posttransplant imatinib administration may not be an ideal prophylactic treatment for Ph+ALL patients. In contrast, Ottmann et al. [30] demonstrated that all Ph+ALL patients who received imatinib upon appearance of BCR-ABL and promptly achieved molecular response remained in remission for the duration of imatinib treatment.

Bortezomib

Recently, both conventional chemotherapy and autologous and alloHSCT combined with new agents, such as thalidomide, lenalidomide, and bortezomib, have improved the depth of response and survival of multiple myeloma patients. However, after transplantation, most patients still harbor residual disease. Ladetto et al. [31] reported the effect of posttransplant consolidation including bortezomib on MRD detected by PCR using tumor-clone–specific primers. Molecular remissions were achieved in 3% of patients after autologous HSCT and 18% after consolidation with bortezomib. It has been proposed that bortezomib increases the expression of Fas and DR5 and enhances GVT effects, and that this agent also suppresses the activity of NFkB, resulting in reduction of inflammatory cytokines related to graft-versus-host activity [32].

Lenalidomide

Lenalidomide is an immunomodulatory drug (IMiD) that has multiple effects on myeloma cells and their microenvironment. Administration of IMiDs for postautologous HSCT maintenance resulted in prolonged progression-free survival (PFS) even in patients who achieved very good partial response or complete response before lenalidomide administration. In the alloHSCT setting, lenalidomide plus low-dose dexamethasone combination therapy have shown significant disease and chronic GVHD (cGHVD) control for myeloma patients, who relapsed after transplantation [33]. GVHD control with IMiD is still controversial but a very attractive issue for investigation [34].

Hypomethylating agents

Low-dose 5-azacitidine (5-Aza) was used by investigators at the M.D. Anderson Cancer Center for patients with AML/MDS as a maintenance therapy or salvage therapy upon relapse after alloHSCT; an overall survival rate of 90% at 1 year was reported [35]. Additive effects of DLI to 5-Aza were also reported. The administration of 5-Aza was not associated with an increased incidence of GVHD. Sanchez-Abarca et al. [36] reported that 5-Aza inhibits T cell proliferation and activation, blocking the cell cycle in the G0 to G1 phase and decreasing the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). They also reported that administration of 5-Aza after transplantation prevented the development of GVHD, leading to a significant increase in survival in a fully mismatched bone marrow transplantation mouse model. Recently, decitabine, another DNA hypomethylating agent, was reported to be used in patients experiencing cytogenetic relapse after alloHSCT [37].

Humanized monoclonal antibodies

Rituximab (anti-CD20 monoclonal antibody) was used for 9 chronic lymphocytic leukemia patients who had persistent disease after alloHSCT and underwent immuno-manipulation to augment GVT effects including immunosuppression withdrawal and DLI with rituximab treatment, and 8 patients had a complete response [38]. Alemtuzumab (anti-CD52 monoclonal antibody), as well as antithymocyte globulin (ATG), has been used as a T cell depletion method in alloHSCT. Because it is reported that the majority of precursor B-ALL blasts express CD52, and CD52 is expressed on other ALL cells, alemtuzumab is considered to potentially contribute to the eradication of MRD [39].

Summary on the Treatment of MRD

For decades, interventions for relapsed patients have been performed using DLI and chemotherapies; however, they are a 2-edged sword, hampering normal hematopoietic cells as well as tumor cells. Recently, the emergence of new strategies using tumor-specific DLI and tumor-specific new agents has prompted us to use these methods before clinical relapse. Some of them are used as prophylaxis, and some of them are used upon tumor emergence at molecular level. Trials confirming these strategies are just beginning, and there is a need for the definition of MRD. Thus, it is becoming more and more important that the measurement of MRD becomes standard practice; otherwise, clinical studies will be somewhat meaningless.

NATIONAL CANCER INSTITUTE FIRST INTERNATIONAL WORKSHOP ON THE BIOLOGY, PREVENTION, AND TREATMENT OF RELAPSE AFTER ALLOHSCT

As stated above, there is a strong association of MRD with relapse following alloHSCT. The growing recognition of relapse as one of the most significant posttransplant problems led to the organization and convening of the National Cancer Institute First International Workshop on the Biology, Prevention, and Treatment of Relapse after AlloHSCT [40]. The primary objectives of the Workshop were to review the current "state-of-the-science" relative to the biology, natural history, prevention, and treatment, and identify the most important biological and clinical questions that need to be addressed relative to relapse following alloHSCT.

