recently, hyperfractionated TBI has become one of the widespread standard precautions for HSCT-associated complications, and lens protection with custom-made shielding blocks for each patient is also expected to decrease the incidence of cataracts following HSCT [6,7]. Furthermore, cataract surgery has recently become a low-risk procedure and improves visual acuity in more than 90% of eyes in normal healthy subjects [8–11] but the long-term outcome of surgical repair in patients who undergo HSCT remains to be clarified [3].

Thus, in this study, the characteristics, risk factors, and long-term outcomes of cataract following HSCT were retrospectively analyzed.

The patients' characteristics are summarized in Table I. The median follow-up time was 836 days (range, 0–7,316 days). Of the 561 patients who had no preceding history of ocular complications, 520 (93%) underwent HSCT for hematological malignancies. HLA-matched and unrelated transplantation was performed in 435 (78%) and 276 (49%) patients, respectively. For conditioning regimens, 316 (56%) patients received radiation-containing regimens. Cataracts developed in 45 patients. The 10-year estimated cataract incidence was 14% (Fig. 1). Age (P value, 0.001), acute (0.027) and chronic (<0.001) GVHD, other ocular complications (0.007), and systemic infections (0.009) were significantly higher in patients with cataract. For systemic infections, total 340 episodes were documented in whole patient and 42 episodes were in patients with cataract, 11 bacterial, 9 mycoses, 13 viral, 2 pneumocystis, and 7 pathogen not identified. Chronic GVHD was apparent in 38 (84%) of 45 cataract patients, and 29 patients (64%) were classified as extensive type. The steroid dose and radiotherapy for the conditioning regimen were not different between the two groups (Table I). For ocular complications, 196 patients (34.9%) had a total of 253 ocular lesions during the study period. Ocular lesions other than cataract were dry eye (13.0%), keratoconjunctivitis (12.4%), ocular infection (6.2%), retinal bleeding (2.1%), and others (3.2%), including glaucoma, intraocular relapse, and uveitis. Cataract occurred at a median of 498 days (range, 38–4,535 days); most (91%)

TABLE I. Patients' Characteristics

		Patients n = 561	Patients without Cataract n = 516	Patients with Cataract n = 45	P value
Age, median (range) (years)		36 (0–66)	36 (0–66)	42 (17–61)	0.001
Sex	Male	335	311	24	0.363
	Female	226	205	21	
Donor	Related	285	265	20	0.374
	Unrelated	276	251	25	
HLA	Matched	435	403	32	0.281
	Mismatched	126	113	13	
Diagnosis	Acute myeloid leukemia	154	145	9	0.074
	Chronic myelogenous leukemia	132	117	15	
	Myelodysplastic syndrome	76	71	5	
	Acute lymphoblastic leukemia	116	111	5	
	Other lymphoid malignancies*1	42	38	4	
	Aplastic anemia and Myelofibrosis	41	34	7	
Steroid use, maximum dose	mPSL−	204	197	7	0.103
	mPSL ≤ 1.0 mg/kg	138	120	18	
	1.0 < mPSL ≤ 2.5 mg/kg	113	99	14	
	mPSL > 2.5 mg/kg	106	100	6	
Conditioning regimen	Radiation-containing regimen	316	291	25	0.913
	Non radiation-containing regimen	245	225	20	
Acute GVHD	Grade II to IV (%)	225 (40.1)	200 (38.8)	25 (55.6)	0.027
Chronic GVHD	Limited (%)	78 (13.9)	69 (13.4)	9 (2)	<0.001
	Extensive (%)	150 (26.7)	121 (23.4)	29 (64.4)	
Other ocular complications, n (%)		175 (31.2)	152 (29.5)	23 (51.1)	0.007
Systemic infection, n (%)		282 (50.3)	251 (48.6)	31 (68.9)	0.009

