

Table 2.
Transplantation Outcomes*

Patient No.	Time to		Acute GVHD					mPSL, mg/kg	Response	Chronic GVHD (Involved Organs)	Follow-up, d	Current Disease Status	Cause of Death
	ANC >0.5 × 10 ⁹ /L, d	Platelets >20 × 10 ⁹ /L, d	Grade	Skin	Liver	Gut							
1	16	26	IV	3	4	4	2	PG	NE	121	Dead	Acute GVHD	
2	35	30	0	0	0	0	—	—	NE	133	Dead	Relapse	
3	17	—	0	0	0	0	—	—	NE	56	Dead	Relapse	
4	14	15	IV	4	0	0	2	CR	Ext (skin, mouth, eyes, liver, lung)	439	Dead	BO	
5	15	22	I	1	0	0	—	—	Ext (skin, mouth, liver)	286	Dead	IP	
6	21	38	II	3	0	0	—	—	NE	260	Dead	Relapse	
7	14	25	I	2	0	0	—	—	Ext (mouth, liver)	687+	CR, alive		
8	20	30	II	3	1	0	1	PR	Ext (skin)	667+	CR, alive		
9	22	—	II	3	0	0	—	—	Ext (skin, mouth, eyes)	336	Dead	Organizing pneumonia	
10	18†	—	0	0	0	0	—	—	NE	94	Dead	Secondary graft failure	
11	16	23	0	0	0	0	—	—	Ext (skin, mouth)	564+	CR, alive		
12	16†	—	IV	2	4	3	2	PG	NE	69	Dead	Acute GVHD	
13	18	23	I	1	0	0	1	CR	Ext (mouth, eyes, liver)	525+	CR, alive		
14	18	—	IV	3	4	2	1	UE	NE	64	Dead	Relapse	
15	14	16	0	0	0	0	—	—	Ext (mouth, eyes)	511+	CR, alive		
16	26	112	0	0	0	0	—	—	Lim (mouth)	463+	CR, alive		
17	22	38	IV	4	0	0	2	CR	Ext (skin, mouth, eyes, liver, lung)	276	Dead	BO + aspergillosis	

*ANC indicates absolute neutrophil count; GVHD, graft-versus-host disease; mPSL, methylprednisolone; PG, progressive response; NE, not evaluable; CR, complete response; Ext, extensive disease; BO, bronchiolitis obliterans; IP, interstitial pneumonitis; PR, partial response; UE, unevaluated; Lim, limited disease.

†Secondary graft failure occurred after neutrophil recovery.

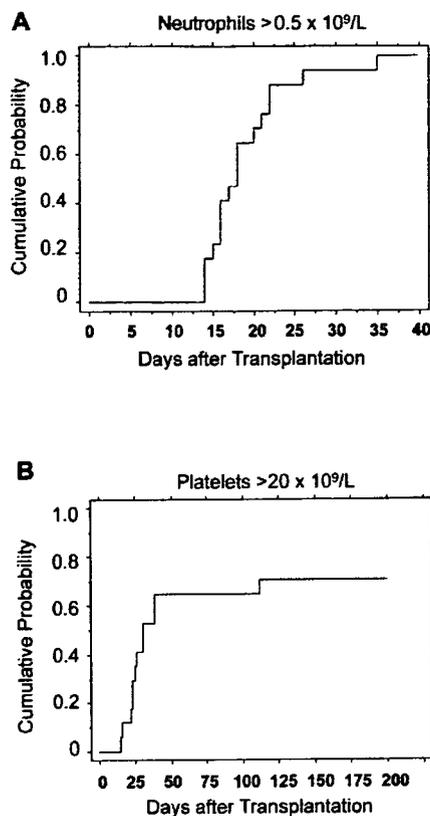


Figure 1. Engraftment after unrelated-donor bone marrow transplantation following reduced-intensity conditioning expressed as the cumulative probability of a neutrophil count $>0.5 \times 10^9/L$ (A) and a platelet count $>20 \times 10^9/L$ (B). All patients achieved neutrophil recovery, but 5 patients did not achieve platelet recovery. The median times until neutrophil and platelet recoveries were 18 days (range, 14-35 days) and 26 days (15-112 days), respectively. Late graft failure was observed in 2 patients.

marrow from a donor with allele-level mismatches at 3 HLA loci. Two patients with grade IV acute GVHD involving only the skin were successfully treated with methylprednisolone. Grade II acute GVHD involving only the skin was treated solely with CsA in 2 patients (Table 2). In 7 patients without relapse or secondary graft failure, CsA was tapered from a median of day 120 (range, day 96-169). Only 2 of the 7 patients were able to discontinue CsA (at days 203 and 288). Chronic GVHD was documented in all patients who

survived beyond day 100 (1 with limited GVHD, 9 with extensive disease). There was no significant correlation between HLA disparity at the allele level and the incidence of GVHD, although it was difficult to analyze the data statistically because of the small number of patients in this study.

3.5. Survival and Causes of Death

The median follow-up period was 286 days (range, 56-687 days). Overall, 11 patients died, but 6 patients are currently in remission (2 in remission and 4 not in remission at the time of transplantation). The estimated 100-day and 1-year nonrelapse mortality rates were 14% (95% CI, 12%-17%) and 46% (95% CI, 33%-57%), respectively (Figure 2B). Estimated 1-year overall survival and progression-free survival rates were both 41% (95% CI, 32%-51%; Figure 3). There were 4 deaths due to recurrent or progressive disease at a median time of 55 days (range, 32-93 days). The causes of the 7 treatment-related deaths included acute GVHD ($n = 2$), secondary graft failure with sepsis ($n = 1$), interstitial pneumonia ($n = 1$), organizing pneumonia ($n = 1$), bronchiolitis obliterans ($n = 1$), and bronchiolitis obliterans with invasive aspergillosis ($n = 1$).

4. Discussion

In our previous study in an unrelated-donor BMT setting, 5 patients underwent conditioning with a combination of Flu (30 mg/m^2 for 6 days) or cladribine (0.11 mg/kg for 6 days), BU (4 mg/kg for 2 days), and antithymocyte globulin (2.5 mg/kg for 4 days) without TBI, but secondary graft failure in 2 of these patients alerted us to a possible higher risk of graft rejection when we used bone marrow instead of peripheral blood cells as the stem cell source. In this study, we demonstrated that the addition of 4 Gy of TBI to the widely applied combination of Flu (30 mg/m^2 for 6 days) and BU (4 mg/kg for 2 days) reduces the risk of graft failure and enables the rapid achievement of full donor chimerism without donor lymphocyte infusion (DLI) and that the regimen-related toxicity was acceptable. Nevertheless, a relatively high incidence of nonrelapse mortality was observed. We lost 4 patients who developed extensive chronic GVHD and subsequent pulmonary complications in the later phase, more than 6 months after transplantation. Because many patients develop extensive GVHD, we assume that the pulmonary complications were primarily due to GVHD and not the consequence of our reduced-intensity stem cell transplantation (RIST) regimen incorporating 4 Gy of TBI. However, Deeg et al reported that more pulmonary compli-

Table 3.

Maximum Toxicities (N = 17)*

Grade	Cardiac, n	Mucositis, n	GI, n	Hepatic, n	CNS, n	Hyponatremia, n	Pulmonary, n	Renal, n
0	12	0	9	1	16	6	11	15
I	4	0	3	2	0	7	2	0
II	0	2	4	7	0	0	0	2
III	1	15	1	5	1	4	4	0
IV	0	0	0	2	0	0	0	0

*GI indicates gastrointestinal tract; CNS, central nervous system.