The Workshop, which took place on November 2 and 3, 2009, in Bethesda, Maryland, USA, brought together an international group of more than 200 basic and clinical researchers. Over 50 formal presentations were made by the Workshop committee members that addressed both GVT and non-GVT biology, relapse epidemiology, and natural history, strategies, and therapies for prevention, disease-specific methods, and strategies for monitoring, and disease-specific treatment of relapse following alloHSCT. These presentations are available for viewing at https://ccrod.cancer.gov/confluence/display/NCIRelapse/Presentations+from+Workshop. Each of the 6 workshop committees subsequently prepared a "state-of-the-science" manuscript, which contained their commended research priorities; these manuscripts were published sequentially during 2010 in the *Biology of Blood and Marrow Transplantation* [1,14,19,41-44].

The central Workshop theme was that in its most simplistic form, relapse occurs because tumor cells are first able to resist the cytotoxic effects of the conditioning regimen. These surviving cells either never respond to initial GVT or they subsequently escape from GVT effects after initial control.

Central and recurrent research themes included the necessity to establish biorepositories to collect and store tumor samples before transplant when possible, and after transplant, store samples from allografts for analysis, and collect blood and serum samples at set posttransplant time points and at the time of relapse for study of immunology related to relapse. Second, there is a need for more careful study of the natural history of relapse for specific diseases, particularly in regard to MRD. To perform such studies, there needs to be international acceptance of standard definitions and techniques; it is hoped that the definitions and techniques proposed by the Workshop will be considered for this purpose. Finally, there needs to be multiinstitutional collaboration in regard to prevention and treatment of relapse after alloHSCT. A formal summary of the workshop recommendations will be presented during the 2011 Tandem Transplant Meetings Educational Sessions.

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To the editor:

Reduced-intensity versus conventional myeloablative conditioning for patients with Philadelphia chromosome–negative acute lymphoblastic leukemia in complete remission

We read with interest the results of the comparison between reduced-intensity conditioning (RIC) and conventional myeloablative conditioning (MAC) allogeneic stem cell transplantation (allo-SCT) for patients with acute lymphoblastic leukemia (ALL) in complete remission (CR), reported by Mohty et al.[1] They concluded that RIC allo-SCT was a potential therapeutic option for ALL.

Although we agree in general with their conclusion, our major concern is that the cytogenetic background between MAC and RIC might differ. Adjustment for cytogenetic risk groups was not performed in a multivariate analysis, because there was no difference in the cytogenetic distribution between MAC and RIC when analyzed among 3 risk groups: t(9;22), t(4;11), or other ($P = .10$). However, when analyzed among 4 groups, including NA/failed as one group, a significant difference was noted between MAC and RIC ($P = .02$). In fact, the number of Philadelphia chromosome–positive [Ph$^+$] ALL was smaller in MAC than in RIC [104/449 (23%) vs 41/127 (32%), $P = .049$]. Since the relapse incidence (RI) was higher among Ph$^+$ ALL patients than in the whole study population (40% ± 5% vs 31% ± 2% in MAC, and 49% ± 9% vs 47% ± 5% in RIC), lower RI in MAC might be associated with a smaller number of Ph$^+$ ALL. Allo-SCT has been recognized as the only curative therapy for Ph$^+$ ALL,[2] and there are already several reports of RIC allo-SCT for Ph$^+$ ALL.[3,4] It may be better to treat Ph$^+$ ALL and Philadelphia chromosome-negative (Ph$^-$) ALL as different diseases because their treatment would differ in an era of tyrosine kinase inhibitors. Therefore, it would be more practical to present data only from patients with Ph$^-$ ALL.[5]

The results of our 121 HLA-matched allo-SCT for adult Ph$^-$ ALL in first (81 MAC, 21 RIC) or second (14 MAC, 5 RIC) CR for patients aged ≥ 45 years (between 1998 and 2007 using the Japan Society for Hematopoietic Cell Transplantation and the Japan Marrow Donor Program database) were comparable between MAC and RIC (Figure 1A-D). In a multivariate analysis, RIC was not a significant risk factor for relapse (Hazard ratio [HR] 1.66, 95% confidence interval [CI] 0.63-4.37, $P = .30$). The variables considered in our multivariate analyses were conditioning (MAC vs RIC), age (> 50 years vs ≤50 years), sex, white blood cell counts (< 30 000/μL vs ≥30 000/μL), lineage (T vs B), disease status (first vs second CR), donor source (sibling vs unrelated), and graft-versus-host disease prophylaxis (cyclosporine-based vs tacrolimus-based). Similarly, RIC posed no significant risk factor for leukemia-free survival (HR 1.00, 95% CI 0.51-1.96, $P = .99$), nonrelapse mortality (HR 1.05, 95% CI 0.53-2.05, $P = .89$), and overall survival (HR 1.06, 95% CI 0.64-2.07, $P = .87$).