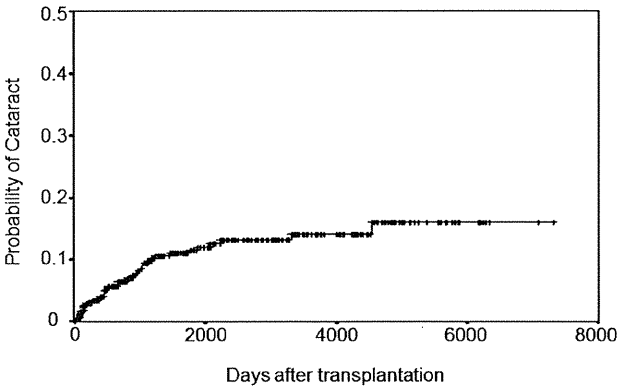

Figure 1. Cumulative incidence of cataract.

developed over 100 days after HSCT, whereas other ocular lesions occurred earlier (keratoconjunctivitis at 97 days, dry eye at 206 days, ocular infection at 139 days, and retinal bleeding at 116 days, respectively).

For clinical management and outcome of cataract, 13 of 45 (29%) patients with cataract underwent phacoemulsification and intraocular lens implantation at a mean of 1,028 days (range, 375–4,549 days) after HSCT. The median age at surgery was 42 (range, 17–52) years. The median follow-up period after cataract surgery was 1,415 (range, 252–2,277) days. During this period, six patients developed posterior capsule opacification (PCO). YAG (yttrium-aluminum-garnet) laser posterior capsulotomy was performed in five patients and successfully improved their visual acuity without any sequelae. The median period from cataract surgery to YAG laser therapy was 983 (range, 727–2,120) days.

On univariate analysis, age, extensive chronic GVHD, use of steroids, acute GVHD (grade II to IV), other ocular complications, and systemic infection were significantly related to the incidence of cataract. TBI-containing regimen did not affect the development of cataract. On multivariate analysis, age, acute and extensive chronic GVHD, and systemic infection were identified as independent factors that significantly affected the development of cataract (Table II).

In this study, the 10-year estimated cataract incidence was lower (14%, Fig. 1) than in previous reports [4–6,12–17]. This might be due to several reasons. First, ocular screening tests were not performed until patients noticed ocular symptoms such as impaired vision, pain, or dryness; thus, asymptomatic patients may have been missed, underestimating the incidence of cataract. Second, the difference in TBI methods might affect morbidity. In our hospital, all patients receive the TBI regimen in a hyperfractionated scheme at a maximum total dose of 12 Gy, and ocular shielding is performed when the TBI dose exceeds 8 Gy. In many previous reports, some patients received single or high dose (>12 Gy) TBI [4,6,13–16]. Moreover, TBI was performed without eye shielding [15,18]. Intraocular relapses were observed in four patients, and two patients did not receive TBI (data not shown), which indicates ocular shielding did not increase intraocular relapse. A total dose >8 Gy has been reported to be a significant risk factor for cataract in single-dose TBI [5]. Thus, our TBI regimen seems to be safe, without increasing the risk for cataract and intraocular relapse.

Although the use of steroids and TBI was a significant risk factor for cataracts in many reports [4–6,12–14,16,18–20], neither affected cataract formation in this study. On multivariate analysis, age, acute and extensive chronic GVHD, and systemic infection were identified as the risk factors for cataract. The type of infection did not significantly differ between the two groups ($P = 0.5$, data not shown), which indicates that any systemic infection could be a risk factor for cataract. Although the precise mechanism of cataract formation is not fully understood, oxidative stress has been reported to be an important factor for the development of cataract [21,22]. The reactive oxygen species are generated in various cells in different settings, including infection and GVHD [23,24]. Patients who underwent HSCT would be subjected to oxidative stress by various HSCT-associated complications, which may promote the development of cataract. Physical stimuli such as eye rubbing or tapping may also lead to cataract formation, especially in patients with chronic GVHD (e.g., dry eye), just like in patients with atopic dermatitis [25].