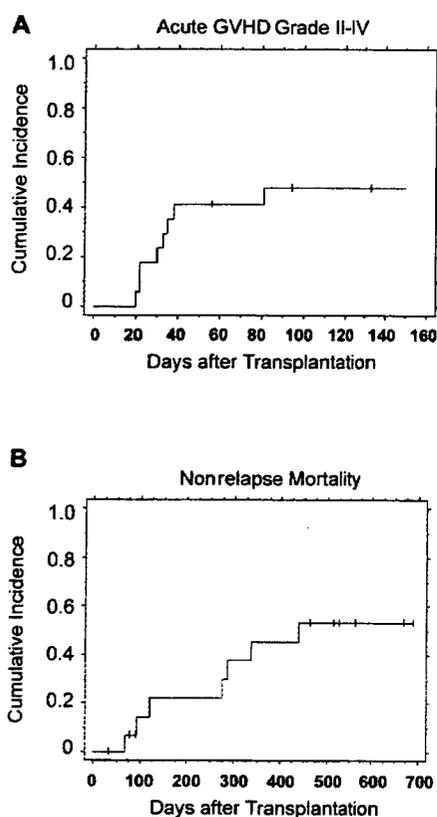


Figure 2. Cumulative incidence of acute GVHD (grades II-IV) (A) and nonrelapse mortality (B) after unrelated bone marrow transplantation following reduced-intensity conditioning. Acute GVHD (grades II-IV) was diagnosed in 8 patients (48%) (grade II in 3 patients and grade IV in 5) at a median of day 32 (range, day 20-81). The estimated 100-day and 1-year nonrelapse mortality rates were 14% and 46%, respectively.

cations developed in patients with aplastic anemia who received 4 to 6 Gy of TBI in combination with cyclophosphamide/antithymocyte globulin for unrelated-donor BMT than in patients who received 2 Gy TBI [20]. These investigators recommended that a 2-Gy TBI dose is sufficient to allow stable engraftment without increased toxicities, and this proposal should be evaluated in future studies. On the other hand, Maris et al described a nonmyeloablative conditioning regimen consisting of 2 Gy TBI and Flu (90 mg/m²) for unrelated-donor HSCT [8]. In their study, the use of bone marrow rather than G-CSF-mobilized peripheral blood cells as the source of hematopoietic stem cells led to a lower engraftment rate (56% versus 85%), as well as lower rates of overall survival (33% versus 57%) and progression-free survival (17% versus 44%). Because bone marrow is currently the only stem cell source available from volunteer donors in Japan, we may need a more intensified regimen than the combination of 2 Gy TBI and 90 mg/m² Flu.

In this study, the rates of acute GVHD of grades II to IV and extensive chronic GVHD in patients who survived for more than 100 days were 48% and 90%, respectively. Grade IV acute GVHD was the primary cause of death in 2

patients. Moreover, the quality of life of patients who develop extensive chronic GVHD rapidly deteriorates, particularly in elderly patients. Although CsA was tapered from a median of day 120 in this series, it might be better to delay the start of CsA tapering in elderly patients, who are associated with higher GVHD rates. Studies have incorporated in vivo T-cell depletion through the addition of antithymocyte globulin or alemtuzumab in order to reduce the risk of GVHD [21-26]. In the study reported by Chakraverty et al, severe GVHD following RIST from an unrelated donor was decreased with in vivo use of alemtuzumab in the preparative regimen [23]. In their study, the rates of acute GVHD (grades II to IV) and chronic GVHD were 21% and 8%, respectively. The long half-life of alemtuzumab (15-21 days) may disturb the induction of full donor chimerism, however. If patients cannot achieve full donor chimerism, the usual option is DLI, which carries a risk of GVHD [26]. Moreover, lymphocytes for DLI are not always available for every patient, particularly in unrelated-donor transplantation settings. In this regard, we think that a regimen that routinely involves DLI after transplantation cannot be considered a universal strategy. In the present study, 2 patients who had

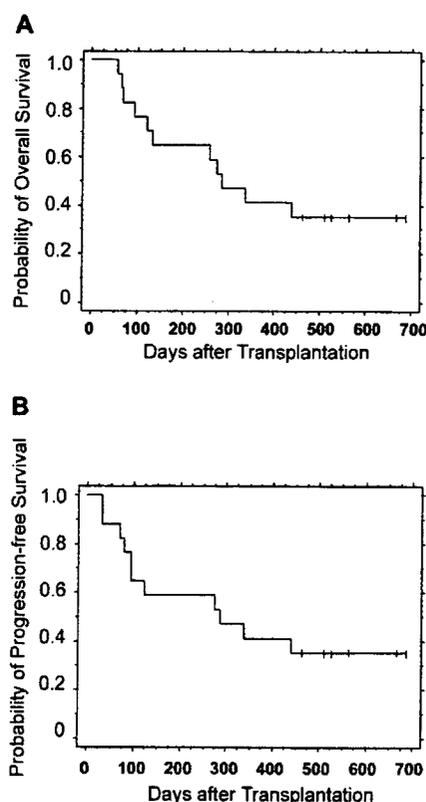


Figure 3. Kaplan-Meier actuarial probability of overall survival (OS) (A) and progression-free survival (PFS) (B) after unrelated-donor bone marrow transplantation following reduced-intensity conditioning. The median follow-up was 286 days (range, 56-687 days). The 1-year OS and PFS rates were both 41%. All 6 of the surviving patients (2 in remission and 4 not in remission at transplantation) remain in remission.

secondary graft failure did not receive DLI, because of grade IV acute GVHD in 1 patient and a reduced performance status in the other. Another approach to preventing severe GVHD is the use of novel immunosuppressive regimens. Several combinations of agents for GVHD prophylaxis, including CsA/mycophenolate mofetil [8,14,16] and tacrolimus/methotrexate [10,15,27], have been reported previously, and their value should be tested in prospective trials.

The induction of adequate antileukemic activity is another primary concern with a RIST procedure, particularly for patients with refractory diseases. de Lima et al reported a promising regimen that consisted of once-daily intravenous BU (130 mg/m² for 4 days) and Flu (40 mg/m² for 4 days) for patients with AML or MDS [27]. Replacement of oral BU with an intravenous preparation may result in an improved toxicity/survival profile. In our series, 4 patients achieved remission after RIST, although they were not in remission at the time of transplantation. Hence, it is likely that the antileukemic effect exerted by 4 Gy TBI in combination with Flu and BU is valuable even for the immediate control of leukemic blasts, although this possibility needs to be confirmed in further studies. The use of DLI has allowed the rescue of relapsed patients after allogeneic HSCT. In this study, however, we did not give DLI to 4 patients with progressive or relapsed diseases after transplantation because the relevance of the graft-versus-leukemia effect in rapidly proliferating diseases was not fully established and 2 of the patients had developed acute GVHD.

In conclusion, our regimen of 4 Gy TBI, Flu (180 mg/m²), and BU (8 mg/kg) was effective in reducing the risk of graft failure following unrelated-donor transplantation. We confirmed, however, that a high incidence of nonrelapse mortality, primarily due to GVHD and/or pulmonary complications, still remains a major obstacle for the wider application of this procedure to elderly or medically infirm patients. Further studies to identify ways to ameliorate transplantation-related toxicities are urgently required.

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Comparable Antileukemia/Lymphoma Effects in Nonremission Patients Undergoing Allogeneic Hematopoietic Cell Transplantation with a Conventional Cytoablative or Reduced-Intensity Regimen

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ABSTRACT

To evaluate the potential of allogeneic hematopoietic cell transplantation (HCT) with a reduced-intensity conditioning regimen (RIST) for the treatment of patients with hematologic malignancies not in remission, we retrospectively reviewed the medical records of 132 patients (89 leukemia or myelodysplastic syndrome, 40 malignant lymphoma, and 3 others) who received conventional myeloablative HCT (CST, $n = 52$) or RIST ($n = 80$). The median age of the RIST group was significantly higher than that of the CST group (53 years versus 40 years, $P < .01$). The RIST group also included a higher proportion of patients with an HCT-specific comorbidity index (HCT-CI) of 1 or more than the CST group (65% versus 37%, $P = .03$). The probabilities of achieving complete remission and the incidences of grades II-IV and III-IV acute graft-versus-host disease (aGVHD) in the CST and RIST groups were, respectively, 77% and 64%, 50% and 50%, and 23% and 28%, with no significant differences. Similarly, there was no difference in the 2-year probabilities of nonrelapse mortality (NRM, 36% and 38%), progressive disease or relapse (PD 51% and 49%), overall survival (OS, 31% and 38%), and progression-free survival (PFS, 28% and 29%). Multivariate analyses revealed that a higher HCT-CI score and transplant from donors other than HLA-matched relatives were associated with increased risks of NRM and poor OS, and patients who received chemotherapy within 2 months before HCT were associated with increased risks of PD, poor OS, and PFS after transplantation. After adjusting for these variables, the risks of NRM, PD, OS, and PFS in the RIST group were not significantly different from those in the CST group. In conclusion, these results suggest that the antileukemia/lymphoma effect associated with RIST is comparable to that associated with CST. RIST appears to be feasible for the treatment of hematologic malignancies not in remission.

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KEY WORDS

Transplantation • Leukemia • Lymphoma

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) has the potential to achieve long-term cure of hematologic malignancies by pretransplant conditioning and a graft-versus-leukemia/lymphoma (GVL) effect. It has been well established that the disease status

at the time of transplantation is the most important prognostic factor, and the rates of relapse and nonrelapse mortality (NRM) significantly increase in patients with hematologic malignancies who were not in remission. Therefore, conventional stem cell transplantation (CST) using a myeloablative conditioning

regimen has been universally used in the hope of maximally reducing the tumor burden before HCT in patients not in remission. However, CST may not be an option for many patients because of their older age or associated comorbidities. Alternatively, over the past few years, nonmyeloablative and reduced-intensity conditioning stem cell transplantation (RIST) have been offered to these patients undergoing HCT, on the assumption that RIST would be better tolerated [1-4].