There are several ways to deal with missing data.[6] Given that the cytogenetics of 54% (244/449) for MAC and 43% (55/127) for RIC were missing in the study by Mohty et al, differences in how to handle missing data may produce different results. Because there was a difference in the cytogenetic distribution between MAC and RIC when analyzing missing data as one category, data adjusted for cytogenetic risk groups, or those of Ph$^-$ ALL, are of considerable interest.

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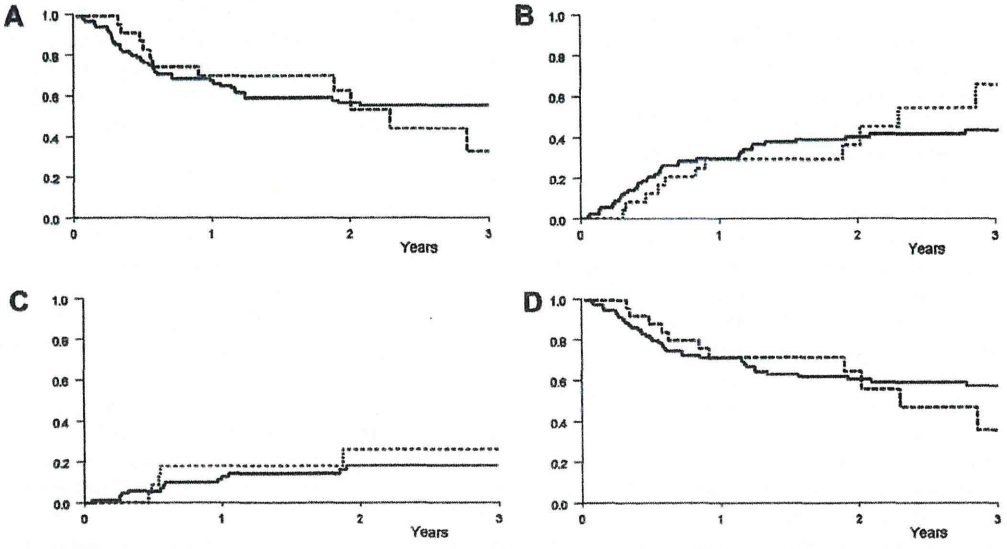

Figure 1. Survival probabilities. (A) Leukemia-free survival according to conditioning regimen: 58% ± 5% in myeloablative conditioning (MAC) vs 63% ± 11% in reduced-intensity conditioning (RIC) at 2 years ($P = .90$). (B) Nonrelapse mortality according to conditioning regimen: 40% ± 5% in MAC vs 36% ± 11% in RIC at 2 years ($P = .79$). (C) Relapse incidence according to conditioning regimen: 18% ± 5% in MAC vs 26% ± 11% in RIC at 2 years ($P = .27$). (D) Overall survival according to conditioning regimen: 59% ± 5% in MAC vs 63% ± 11% in RIC at 2 years ($P = .82$). Solid curve indicates MAC; dashed curve, RIC; x-axis, years after transplantation; and y-axis, probability.

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To the editor:

Dysregulation of the HIF pathway due to *VHL* mutation causing severe erythrocytosis and pulmonary arterial hypertension

Hereditary erythrocytosis can be caused by mutations in genes involved in the hypoxia-inducible factor (HIF) pathway.[1-3] For example, Chuvash polycythemia is caused by an R200W substitution in the von Hippel–Lindau protein (VHL).[1] There is increasing evidence linking VHL-HIF dysregulation to altered vascular physiology, and a mouse model of Chuvash polycythemia develops pulmonary arterial hypertension (PAH).[4-6] Recently, we reported an autosomal dominant erythrocytosis associated with an activating *EPAS-1* (*HIF-2A*) mutation in which there was late-onset PAH in some family members.[7] We now report a patient with severe erythropoietic dysregulation and PAH who is a compound heterozygote for novel *VHL* mutations.

A 2-month-old boy presented with increasing dyspnea and hypoxia requiring emergency ventilation and inotropic support. Echocardiography showed right ventricular dysfunction and hypertrophy. Severe PAH was confirmed by cardiac catheterization. Pulmonary artery systolic pressure was 91 mm Hg (approximately twice systemic values). Infusions of nitric oxide, prostacyclin, and sildenafil were required to allow discontinuation of ventilation. Treatment with vasodilators, diuretics, and bosentan was continued on eventual discharge from hospital.