Visual acuity improved in all patients who underwent cataract surgery. However, six suffered from visual impairment again because of the development of PCO, and five patients underwent YAG laser capsulotomy. PCO is a common long-term complication of cataract surgery and is reported to occur in 20 to 50% of patients 2 to 5 years after surgery [26,27]. Although PCO has been reported as one of the most frequent late complications in HSCT recipients [6,13], the incidence of PCO was not high in our patients compared with that in the general population. All five patients receiving YAG laser capsulotomy improved their visual acuity without sequelae. Thus, these results indicate that the clinical outcomes of cataract surgery following HSCT might be favorable. However, in view of the relatively small number of cataract patients in this study, further investigations will be necessary to confirm the risk factors and the patients' clinical outcomes with a large patient population.

TABLE II. Univariate and Multivariate Analyses of Risk Factors for the Development of Cataract

		Multivariate analysis	
	Univariate *P*	*P*	Hazard ratio (95% CI)
Age (≤36 vs. ≥ 37 years)	0.002	0.003	2.813 (1.423–5.562)
Chronic GVHD (extensive vs. none or limited)	≤0.001	0.001	3.156 (1.560–6.388)
Use of steroids (mPSL > 1.0 mg./kg vs. ≤ 1 mg/kg at maximum dose)	0.038	0.876	0.941 (0.437–2.024)
Acute GVHD grade II–IV	0.001	0.034	1.511 (1.032–2.211)
Other ocular complication	0.029	0.677	1.030 (0.536–1.979)
Systemic infection	<0.001	0.01	2.508 (1.270–4.955)
Use of radiotherapy	0.7438		
Use of TBI	0.6759		

Patients and Methods

Patient characteristics. A total of 622 patients underwent allogeneic HSCT in our hospital for hematological diseases from 1986 to 2006. Of these, the records of 561 patients were retrospectively reviewed to investigate the clinical features of newly developed ocular complications after HSCT; 61 patients were excluded because of a prior history of ocular complications such as cataract, glaucoma, diabetic retinopathy, or intraocular infection. Ophthalmological examinations were carried out when patients had any ocular symptoms. For the 29 patients who received two or more allogeneic transplantations during the study period, only the first transplantation period was analyzed.

Preparative regimen and graft-versus-host disease prophylaxis. Preparative therapies were performed according to the primary disease and type of transplantation. Generally, patients with lymphoid malignancy were conditioned with a fractionated total body irradiation (TBI, 12 Gy) -containing regimen, including cytarabine (8 g/m^2) and cyclophosphamide (CY, 120 mg/kg). Fractionated TBI was performed with partial transmission anterior–posterior eye 33% shielding. Patients with myeloid malignancy were conditioned using a non-TBI-containing regimen including busulfan (BU, 16 mg/kg orally or 12.8 mg/kg intravenously) and CY (120 mg/kg). Plasma BU concentrations were not monitored. Total lymphoid irradiation (TLI, 7 Gy) was added to the BU/CY regimen in cases of human leukocyte antigen (HLA)-mismatched or unrelated transplantation. Patients with severe aplastic anemia were also conditioned using a TLI-containing regimen. Prophylaxis for acute GVHD consisted of a short course of methotrexate and cyclosporine A or tacrolimus (FK). FK was used in cases of either unrelated or HLA-mismatched transplantation. Acute and chronic GVHD were diagnosed and graded according to previously established criteria [28,29].

Statistical analysis. Continuous baseline characteristics were compared between patients with cataract and patients without cataract using the Mann-Whitney test. Categorical characteristics were compared using the χ^2 test. Analyses of risk factors for cataract and the probability of cataract were calculated according to the Kaplan-Meier method. For univariate analysis, the log-rank test was used to compare risk groups for cataract. Multivariate analysis was performed using a Cox's proportional hazard model. The statistical data were obtained using SPSS software (SPSS 11.0, Chicago, IL). All statistical tests were two-sided, and a value of $P < 0.05$ was considered statistically significant.