There have been several reports that the outcome of older patients who underwent RIST while in remission was comparable to that of patients who received CST [5-9], which suggests that the GVL effect associated with RIST might be adequate for controlling chemosensitive or slowly progressing disease. On the other hand, it still remains controversial whether RIST is feasible for patients not in remission, although small pilot studies have shown that RIST was unsuccessful for advanced hematologic malignancies [3,10-14]. To address this issue, we retrospectively analyzed 132 patients who were not in remission at the time of CST or RIST.

PATIENTS AND METHODS

Study Patients

We retrospectively reviewed the medical records of 132 patients with various hematologic malignancies who underwent allogeneic HCT (CST, $n = 52$; RIST, $n = 80$) while not in remission at our institution from January 2000 to December 2004. Patients with chronic myelogenous leukemia (CML) in the chronic phase, myelodysplastic syndrome (MDS)-refractory anemia, and those with lymphoma in partial remission (PR) were not included because the response to treatment and the outcome of these patients is generally considered to be similar to those in patients who are in complete remission (CR). Bone marrow or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC) were harvested from donors according to protocols approved by the guidelines of the Japan Marrow Donor Program, the Japanese Society for Hematopoietic Cell Transplantation, and the Japanese Society of Blood Transfusion. Informed consent was obtained according to the Declaration of Helsinki.

Transplantation Procedures

The conditioning regimens used in CST included the combination of cyclophosphamide (CY; 60 mg/kg i.v. daily for 2 days) and fractionated total body irradiation (TBI; 12 Gy in 6 fractions over 3 days) in 34 patients, CY and oral busulfan (BU; 16 mg/kg divided over 4 days) in 13 patients, and other combinations in 5 patients (Table 1). Targeted dose adjustment of BU

was not performed. Patients who underwent RIST were older than 50 years of age or those who had comorbidities or prior transplantation. The conditioning regimens for RIST consisted of fludarabine (30 mg/m² i.v. daily for 6 days) or cladribine (0.11 mg/kg i.v. daily for 6 days) plus 8 mg/kg of oral BU [15] with ($n = 27$) or without ($n = 53$) 4 Gy TBI. In Japan, only bone marrow is permitted as a stem cell source in transplantation from an unrelated healthy volunteer donor. In the setting of nonmyeloablative stem cell transplantation from an unrelated donor, the sustained engraftment rate has been reported to be lower for recipients of bone marrow than for those given PBSC [13]. Therefore, low-dose TBI was also added to the conditioning regimen for RIST from an unrelated donor to facilitate engraftment.

Day 0 was defined as the day of stem cell infusion. G-CSF was administered after transplantation in all patients until neutrophil engraftment. Most patients who underwent CST were given cyclosporine (CSP) with methotrexate (MTX) [16], and all patients who underwent RIST were given CSP with or without MTX for graft-versus-host disease (GVHD) prophylaxis (Table 1). GVHD was treated with 1 to 2 mg/kg/day prednisolone equivalents, resumption of full-dose CSP administration if applicable, or both. Initial doses of corticosteroids and tapering schedules of immunosuppressive medications were modified at the discretion of the attending physicians according to the presence or absence of malignant cells and the severity of GVHD. Treatment for relapse after transplantation was left to the discretion of the attending physicians.

All patients received ciprofloxacin (200 mg orally 3 times daily) for bacterial prophylaxis until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis. Patients who had positive serologic test results for herpes simplex virus or varicella zoster virus received prophylactic low-dose acyclovir until the cessation of immunosuppressive agents [17]. Prophylaxis against *Pneumocystis jirovecii* infection was provided with trimethoprim-sulfamethoxazole from the first day of conditioning to day -3 of transplantation, and from day 28 until day 180 or the cessation of immunosuppressive agents. Patients were monitored with weekly cytomegalovirus (CMV) pp65 antigenemia testing, and positive antigenemia was treated with ganciclovir as described previously [18,19].

Definitions

Chemotherapy within 2 months before HCT was defined as chemotherapy to control the disease except for rituximab alone for lymphoma and imatinib mesylate alone for CML. Pretransplantation comorbidities were determined by the HCT-specific comorbidity index (HCT-CI) [20] with a minor modification [21]. Neutrophil engraftment was defined as the first

Table 1. Patient Characteristics

	CST	RIST	P-Value
No. of patients	52	80	
Sex, male/female	25/27	50/30	
Median age, years (range)	40 (3-55)	53 (20-68)	<.01
Disease status at conditioning, N (%)			
AML	20 (38)	15 (19)	
Relapse 1	12	6	
Relapse \geq 2	5	5	
Primary refractory	3	4	
MDS (including overt AML)	15 (29)	24 (30)	
Relapse 1	1	2	
Untreated	6	11	
Primary refractory	8	11	
ALL	5 (10)	2 (3)	
Relapse 1	4	1	
Relapse 2	1	1	
CML	5 (10)	3 (4)	
Accelerated phase	3	0	
Blastic crisis	2	3	
NHL	7 (13)	33 (40)	
Relapse 1	2	6	
Relapse \geq 2	2	16	
Primary refractory	3	11	
Others*	0	3 (4)	
Chemotherapy within 2 months before HCT, N (%)	33 (63)	52 (65)	
Leukemia/MDS	30	23	
Lymphoma	3	26	
Others*	0	3	
HCT-CI score, N (%)			.03
0	33 (63)	28 (35)	
1-2	11 (21)	31 (39)	
\geq 3	8 (16)	21 (26)	
Conditioning regimen, N (%)			
TBI/CY	34 (65)	0	
BU/CY	13 (25)	0	
Fludarabine-based (\pm TBI)†	0	68 (85)	
Cladribine-based (\pm TBI)‡	0	12 (15)	
Others	5 (10)	0	
Donor type, N (%)			.1
HLA-matched related donor	17 (33)	41 (51)	
HLA-mismatched related donor	5 (9)	7 (9)	
Unrelated donor	30 (58)	32 (40)	
Stem cell source, N (%)			<.01
G-CSF mobilized PBSC	21 (40)	49 (61)	
BM	27 (52)	20 (25)	
CB	4 (8)	11 (14)	
GVHD prophylaxis, N (%)			<.01
Cyclosporine§	1 (2)	52 (65)	
Cyclosporine/MTX¶	49 (94)	28 (35)	
Tacrolimus	1 (2)	0	
Tacrolimus/MTX	1 (2)	0	
Prior HCT, N (%)	4 (8)	8 (10)	.65

CST indicates conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; NHL, non-Hodgkin's lymphoma; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; TBI, total-body irradiation; CY, cyclophosphamide; BU, busulfan; HLA, human leukocyte antigen; G-CSF, granulocyte colony-stimulating factor; PBSC, peripheral blood stem cell; BM, bone marrow; CB, cord blood; GVHD, graft-versus-host disease; MTX, methotrexate.

*Others included 1 chronic lymphocytic leukemia and 2 multiple myeloma patients.

†Twenty-three patients received 4 Gy TBI.

‡Four patients received 4 Gy TBI.

§Including 7 with antithymocyte globulin.

¶Including 10 with antithymocyte globulin.

of 3 consecutive days after transplantation that the absolute neutrophil count exceeded $0.5 \times 10^9/L$ of peripheral blood. The diagnosis and clinical grading of acute and chronic GVHD (aGVHD, cGVHD) were performed according to established criteria [22-24]. CR was defined as lower than 5% blasts in the bone marrow, with a neutrophil count $>1.5 \times 10^9/L$ and a platelet count $>100 \times 10^9/L$ in leukemia/MDS patients, and according to the International Workshop Criteria [25] in lymphoma patients.

Statistical Analysis

The endpoints of the study were progressive disease/relapse (PD), NRM, overall survival (OS), and progression-free survival (PFS). OS, NRM, and PD were defined as the time between stem cell infusion to the event. PFS was defined as the time between stem cell infusion to PD or death from any cause, whichever occurred earlier. OS and PFS were estimated by the Kaplan-Meier method [26]. NRM and PD were estimated by the cumulative incidence. The chi-square test or Fisher's exact test was used to evaluate the differences in the clinical characteristics of the CST and RIST groups. The log-rank test and the generalized Wilcoxon test were used to compare the probabilities of survival, NRM, and PD after HCT over time across patient subgroups.