Consistently raised hemoglobin (Hb) concentrations (> 21 g/dL) prompted further investigation. Serum erythropoietin (EPO) concentration was grossly elevated at 4120 IU/L. Diagnostic imaging and selective venous sampling provided no evidence of an EPO-secreting lesion. We hypothesized that this unusual phenotype was explicable by congenital dysregulation of the HIF pathway. Gene sequencing revealed heterozygous mutations in exon 2 (376 G>A) and exon 3 (548 C>T) of *VHL* (Figure 1A), predicting the amino acid changes Asp126Asn (D126N) and Ser183Leu (S183L), respectively.

To examine the functional consequences of the mutations, VHL-null renal carcinoma cells were transfected to generate cell pools stably expressing wild type (WT) or mutant proteins (Figure 1C). Function was assessed by measurement of the pH of cell culture media. Impaired or absent VHL function results in more rapid acidification because of HIF-mediated enhancement of glycolysis and suppression of mitochondrial respiration.[8,9] As expected, expression of WT VHL increased media pH while an inactivating VHL mutation (N78S) had no effect. In contrast, each of the D126N and S183L mutants exhibited an intermediate effect (Figure 1B). Pools expressing mutant proteins consumed more glucose and produced more lactate compared with WT, consistent with enhanced glycolytic metabolism (Figure 1B). To confirm that D126N and S183L mutations impair the ability of VHL to regulate HIF, we examined HIF-1α protein levels (Figure 1C) and the expression of HIF target genes *PHD3* and *GLUT-1* (Figure 1D), all of which were elevated in comparison to WT.

Thus, our patient has compound heterozygosity for novel mutations in VHL, which impair the ability to regulate HIF. Strikingly, EPO levels are greatly in excess of those observed in previous patients with inherited VHL-HIF dysfunction, suggesting that this patient has a more severe defect in HIF regulation. We observed that D126N and S183L were expressed at lower levels in transfected cells compared with WT. Because stably transfected cell pools exhibit a range of expression of the introduced protein, we examined this in multiple clonal sublines, with similar results. We hypothesized that this could reflect intrinsic differences in the

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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).

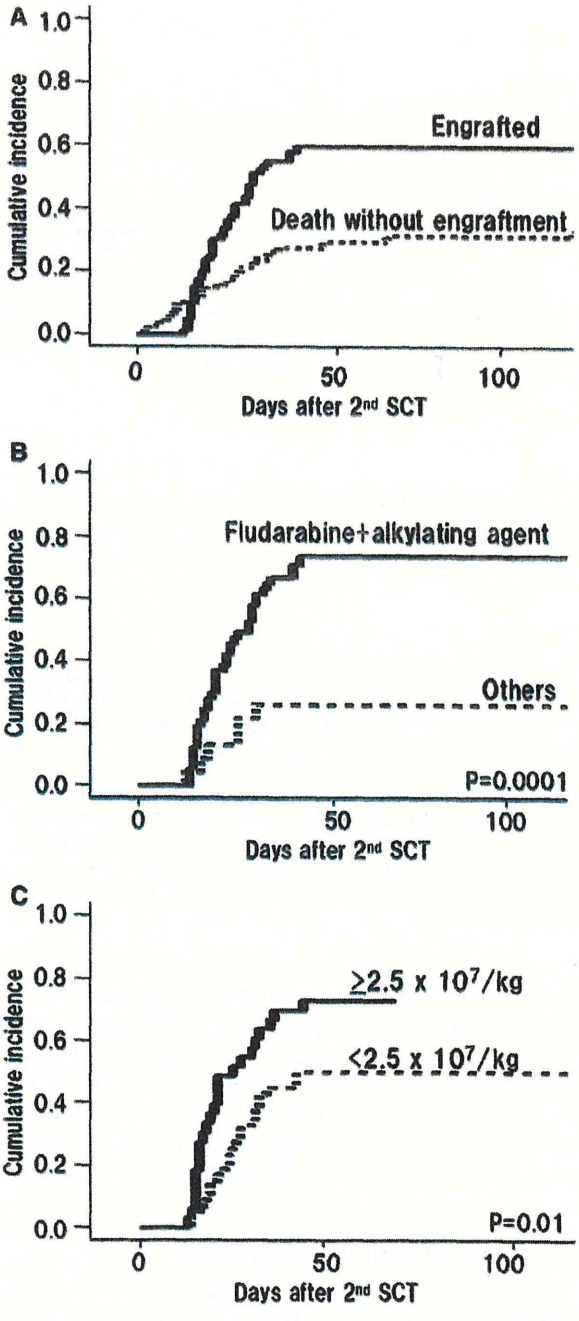

Figure 1. Cumulative incidence of neutrophil engraftment. (A) The cumulative incidences of neutrophil engraftment (solid line) and death without engraftment (dotted line) are shown. (B) The cumulative incidence of neutrophil engraftment was higher in patients who received alkylating agent–containing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