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Active cytomegalovirus infection in patients with acute venous thrombosis: A case-control study

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Infectious diseases have been associated with venous thrombosis (VT) [1]. Several case-studies described active cytomegalovirus infection in patients with VT [2–6]. In this case-control study, we detected five cases with active cytomegalovirus infection and VT, and none in the control group. All were female, below 37 years of age, and had another acquired risk factor for VT. This may imply that active cytomegalovirus infection contributes to a procoagulant state in young subjects with other mild risk factors for VT.

Recent prospective cohort studies showed that an increase of the inflammatory marker C-reactive protein (CRP) was associated with a 1.5–2.5-fold increased risk of VT [7,8]. Interestingly, this risk was highest when CRP levels were elevated closely before the onset of VT [7]. The latter observation supports the hypothesis that activation of the coagulation system is stimulated by an infectious process [9,10]. It is possible that the procoagulant response induced by active cytomegalovirus infection [6,11–13], in some persons exceeds the thrombosis threshold leading to VT [14]. Although active cytomegalovirus infection may play a role in VT onset, it cannot be ruled out that case-reports reporting on this issue emphasized the rare exception. Therefore, we decided to conduct a case-control study in consecutive patients with suspicion of VT to determine the prevalence of acute cytomegalovirus infection and the concurrence of procoagulant abnormalities in patients with VT.

Of 397 patients in the study, 258 (65%) were cases and 139 (35%) were controls (Table I).Compared to controls, patients with VT were more often male, had less often a history of VT, more often experienced infectious signs in the preceding 4 weeks, and had higher CRP, leukocytes and factor VIII levels. Female patients were more frequent on oral contraceptives. Seroprevalence of cytomegalovirus IgG was not higher in the case group (n = 126, 55% in cases, and n = 77, 60% in controls). Levels of cytomegalovirus IgM were found in 4% of cases and 4% of controls.

In five patients with VT (2%) a positive viral load of cytomegalovirus DNA was detected, compared to none in the controls. All were younger than 37 years of age and female. As 31 of 258 patients with VT (12%) were younger than 37 years and

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IMAGES IN HEMATOLOGY

Ocular palsy associated with aggressive NK-cell leukemia

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A 22-year-old woman was referred to our hospital with a 1-month history of recurrent fever, subacute onset of bilateral orbital pain, and diplopia. Physical examination revealed the limits of supraduction of her right eye and infraduction of her left eye. A bone marrow aspirate from the ilium showed scattered large immature-looking lymphocytes (15.8%) with pale cytoplasm, fine nuclear chromatin, and nucleoli (Fig. 1). These cells were stained positively with CD56, TIA-1, granzyme, and perforin, and were stained negatively with CD3. In situ hybridization for EBER demonstrated that the cells were EBV-positive. A diagnosis of aggressive NK-cell leukemia was made.

Magnetic resonance imaging of orbital cavity showed marked enlargement, and diffuse high intensity signals in the right superior rectus muscle and left inferior rectus muscle on fat-saturated T2-weighted image (Fig. 1). These lesions were diffusely contrast-enhanced on fat-saturated T1-weighted image (Fig. 2).

After two courses of dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide chemotherapy, she underwent cord blood transplantation with a preparative regimen comprised of etoposide, cyclophosphamide, and total body irradiation. She achieved complete remission, and her ocular manifestations completely resolved.

We could not perform biopsy of the lesions due to the poor general condition of the patient, and the exact etiology

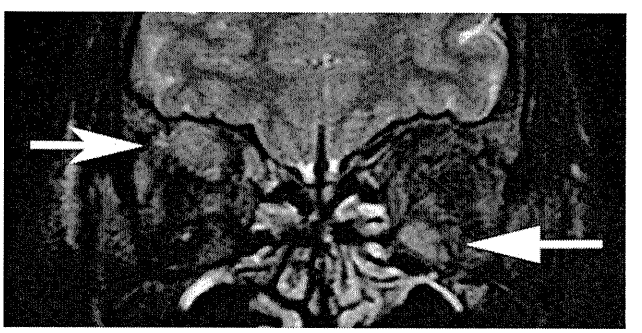

Fig. 1 MRI of orbital cavity showed the marked enlargement, and diffuse high intensity signal in the right superior rectus muscle and left inferior rectus muscle on fat-saturated T2-weighted image