Multiple Cox regression models were used for multivariate risk factor analysis for PD, NRM, OS, and PFS after HCT. Clinical factors evaluated in the PD, NRM, OS, and PFS analyses were patient age at the time of HCT (continuous), HCT-CI (0, 1-2, 3 or more), conditioning (CST, RIST), donor (HLA-matched related, HLA-mismatched related or unrelated), disease type (leukemia/MDS, lymphoma), and chemotherapy within 2 months before HCT (yes, no). Logistic regression analysis was performed to identify prognostic factors that were associated with the achievement of CR. In addition to the variables examined in the Cox analysis, blast percentage ($\geq 20\%$, $<20\%$) in the bone marrow or peripheral blood and the serum lactate dehydrogenase (LDH) level (normal, elevation) before HCT were included for the analysis of CR in patients with leukemia/MDS and those with lymphoma, respectively. We considered 2-sided *P*-values of $<.05$ to be statistically significant. Statistical analyses were performed with the SAS version 8.2 (SAS Inc, Cary, NC).

RESULTS

Patient Characteristics

The characteristics of all patients who underwent CST ($n = 52$) or RIST ($n = 80$) are summarized in Table 1. The median age of the RIST group was significantly higher than that of the CST group (53

years versus 40 years, $P < .01$). A large number of patients in both groups had acute myeloid leukemia (AML) or MDS (CST 67%, RIST 49%), and the RIST group included a higher population of patients with malignant lymphoma (CST 13%, RIST 40%). All malignant lymphomas ($n = 40$) were non-Hodgkin's lymphoma, including aggressive ($n = 16$), highly aggressive ($n = 15$), and indolent ($n = 9$) lymphomas. The distribution of lymphoma subtypes was similar between the 2 groups. Disease status at transplantation included primary refractory ($n = 42$), refractory relapse ($n = 65$), blastic crisis, or accelerated phase of CML ($n = 8$) and untreated disease ($n = 17$). The distribution of disease status and the proportion of patients who received chemotherapy within 2 months before HCT were similar between the 2 groups. The RIST group contained higher proportions of patients with an HCT-CI score of 1 or more (CST 37%, RIST 65%) and those who received G-CSF-mobilized PBSC (CST 40%, RIST 61%) than the CST group.

In the leukemia/MDS patients ($n = 89$), the median percentage of blasts (82 patients in bone marrow and 7 patients in peripheral blood) in both groups were similar (CST 29%, RIST 30%). In patients with malignant lymphoma, serum LDH was elevated above the upper normal limit in 3 of 7 (43%) in the CST group compared to 23 of 33 (70%) in the RIST group.

Engraftment and GVHD

The clinical course and response are detailed in Table 2. The median duration of follow-up in surviving patients is 1123 days (range: 367-2044 days) in the CST group and 899 days (range: 334-1961 days) in the RIST group. Neutrophil engraftment was observed in 48 patients (92%) and 75 patients (94%), at a median of 17 days and 12 days, respectively. Engraftment was not confirmed in the remaining 9 patients because of death or PD within 28 days after HCT. The incidences of grade II-IV and grade III-IV aGVHD were similar in the CST and RIST groups (50% versus 50% and 23% versus 28%, respectively). The incidences of cGVHD and chronic extensive GVHD were also similar (46% versus 49% and 34% versus 38%, respectively).

Disease Response

The probabilities of achieving CR as the best response were similar after CST and RIST (77% and 64%, respectively) (Table 2). To examine the possible risk factors for achieving CR, we separately analyzed patients with leukemia/MDS and those with lymphoma using a logistic regression analysis (Table 3). Conditioning regimen (RIST) did not influence the CR rate in patients with leukemia/MDS (odds ratio [OR] 1.11, 95% confidence interval [CI] 0.40-3.07,

Table 2. Clinical Course and Response

	CST (N = 52)	RIST (N = 80)
Median follow-up of surviving patients, days	1123 (367-2044)	899 (334-1961)
Engraftment of neutrophils, N (%)	48 (92)	75 (94)
Median day (range)	17 (10-35)	12 (5-43)
Acute GVHD, N (%)		
Grade II-IV	26 (50)	40 (50)
Grade III-IV	12 (23)	22 (28)
CR*, N (%)	40 (77)	51 (64)
Leukemia/MDS (n = 89), CR/total	35/45	35/44
Lymphoma (n = 40), CR/total	5/7	14/33
Causes of NRM, N (%)	15 (29)	26 (33)
GVHD	6	11
Infection		
fungus	0	4
CMV	0	1
bacterial	4	7
Interstitial pneumonitis	2	1
Others†	3	2

CST indicates conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; GVHD, graft-versus-host disease; MDS, myelodysplastic syndrome; CR, complete remission; NRM, nonrelapse mortality; CMV, cytomegalovirus.

*CR as the best response after transplantation.

†Others included acute myocardial infarction, subarachnoid hemorrhage, and pulmonary alveolar haemorrhage in the CST group, and cerebral hemorrhage and unknown in the RIST group.

$P = .84$) or in those with lymphoma (OR 0.29, 95% CI 0.05-1.75, $P = .18$). In the leukemia/MDS patients, those who received chemotherapy within 2 months before HCT (OR 0.32, 95% CI 0.09-1.05, $P = .06$) and transplant from donors other than an HLA-matched relative (OR 0.28, 95% CI 0.08-1.06, $P = .06$) tended to have a lower CR rate, whereas the

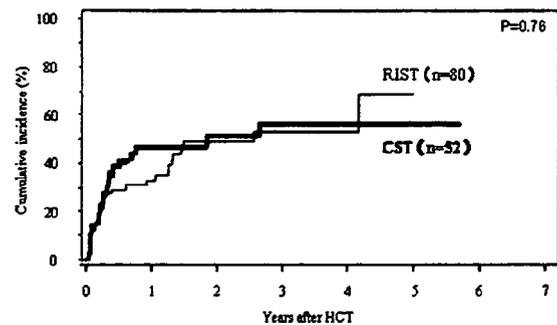


Figure 1. Cumulative incidence of PD. The 2-year probabilities of PD in the CST (51%) and RIST (49%) groups were not significantly different ($P = .76$).

blast percentage ($\geq 20\%$) of bone marrow or peripheral blood was not associated with the CR rate. In lymphoma patients, chemotherapy within 2 months before HCT was the only factor that was significantly associated with a low CR rate (OR 0.04, 95% CI 0.005-0.40, $P < .01$), whereas serum LDH elevation did not influence the CR rate.

As shown in Figure 1, the cumulative incidence of PD was not significantly different between the CST and RIST groups. The 2-year probabilities of PD were 51% in the CST group and 49% in the RIST group, which were not significantly different ($P = .76$). Cox regression analysis was performed to identify factors that were associated with PD. Multivariate analyses in all patients showed that those who received chemotherapy within 2 months before HCT were associated with an increased risk of PD (hazard ratio [HR] 3.93, 95% CI 1.97-7.83, $P < .01$) (Table 4). After adjusting for these variables, the intensity of conditioning (CST or RIST) did not influence the rate of PD in any of the patients. To further evaluate the association between risk factors and outcome, we performed a subset analysis in patients who underwent CST or RIST. As a result, chemotherapy within 2

Table 3. Logistic Analysis of CR Rate in Leukemia/MDS and Lymphoma Patients

		Leukemia/MDS (N = 89)		Lymphoma (N = 40)	
		Odds Ratio (95% CI)	P	Odds Ratio (95% CI)	P
HCT-CI	0	1.00		1.00	
	1-2	1.44 (0.43-4.87)	.56	3.33 (0.66-16.7)	.14
	3 or more	0.96 (0.28-3.35)	.95	2.22 (0.40-12.3)	.36
Age		1.00 (0.97-1.04)	.70	1.02 (0.97-1.07)	.49
Conditioning	RIST	1.11 (0.40-3.07)	.84	0.29 (0.05-1.75)	.18
Donor	Alternative*	0.28 (0.08-1.06)	.06	0.95 (0.26-3.42)	.93
Chemotherapy within 2 months before HCT	Yes	0.32 (0.09-1.05)	.06	0.04 (0.005-0.40)	<.01
	Blasts†	$\geq 20\%$	0.62 (0.21-1.80)	.38	
Serum LDH level	Elevation			0.35 (0.09-1.34)	.12

MDS indicates myelodysplastic syndrome; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; RIST, reduced-intensity stem cell transplantation; LDH, lactate dehydrogenase; CI, confidence interval.

*Non-HLA-matched related donor.

†Blast counts in bone marrow (N = 82) or peripheral blood (N = 7).

Table 4. Multivariate Analysis of PD, NRM, OS, and PFS in All Patients

Covariates*	N	PD		NRM		OS		PFS	
		HR (95% CI)	P						
Conditioning									
CST	52	1.00		1.00		1.00		1.00	
RIST	80	0.91 (0.53-1.55)	.72	0.99 (0.51-1.96)	.99	0.95 (0.60-1.51)	.83	0.95 (0.63-1.43)	.79
HCT-CI score									
0	65			1.00		1.00		1.00	
1-2	38			3.25 (1.43-7.40)	<.01	1.76 (1.08-2.89)	.02		
3 or more	29			6.61 (2.88-15.2)	<.01	2.62 (1.51-4.56)	<.01	1.63 (1.02-2.62)	.04
Donor									
MRD	58			1.00		1.00			
Alternative†	74			2.77 (1.39-5.54)	<.01	1.80 (1.15-2.82)	.01		
Chemotherapy within 2 months before HCT									
No	47	1.00				1.00		1.00	
Yes	85	3.93 (1.97-7.83)	<.01			1.73 (1.10-2.72)	.02	2.23 (1.44-3.45)	<.01

PD indicates progressive disease or relapse; NRM, nonrelapse mortality; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; MRD, HLA-matched related donor; HCT, hematopoietic cell transplantation.

*Factors analyzed included age at the time of HCT (continuous), HCT-CI (0, 1-2, 3, or more), conditioning (CST, RIST), donor (MRD, Alternative), disease type (leukemia/MDS, lymphoma) and chemotherapy within 2 months before HCT (yes, no).

†Non-HLA-matched related donor.

months before HCT was associated with an increased risk of PD only in the RIST group, and not in the CST group (Table 5).

NRM

Major causes of NRM for patients in both groups were GVHD and infection (Table 2). More patients died of fungal infection in the RIST group compared to the CST group, but the 2-year probabilities of NRM were not significantly different (36% and 38%, $P = .50$, Figure 2). A Cox regression analysis was performed to identify factors associated with NRM. Multivariate analyses in all patients showed that a higher HCT-CI score (1 or more) and transplant from an HLA-mismatched related or unrelated donor (al-

ternative donor) were associated with an increased risk of NRM (Table 4). After adjusting for these variables, the intensity of conditioning (CST or RIST) did not influence the rate of NRM in any of the patients. A subset analysis revealed that a higher HCT-CI score (1 or more) was associated with increased NRM in the CST group, but not in the RIST group (Table 5). In contrast, transplant from an alternative donor was associated with increased NRM in the RIST group, but not in the CST group.

Survival

The 2-year probabilities of OS and PFS were not significantly different between the CST and RIST groups (31% and 38%, $P = .98$, for OS; 28% and

Table 5. Multivariate Analysis of Outcomes after HCT in the CST and RIST Groups

Covariates	CST (N = 52)		RIST (N = 80)	
	HR (95% CI)	P	HR (95% CI)	P
PD				
Chemotherapy within 2 months before HCT		NS	6.16 (2.15-17.7)	<.01
NRM				
HCT-CI (1-2)	4.48 (1.26-16.0)	.02		NS
HCT-CI (3 or more)	10.2 (2.91-35.7)	<.01	2.41 (1.14-5.10)	.02
Alternative donor*		NS	4.63 (1.96-10.9)	<.01
OS				
HCT-CI (1-2)	2.69 (1.23-5.90)	.01		NS
HCT-CI (3 or more)	4.84 (1.97-11.9)	<.01		NS
Alternative donor*		NS	3.04 (1.73-5.35)	<.01
PFS				
HCT-CI (3 or more)	2.26 (1.01-5.04)	.04		NS
Chemotherapy within 2 months before HCT	2.10 (1.05-4.19)	.03	2.10 (1.19-3.70)	.01
Alternative donor*		NS	1.79 (1.06-3.00)	.03

PD, indicates progressive disease or relapse; NRM, nonrelapse mortality; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; NS, not significant.

*Non-HLA-matched related donor.

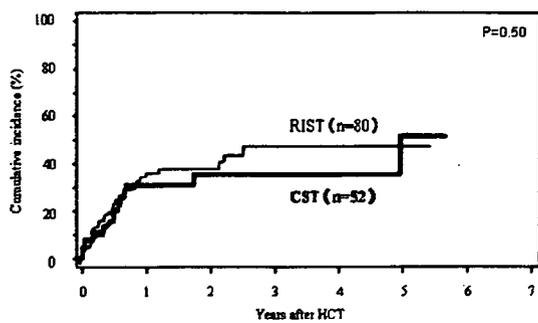


Figure 2. Cumulative incidence of NRM. The 2-year probabilities of NRM in the CST (36%) and RIST (38%) groups were not significantly different ($P = .50$).

29%, $P = .89$, for PFS), as shown in Figure 3 and Figure 4. The 2-year probabilities of PD, OS, and PFS were not significantly different between patients who developed grade III-IV aGVHD and those who did not (37% and 44%, $P = .39$, for PD; 33% and 50%, $P = .07$, for OS; 27% and 41%, $P = .24$, for PFS). On the other hand, the 2-year probability of NRM in patients who developed grade III-IV aGVHD was significantly higher than that in those who did not (56% and 21%, $P = .004$). We also evaluated outcomes in patients who had AML or MDS (CST, $n = 35$; RIST, $n = 39$). There was no significant difference in the 2-year probabilities of PD (50% and 51%), OS (37% and 33%), and PFS (34% and 22%) between the CST and RIST groups. On the other hand, the 2-year probability of NRM in the RIST group was significantly higher than that in the CST group (52% and 23%, $P = .03$).

Multivariate analyses in all patients showed that a higher HCT-CI score (1 or more) and transplant from an alternative donor were associated with poor OS, and patients who received chemotherapy within 2 months before HCT were associated with poor OS and PFS (Table 4). After adjusting for these variables, the risks of OS and PFS were not significantly different between the CST and RIST groups. Disease type (leukemia/MDS or lymphoma) was not a significant

factor for OS or PFS. Furthermore, subset analyses revealed that a higher HCT-CI score (1 or more) was associated with poor OS and PFS in the CST group, but not in the RIST group (Table 5). In contrast, transplant from an alternative donor was associated with increased NRM in the RIST group, but not in the CST group. Patients who received chemotherapy within 2 months before HCT had a poor PFS in both groups.

DISCUSSION

Our results suggest that the antileukemia/lymphoma effect of RIST might be comparable to that of CST for hematologic malignancies that are not in remission. We found that a higher HCT-CI score and transplant from an alternative donor were associated with increased risks of NRM and poor OS, and patients who received chemotherapy within 2 months before HCT because of the acceleration of disease progression were associated with increased risks of PD, poor OS, and PFS. The estimated rates of NRM, PD, OS, and PFS in the RIST group were not significantly different from those in the CST group even though the patients who received RIST were significantly older and had significantly higher HCT-CI scores than those who received CST. Several reports have described a similar OS rate in older patients who underwent RIST and CST because the lower NRM rate was offset by a higher PD [5,27,28]. In contrast, Scott et al. [7] found no significant differences in OS, PFS, PD, or NRM between CST and RIST in patients with MDS/AML.

In this study, disease response to the transplantation procedure was similar between the CST and RIST groups when the CR rate is considered the best response, as were the rate and timing of PD. Whereas some reports have shown that PD after HCT was increased in patients who underwent RIST compared to CST [3,5,11], others have found no significant difference [6-8,29]. This discrepancy might result from the differences in disease status at the time of

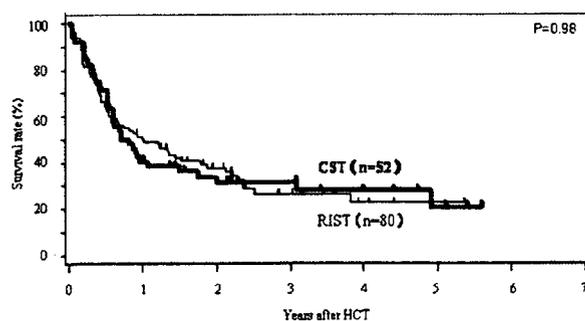


Figure 3. Estimated OS according to the conditioning regimen. The 2-year probabilities of OS in the CST (31%) and RIST (38%) groups were not significantly different ($P = .98$).

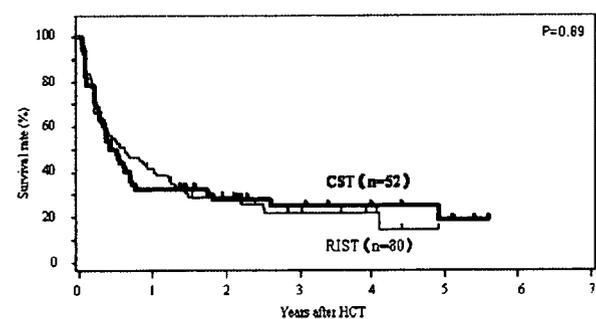


Figure 4. Estimated PFS according to the conditioning regimen. The 2-year probabilities of PFS in the CST (28%) and RIST (29%) groups were not significantly different ($P = .89$).

transplantation and the intensity of the conditioning regimens. In our study, the median percentage of blasts in leukemia/MDS patients and the distribution of serum LDH levels in lymphoma patients were comparable between the CST and RIST groups. The proportion of patients who required chemotherapy within 2 months before HCT was similar in the 2 groups. Overall, the risk of disease progression was comparable. The lack of a significant difference in PD between the CST and RIST groups in our study may be because the reduced-intensity regimens used in our study were more intense than those in previous reports. Nevertheless, our results suggest that RIST has a comparable antileukemia/lymphoma activity through a GVL effect compared to CST.