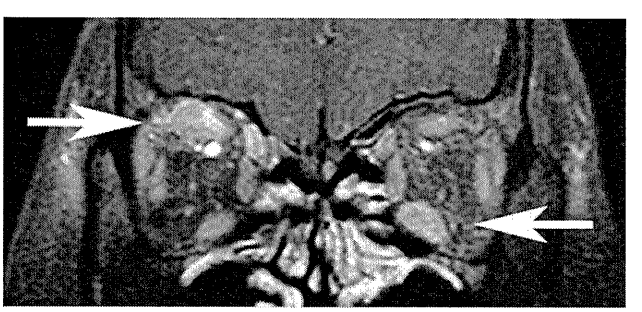

Fig. 2 Eye lesions were diffusely contrast-enhanced on fat-saturated T1-weighted image

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of her ocular manifestation remains to be elucidated. While her ocular symptoms occurred in parallel to the progression of the leukemia, a number of possibilities for the cause of her ocular involvement can be raised, including tumor infiltration, infection, or paraneoplastic myositis. An accumulation of cases of ocular involvement associated with NK-cell leukemia is required.

Conflict of interest None declared.

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ORIGINAL ARTICLE

Outcome of medium-dose VP-16/CY/TBI superior to CY/TBI as a conditioning regimen for allogeneic stem cell transplantation in adult patients with acute lymphoblastic leukemia

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Abstract The choice of conditioning regimen before allogeneic stem cell transplantation (SCT) in patients with acute lymphoblastic leukemia (ALL) is important. We retrospectively compared outcomes of medium-dose VP-16/cyclophosphamide/total body irradiation (VP/CY/TBI) regimen and CY/TBI. Five hundred and twenty-nine patients (VP/CY/TBI: $n = 35$, CY/TBI: $n = 494$) who met all of the following criteria were compared: first time for SCT, aged 15–59 years; first or second complete remission at SCT; bone marrow or peripheral blood as stem cell source; and HLA phenotypically matched donor. Median age of the patients was 34 years, and patients who received VP/CY/TBI were younger (28 vs. 34 years, $P = 0.02$). Cumulative incidences of relapse and non-relapse mortality (NRM) were higher for patients who

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received CY/TBI ($P = 0.01$ for relapse, $P < 0.01$ for NRM). After a median follow-up period of 36.9 months, 5-year overall survival (OS) rates were 82.2% in the VP/CY/TBI group and 55.2% in the CY/TBI group. OS, and disease-free survival (DFS) in the VP/CY/TBI group were shown to be significantly better by multivariate analysis [hazard ratio: 0.21 (95% confidence interval: 0.06–0.49) for DFS, hazard ratio: 0.25 (95% confidence interval: 0.08–0.59) for OS]. VP/CY/TBI was associated with a lower relapse rate and no increase in NRM, resulting in better survival than that in CY/TBI for adult ALL patients.

Keywords Acute lymphoblastic leukemia · Stem cell transplantation · Conditioning regimen · Medium-dose VP/CY/TBI

1 Introduction

The prognosis of adult acute lymphoblastic leukemia (ALL) is dismal, [1–10] and allogeneic hematopoietic stem cell transplantation (alloSCT) is therefore performed in most cases. However, even in patients who received alloSCT conditioned with a standard regimen of cyclophosphamide with total body irradiation (CY/TBI), the prognosis has not been satisfactory due to a high rate of relapse. [11–16] Although VP-16 (VP) has been used as an alternative to CY or as an agent added to the standard regimen, the dose of VP was high (50–60 mg/kg or 1.5 mg/m^2) and the high rate of non-relapse mortality (NRM) was problematic [17–23]. Recently, we and others have reported excellent outcomes for adult patients with ALL who underwent alloSCT conditioned with 30–40 mg/kg VP added to CY/TBI (VP/CY/TBI). [24–26] In this paper, 30–40 mg/kg VP is called as "medium-dose VP". Although the conditioning regimen is one of the most important factors in alloSCT, there have been few studies in which conditioning regimens for ALL were compared, and there has been no study in which the outcomes of VP/CY/TBI and CY/TBI were compared. We therefore retrospectively compared the outcomes for patients who received VP/CY/TBI and patients who received CY/TBI, and we also investigated risk factors for relapse, NRM, disease-free survival (DFS) and overall survival (OS) to obtain useful information for selecting a conditioning regimen.