Our study found that chemotherapy within 2 months before HCT was the only factor that significantly predicted a lower CR rate in lymphoma patients and tended to be associated with a lower CR rate in leukemia/MDS patients. Furthermore, chemotherapy within 2 months before HCT was also associated with a worse prognosis not only with regard to PD but also for OS and PFS. A subset analysis showed that this negative impact of recent chemotherapy was only seen in RIST patients, and not in CST patients, which suggests that the tempo of the progression of the disease before HCT is especially important in RIST patients. Wong et al. [30] reported that high peripheral blast counts ($\geq 30\%$) in patients with AML/MDS were associated with poor event-free survival and OS after HCT regardless of the conditioning regimen. In our study, however, $\geq 20\%$ of blasts in the bone marrow or peripheral blood and serum LDH level elevation did not have a significant impact on the CR rate in leukemia/MDS and lymphoma patients, respectively.

In our study, there was no significant difference in NRM between the CST and RIST groups, which was in contrast to previous reports showing that reduced-intensity regimens were associated with less organ damage, and thus contributed to less NRM [1,4,5,9,27,31-34]. There are several possible explanations for this discrepancy. First, the patients who received RIST were older and had a higher HCT-CI score than those in the CST group. Second, the reduced-intensity conditioning (RIC) we used was more toxic than "truly nonmyeloablative" conditioning. Finally, we tapered immunosuppressive medications rapidly, especially in the RIST group, in an attempt to induce a more potent GVL effect, which resulted in more severe GVHD and subsequent infectious complications. However, our data showed that grade III-IV aGVHD did not contribute to a reduction in the rate of PD or to an overall improvement in survival, which was consistent with a previous report [14], although a high rate of NRM in patients with severe aGVHD may have masked its competing event (ie, PD).

We confirmed that HCT-CI was a significant risk factor for NRM and OS in patients not in remission. HCT-CI has recently been introduced to evaluate pretransplant comorbidities in HCT recipients, which predict well NRM and OS after allogeneic HCT [20]. In this study, the proportion of patients who were not in remission and were associated with comorbidities was 53%, which was higher than the value (42%) in our previous report [21], probably because these patients tended to be heavily pretreated and were forced to pursue HCT in the hope of a rare cure. Interestingly, this negative impact of HCT-CI was only seen in patients who underwent CST, and not in those who underwent RIST. Our data imply that RIC may be preferable in patients with hematologic malignancies not in remission and with a high HCT-CI score by reducing early NRM after transplantation.

Transplant from an alternative donor was another prognostic factor for NRM and OS in this study, which is consistent with previous reports [12,35-38]. Furthermore, an increased risk of NRM and OS associated with alternative donors was observed only in patients who underwent RIST. There are several possible explanations. First, the Japan Marrow Donor Program allows the donation of bone marrow, but not PBSC, from volunteer donors, which has been reported to be associated with poor engraftment and worse outcomes after nonmyeloablative stem cell transplantation [13]. Second, our conditioning regimen including low-dose TBI for RIST from an alternative donor was more toxic than that for RIST from an HLA-matched related donor. Further studies are required to establish optimized conditioning regimens and GVHD prophylaxis for RIST in unrelated pair settings.

In 27 patients who had all of these risk factors (ie, chemotherapy within 2 months before HCT, HCT-CI score of 1 or more, and transplant from an alternative donor), the 2-year probabilities of NRM, PD, and OS were 56%, 44%, and 21%, respectively, with no significant differences between the CST and RIST groups (data not shown). Therefore, the indications for transplantation in patients with multiple risk factors should be carefully determined.

This study has several inherent limitations. First, the eligibility requirements for CST and RIST were different. Most patients who received RIST were considered ineligible for CST because of age or comorbid conditions. Second, factors other than the conditioning regimen were not entirely comparable between the 2 groups, that is, patient age, underlying diagnosis (leukemia/MDS and lymphoma), donor selection, stem cell source, and GVHD prophylaxis. Third, some of the conventional cytoreductive conditioning regimens we used (ie, use of oral BU and lack of its pharmacologic monitoring) may no longer be considered optimal. Fourth, because the reduced-intensity

regimens used in our study were more intense than those in previous reports, our data may not be generalized to the concept of "reduced-intensity regimen" and there may be circumstances where PD would be more marked. Finally, the follow-up of patients in this study was too short to draw any definite conclusions. Nevertheless, the observed data may still be useful in evaluating the impact of RIST on disease control in patients suffering from a higher risk of disease progression after transplantation.

In conclusion, our results suggest that the antileukemia/lymphoma effect associated with RIST might be comparable to that of CST for hematologic malignancies not in remission, particularly when patients do not require chemotherapy within 2 months before HCT or they had a higher HCT-CI score. To determine the ultimate utility of specific conditioning regimens, controlled prospective trials are needed, with enrolled patients being stratified according to disease activity, hematopoietic stem cell source, and associated comorbidities.

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T-Cell Large Granular Lymphocyte Leukemia of Donor Origin After Cord Blood Transplantation

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Abstract

We report the first case of T-cell large granular lymphocyte leukemia of donor origin after a second cord blood transplantation for acute myeloid leukemia, and review the literature regarding rare cases of T-cell-origin posttransplantation lymphoproliferative disorders.

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Key words: Bone marrow, Epstein-Barr virus, Polymerase chain reaction, Posttransplantation lymphoproliferative disorders, T-cell receptor

Introduction

T-cell large granular lymphocyte leukemia (LGL; LGLL) is characterized by the monoclonal proliferation of CD3⁺, and CD8⁺ LGLs, with abundant cytoplasm and fine or coarse azurophilic granules.^{1,2} Reactive expansion of LGL in the peripheral blood has been occasionally reported during viral infection and in recovery phase of allogeneic hematopoietic stem cell transplantation (HSCT).^{3,4}

Posttransplantation lymphoproliferative disorder (PTLD) is a characteristic lymphoid proliferation or the development of lymphoma in a setting of decreased T-cell immune surveillance, typically in recipients of solid organ transplantation or allogeneic HSCT. Most reported cases of PTLD are of B-cell origin, in association with Epstein-Barr virus (EBV) infection, which leads to monoclonal or, less frequently, polyclonal proliferation of B cells. Most of the rare cases of T-cell PTLD were reported after solid organ transplantation, with very rare cases after allogeneic HSCT.

In this report, we describe the unique clinical and laboratory findings of a patient with $\gamma\delta$ T-cell LGLL of cord donor origin after a second cord blood transplantation for acute myeloid leukemia.

Case Report

A 58-year-old Japanese man with acute myeloid leukemia (French-American-British classification; M2) in second complete remission received allogeneic HSCT from an unrelated female cord blood donor. The conditioning regimen consisted of total body irradiation of 12 Gy in 6 fractions from day -6 to -4, and cyclophosphamide 60 mg/kg once daily intravenously on days -3 to -2 (total dose, 120 mg/kg). He received human leukocyte antigen-loci mismatched (2 by serology and 2 by DNA typing) unrelated cord blood, which contained 3.03×10^7 nucleated cells/kg in January 2003. Cyclosporine and short-term methotrexate were used as graft-versus-host disease prophylaxis. However, hematologic recovery was not observed up to day 40, and we concluded that this was a case of primary graft failure without leukemia relapse because the results of interphase fluorescence in situ hybridization analysis on days 23, 30, and 37 on bone marrow (BM) samples were negative. Because his condition remained good, we planned a second cord blood transplantation with a reduced-intensity regimen, which consisted of fludarabine 30 mg/kg once daily intravenously from days -8 to -3 (total dose 180 mg/kg), busulfan 4 mg/kg orally on days -6 and -5 (total dose 8 mg/kg), and total body irradiation of 4 Gy in 1 fraction on day -1. Cyclosporine and mycophenolate mofetil 15 mg/kg twice daily were administered. On day 51 of the initial transplantation in March 2003, human leukocyte antigen-loci mismatched (2 by serology and 3 by DNA typing) male cord blood, containing 2.6×10^7 /kg nucleated cells, was infused. Neutrophil engraftment was observed by day 33 after second transplantation. Acute and chronic graft-versus-host disease did not develop, and cyclosporine was tapered off in November 2003.

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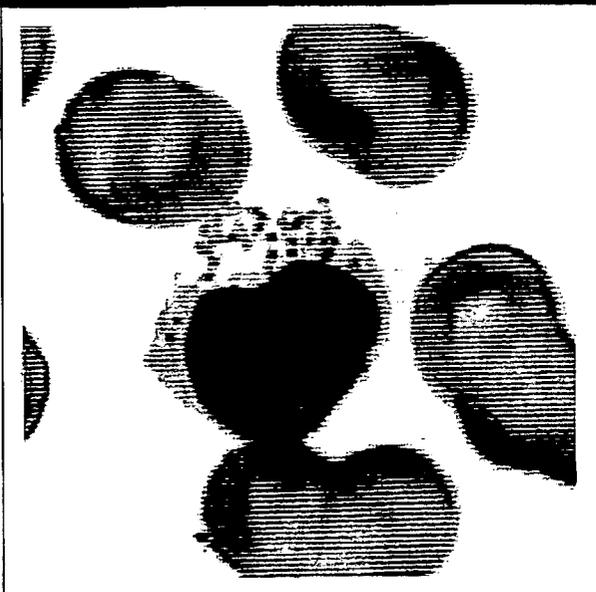
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T-Cell LGLL After Cord Blood Transplantation

Figure 1 T-Cell Large Granular Lymphocyte Leukemia Stained with May-Giemsa on the Peripheral Blood Smear



The predominant cells were typical of LGLs with abundant cytoplasm and fine or coarse azurophilic granules. Hematoxylin and eosin stain; original magnification $\times 1000$.

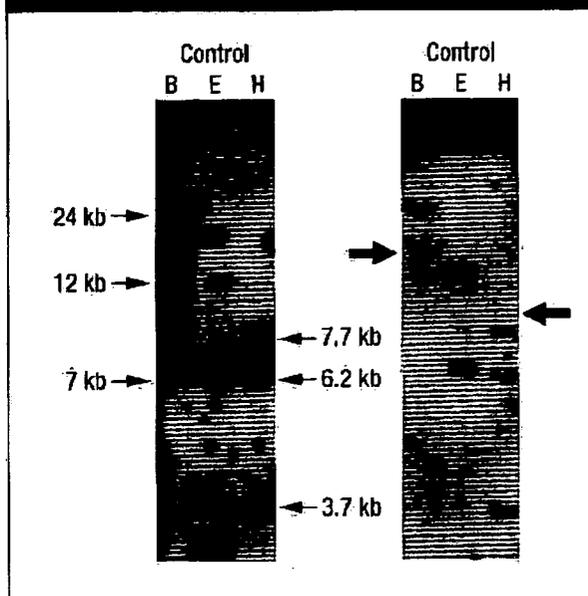
In February 2004, 10 months after the second cord blood transplantation, he developed anorexia, abdominal distention with fluid accumulation, and edema in the lower extremities. A computed tomography scan showed gross ascites and mild pleural effusion but no sign of enlarged lymph nodes or hepatosplenomegaly. The peripheral white blood cell count was $10,300/\mu\text{L}$ ($10.3 \times 10^9/\text{L}$), and 30% of the cells had a morphology of medium to large lymphocytes with abundant azurophilic granules in the cytoplasm, as shown in Figure 1. The hemoglobin level was 8.8 g/dL (88 g/L), and the platelet count was $192 \times 10^3/\mu\text{L}$ ($1.92 \times 10^9/\text{L}$).

A retrospective review of the peripheral blood smears disclosed that the appearance of LGL coincided with the tapering off of immunosuppression 3 months before the admission.

Flow cytometry examination of the peripheral blood mononuclear cells showed a homogeneous population of T-cell LGLs positive for CD2, CD3, CD8, CD56, and T-cell receptor (TCR)- $\gamma\delta$, but negative for CD4 and TCR- $\alpha\beta$. The BM biopsy specimen histologically showed 10% of hypocellular gelatinous marrow with diffuse infiltration of medium to large lymphoid cells. Immunoperoxidase studies on sections of BM showed strong expression of T-cell-restricted intracellular antigen-1, partially positive staining of CD8 and granzyme B, but no expression of CD3 or CD20. Southern blot analysis of the BM cells revealed a clonal rearrangement of the TCR- β chain, as shown in Figure 2 and TCR- δ chain (data not shown).

Abdominal paracentesis was performed with milky chylous fluid, and a flow cytometry examination showed results similar to those in the peripheral blood. Multiprimer-based polymerase chain reaction

Figure 2 Southern Blots of T-Cell Receptor β -Chain Gene Rearrangements



DNA from BM of this patient was hybridized with a TCR β 1 probe. Arrows indicate rearranged bands. Abbreviations: B = Bam HI; E = Eco RI; H = Hind III

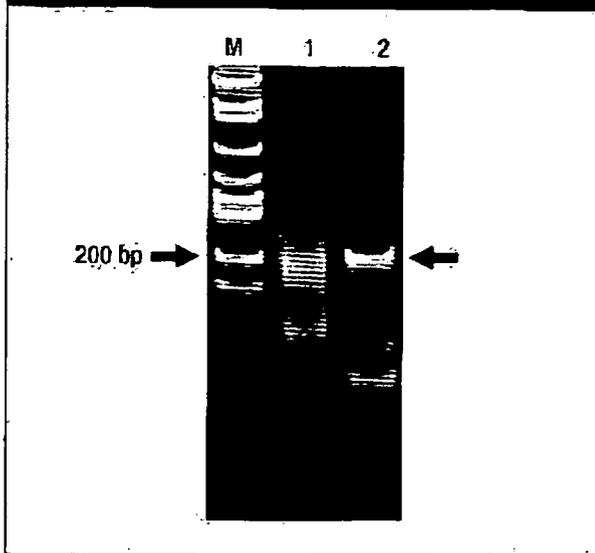
(PCR) analysis of ascitic cells also showed clonal rearrangement of the TCR- δ chain, as shown in Figure 3. The primer sets were used in the following locations: V δ 1, 5'-AAA GTG GTC GCT ATT CTG TC-3'; V δ 2A, 5'-GCA CCA TCA GAG AGA GAT GA-3'; J δ , 5'-TGG TTC CAC AGT CAC ACG GG-3'; D δ 3B, 5'-TTG TAG CAC CGT GCG TAT CC-3'. The amplified 200 base-pair PCR products of the TCR- δ chain were then cloned into the pCR-TOPO vector. The DNA sequences of 3 clones amplified by vectors were identical and had high homology to TCR- δ chain including a 197 base-pair sequence (data not shown). This sequence also involved the forward and reverse primers V δ 1 and J δ , respectively, described previously.

The results of all of the previously mentioned studies indicated the clonal expansion of T cells compatible with a diagnosis of T-cell LGLL with $\gamma\delta$ T-cell phenotype involving peripheral blood, BM, and ascites.

Donor-recipient DNA chimerism was analyzed by comparing the short tandem repeat findings for the donor blood sample and pretransplantation recipient samples. Eleven short tandem repeat loci were analyzed by PCR using an AmpFISTR SGM Plus[®] kit. The peripheral blood sample (containing 30% T-LGL) and the second cord blood sample showed the same peaks at the locus (D16S539), as shown in Figure 4. These results further confirmed that the expanded $\gamma\delta$ T-LGL cells were exclusively of second cord blood transplantation donor origin.

Serologic examination showed no evidence of viral infection. Real-time PCR analysis revealed a high load of EBV (7.9×10^3 copies/ 10^6 cells). However, in situ hybridization studies of BM cells did not reveal EBV-encoded small RNA, and Southern blot analysis of BM cells also showed no band for

Figure 3 Polymerase Chain Reaction for T-Cell Receptor δ Gene Rearrangement



(1) Negative control; and (2) patient's sample of frozen-neoplastic lymphoid cells in ascites. A clonal band was identified at approximately 200 base pairs. Abbreviations: bp = base pairs; M = molecular weight marker

clonal EBV genomes. Chromosome analysis demonstrated a normal 46, XY karyotype in all 20 cells examined.