2 Patients and methods

2.1 Collection of data and data source

Clinical data for patients who received the VP/CY/TBI regimen were collected from six centers in Hokkaido, Japan, and data for patients who received CY/TBI were collected from the Japan Society for Hematopoietic Cell Transplantation database (Transplant Registry Unified Management Program) and the Japan Marrow Donor Program database. [27] Data for 35 patients who received VP/CY/TBI and data for 494 patients who received CY/TBI and who met all of the following criteria were analyzed: SCT performed between 1993 and 2007, first time for SCT, aged 15–59 years, diagnosed as having ALL/lymphoblastic lymphoma or acute biphenotypic leukemia, first or second CR (CR1 or CR2) at SCT, bone marrow (BM) or peripheral blood stem cells (PBSC) as stem cell source, and HLA-phenotypically 6 loci matched (A, B and DR loci) related donor (MRD) or unrelated donor (MUD). Patients who met at least one of the following criteria were excluded: secondary SCT, Burkitt leukemia/lymphoma, cord blood as stem cell source, secondary leukemia or T-cell depletion. Data on use of VP and the dose of VP were lacking in almost all of the patients in the registry data. The dose of VP was a key factor for VP/CY/TBI conditioning and we collected patients for our analysis by precise criteria including the same dose of conditioning. Therefore, we could not analyze the patients who received VP/CY/TBI conditioning from the registry data. This study was conducted with the approval of the Institutional Review Board of Hokkaido University Hospital.

2.2 Conditioning regimens and transplantation procedures

CY/TBI consisted of CY at 60 mg/kg once daily administered intravenously (i.v) for 2 days (total dose: 120 mg/kg) combined with fractionated TBI of 12 Gy (either 4 or 6 fractions). In this group, the days on which CY or TBI were administered differed depending on the center. Medium-dose VP/CY/TBI consisted of VP at a dose of 15 mg/kg once daily i.v. for 2 days (total dose: 30 mg/kg) and CY/TBI. [24, 25] VP, CY and TBI were administered on days −7 to −6, days −5 to −4 and days −3 to −1, respectively. Patients who received ATG, campath-1H or cytotoxic agents other than CY or VP in the conditioning regimen were excluded from the analysis. GVHD prophylaxis and other SCT procedures were performed according to the decision of the clinicians of each center.

2.3 Definitions

Neutrophil engraftment and platelet engraftment were defined as the first of 3 days with absolute neutrophil count $>0.5 \times 10^9$/l and the first of 7 days with an untransfused platelet count $>50 \times 10^9$/l, respectively. Toxicity after SCT was graded by the National Cancer Institute (NCI) common toxicity criteria (NCI, Bethesda,

MD, USA). Acute GVHD (AGVHD) and chronic GVHD (CGVHD) were graded by standard criteria. [28, 29] Relapse was defined as a recurrence of underlying diseases. NRM was defined as death during a continuous remission throughout the duration of the study. OS was calculated from the day of SCT until death or last follow-up. DFS was defined as survival in a state of continuous remission.