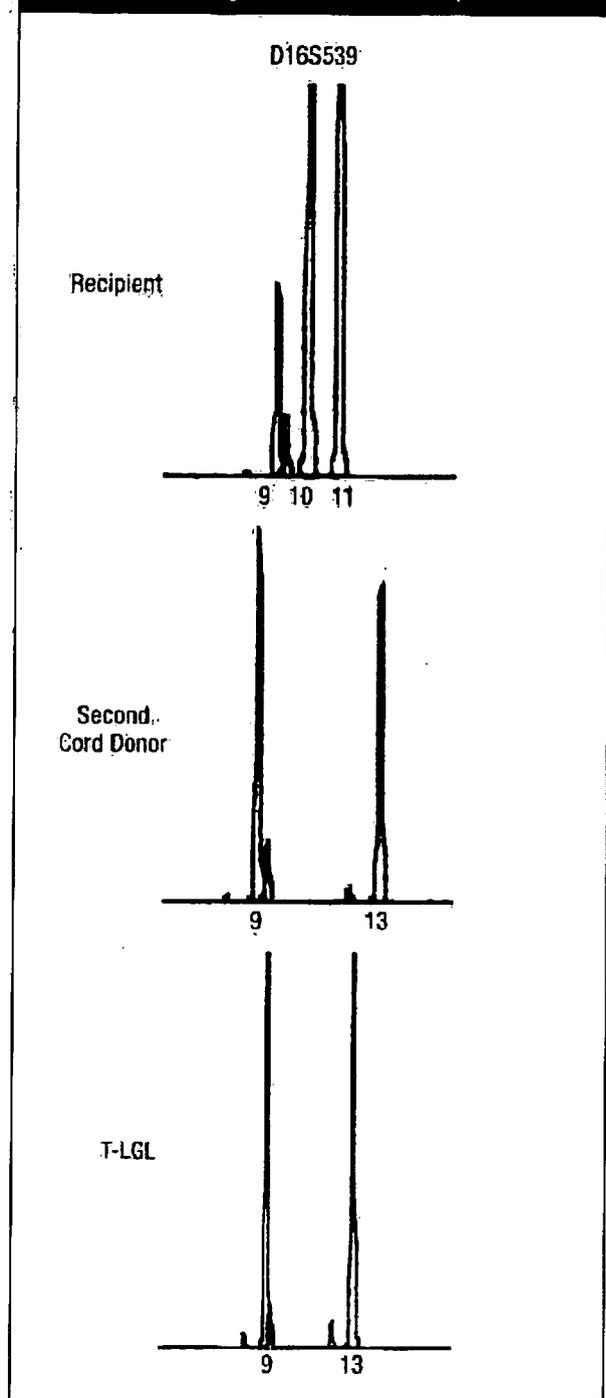
After admission, his abdominal distention and dyspnea with hypoxemia progressed rapidly with spiking fever. A computed tomography scan demonstrated acute respiratory distress syndrome. Because we found no evidence of bacterial or fungal infection or drug-induced pneumonia, cyclosporine and methylprednisolone were started immediately but with no effect, and he died of acute respiratory failure 1 week later. A postmortem lung biopsy showed extensive diffuse alveolar damage without the T-LGL cell's involvement; on the other hand, the leukemic cell involvement in Glisson's sheath was shown by a liver biopsy.

Discussion

In this case, the increase in LGLs developed 7 months after the second cord blood transplantation, and the kinetics of LGLs correlated with the tapering off of immunosuppression, which suggested the possibility that lymphocytosis might have been associated with reactive expansion because of viral infection or an alloimmune reaction. However, our case showed *TCR- β* and *TCR- δ* gene rearrangement by Southern blot analysis and *TCR- δ* gene rearrangement by PCR and cytotoxic T-cell immunophenotype, which were comparable with T-cell LGL.

Most cases of PTLID, usually of B-cell origin, are associated with EBV infection and represent the EBV-induced monoclonal expansion of B cells in conditions with decreased T-cell immune surveillance.^{5,6} Although there have been some reports of EBV-associated PTLID after cord blood transplantation,⁷⁻¹⁰ the incidence of PTLID of T-cell origin has been reported to be only 4%-14% with a less frequent association with EBV.^{6,11}

Figure 4 Donor-Recipient DNA Chimerism Analysis by Comparing the Short Tandem Repeat



The peripheral blood sample (containing 30% T-LGL) and the second cord blood sample showed the same peaks at the locus (D16S539).

In our case, because a high viral load of EBV was detected by real-time PCR analysis, we initially speculated that $\gamma\delta$ T-LGL was EBV-associated PTLID, but this was later denied based on the results of EBV-encoded small RNA in situ

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Table 1A Literature Review of T-Cell Posttransplantation Lymphoproliferative Disorder After Hematopoietic Stem Cell Transplantation^{7,13-16}

Study	Case Number	Age/Sex	Donor	Diagnosis	Origin	Involved Organ
Zutter et al ¹³	1	12/Male	Sibling*	B Lymphoblastic lymphoma	Recipient	Lymph node, BM
Zutter et al ¹³	2	9/Male	Sibling*	Lymphoblastic lymphoma	ND	Pericardium, pleura
Zutter et al ¹³	3	2/Female	Father	NHL (polymorphic)	Donor	Lung, liver, spleen
Wang et al ¹⁴	4	13/Male	Sibling*	NHL (diffuse large)	Recipient	Lymph node
Sirvent et al ¹⁵	5	ND/ND	ND	LGL ($\alpha\beta$)	ND	PB, BM
Collins et al ¹⁵	6	11/Male	ND	NHL (polymorphic)	ND	Lymph node, brain
Au et al ¹⁶	7	39/Male	Unrelated	LGL	Donor	PB, BM
Our Case	8	58/Male	UCB	LGL ($\gamma\delta$)	Donor	PB, BM, ascites, liver

*Human leukocyte antigen-matched sibling.

Abbreviations: ND = not determined; NHL = non-Hodgkin lymphoma; PB = peripheral blood; UCB = unrelated cord blood

Table 1B Literature Review of T-Cell Posttransplantation Lymphoproliferative Disorder After Hematopoietic Stem Cell Transplantation^{7,13-16}

Study	Case Number	Time to PTLD* (Days)	EBER-ISH	Rearrangement	Survival† (Days)
Zutter et al ¹³	1	1290	Not determined	TCR- γ (SB)	85
Zutter et al ¹³	2	630	Not determined	Not determined	180
Zutter et al ¹³	3	39	Not determined	Polyclonal	11
Wang et al ¹⁴	4	601	Negative	TCR- γ (PCR)	> 1170
Sirvent et al ¹⁵	5	300	Negative	TCR- β (SB)	> 690
Collins et al ¹⁵	6	90	Negative	Not determined	29
Au et al ¹⁶	7	180	Negative	TCR- γ (PCR)	134
Our Case	8	330	Negative	TCR- β (SB), TCR- δ (SB, PCR)	30

*Time from transplantation to PTLD.

†Survival time from diagnosis of PTLD.

Abbreviations: EBER-ISH = EBV-encoded small RNA in-situ hybridization; SB = Southern blotting

hybridization stains and Southern blot EBV terminal repeat analysis. Therefore, the clinical significance of EBV infection in this case remains undetermined.

Most previously reported cases of T-cell PTLD developed after solid organ transplantation,¹² and there have been only 7 previously documented cases of T-cell PTLD after allogeneic HSCT, as summarized in Table 1.^{7,13-16} Posttransplantation lymphoproliferative disorder was of donor origin in 3 of 8 total cases, including our case, of recipient origin in 2, and of undetermined origin in the remaining 3. No correlation has been demonstrated between EBV and T-cell PTLD after HSCT.

Generally, most cases of B-cell posttransplantation lymphoproliferative disorder after HSCT develop within the first 5 months, because the balance between proliferating EBV-infected B cells and cytotoxic T cells cannot be controlled with the unrecovered lymphocyte components.¹⁷ In solid organ transplantation, EBV-positive cases tend to occur earlier than EBV-negative cases, ie, a median interval of 6-10 months compared with 4-5 years.^{6,7} Some cases of T-cell PTLD have

a longer interval between the day of transplantation and the occurrence of PTLD than in B-cell PTLD. The donor source of transplantation included sibling (3 cases), father (1 case), unrelated (1 case), cord (our case), and not described (2 cases). Therefore, whereas there has been very little experience with cases after cord blood transplantation, all 8 cases of PTLD in the literature are of B-cell origin.⁸⁻¹¹ Our case is the first report of PTLD of T-cell origin after cord blood transplantation and might reflect very intense immunosuppression passing through consecutive cord blood transplantation.

It has been reported that T-cell PTLD has a worse prognosis than B-cell PTLD in a solid organ transplantation setting. In 1 series of 6 cases presenting with T-cell non-Hodgkin lymphoma as PTLD, pulmonary involvement was reported in 5 cases and marrow infiltration in 4 cases. All patients showed aggressive courses.¹⁸ Of importance is that of 8 patients with T-cell PTLD after HSCT: 3 patients who died within 30 days had extranodal involvement in the lung, liver, spleen, brain, and/or ascites.

Conclusion

We have reported an unusual case of EBV-negative, T-cell PTLID as $\gamma\delta$ T-cell IGLL of donor origin after a second cord blood transplantation. The occurrence of T-cell PTLID after HSCT is extremely rare, and the efficient accumulation of knowledge and further research are needed to establish the oncogenic mechanism and appropriate therapeutic maneuvers in this disease entity.

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