2.4 Endpoint and statistical analysis

The primary endpoint of this study was to compare relapse, NRM and survival in adult patients with ALL who received CY/TBI and those who received VP/CY/TBI and determine prognostic factors for survival. Descriptive statistical analysis was performed to assess patient characteristics and transplantation procedure, using the Chi-square test or Fisher's exact test as appropriate for categorical variables and the 2-sided Wilcoxon rank sum test for continuous variables. The probabilities of OS and DFS were estimated using the Kaplan–Meier method. Relapse rate and NRM rates were estimated using cumulative incidence analysis and considered as competing risks, and the Pepe and Mori test was used for group comparison of cumulative incidence [30]. Data for the day of relapse were not available in 9 patients who relapsed after SCT, and all of those patients who received CY/TBI. For strict assessment of VP/CY/TBI, 1 day before the last follow-up day was used as the day of relapse of these patients in the Kaplan–Meier method and cumulative incidence analysis, and these results were checked using sensitivity analysis. The effects of various patient and disease categorical variables on survival probabilities were studied using the log-rank test. All P values were two-sided and a P value of 0.05 was used as the cutoff for statistical significance. This study was a retrospective analysis that potentially included bias, and we therefore need to adjust the difference of variables using matched-pair analysis or multiple regression analysis. We considered that the latter was statistically better for our analysis for the following reasons: Selection of matching parameters included intentional bias, and if we used "matching", accuracy of parameter estimation would be reduced due to the reduction of the number of control patients. For adjusting the difference of background, probabilities of relapse, NRM, OS and DFS were estimated using the Cox proportional-hazards regression model, with consideration of other significant clinical variables in the final multivariate models. The variables considered were conditioning regimen, year in which SCT was performed, patient's age at SCT, patient's sex, disease status at SCT, donor (MRD or MUD) and HLA-allele matching. HLA-identical siblings were included in the "HLA-allele match" group.

3 Results

3.1 Patients and transplantation characteristics

Patients and SCT characteristics are summarized in Table 1. The median age of the patients was 34 years (range 15–59 years). Cytogenetic study was performed in 475 (89.8%) of the patients, and 270 (56.8%) of the evaluable patients had chromosomal abnormalities, including poor-risk cytogenetics of Philadelphia chromosome (Ph, $n = 148$, 31.2%), MLL-related abnormalities ($n = 7$, 1.5%), t(1;19) ($n = 10$, 2.1%), −7 ($n = 5$, 1.1%) and +8 ($n = 2$, 0.4%). Data on use of tyrosine kinase inhibitors (TKI) for Ph-positive patients before SCT were lacking due to the limitation of registry data. In the 148 Ph-positive patients, 127 patients received SCT after 2001, the year in which imatinib was approved in Japan, suggesting that Ph-positive patients came to be able to receive SCT by administration of TKI. Four hundred and forty-two patients (83.6%) were in CR1 at SCT and 87 patients (16.4%) were in CR2 at SCT. In the 127 Ph-positive patients who were diagnosed after 2001, twenty-three patients received SCT in molecular remission and 34 patients were not in molecular remission, and data on molecular status were not available for 70 patients. Five of the 8 patients with Ph who received VP/CY/TBI were diagnosed after 2001. Four of those five patients were in molecular remission before SCT and the other patient was not in molecular remission. Two hundred and fifty-eight patients (48.8%) underwent SCT from an MRD and 271 patients (51.2%) underwent SCT from an MUD. Four hundred and thirty-three patients (81.9%) received BM and 95 patients (18.0%) received PBSC, and PBSC were from an MRD in all cases because donation of PBSC from unrelated donors is not permitted in Japan. Although patients who received VP/CY/TBI (VP/CY/TBI: median age of 28 years; CY/TBI: median age of 34 years, $P = 0.02$) were younger, other factors such as Ph, SCT in CR2, and donor status were not significantly different between the two groups.

3.2 Transplantation outcomes

3.2.1 Engraftment

Five hundred and twenty-two patients (98.7%) achieved neutrophil engraftment and there was no difference between the groups [CY/TBI: $n = 487$ (98.6%), VP/CY/TBI: $n = 35$ (100%), $P = 0.43$, Table 2]. In both groups, median day of neutrophil engraftment was day 16 [CY/TBI: day 16 (range, days 8–49), VP/CY/TBI: day 16 (range, days 8–26), $P = 0.49$]. Platelet engraftment could be assessed in 472 patients, and 445 patients (94.1%) achieved platelet engraftment. There was no difference