InterScience
Research article

Prospective phase II trial to evaluate the complications and kinetics of chimerism induction following allogeneic hematopoietic stem cell transplantation with fludarabine and busulfan

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This prospective trial assessed the safety and efficacy of allogeneic hematopoietic stem cell transplantation from a HLA-matched donor with a reduced-intensity regimen (RIST) consisting of iv fludarabine 30 mg/m^2 for 6 days and oral busulfan 4 mg/kg/day for 2 days in patients older than 50 years with hematological malignancies. Cyclosporine alone or cyclosporine with short-term methotrexate was randomized for graft-versus-host disease prophylaxis. After 30 patients had been enrolled, an interim analysis was performed, and this report focuses on a precise evaluation of the toxicity profile and chimerism kinetics. Sustained engraftment in all patients, no severe regimen-related toxicity (RRT) within 20 days, and no transplant-related mortality through Day 100 were observed. T-cell (CD3+) full-donor (over 90%) chimerism was observed in 22 of the 30 patients, while the remaining eight had mixed-donor chimerism over 77% on Day 90. Thereafter, five subsequently converted to full-donor chimerism without donor lymphocyte infusion by day 120 (n = 4) or Day 180 (n = 1). Two showed persistent mixed chimerism without relapse through Day 180. Grade III–IV acute graft-versus-host disease and extensive chronic graft-versus-host disease occurred in 10% and 73%, respectively. With a median follow-up of 1.5 years, overall survival and disease-free survival at 1 year was 83% and 62%, respectively. Seven patients hematologically relapsed overall, and five of them had myelodysplastic syndrome with poor prognostic factors. In older patients, RIST with fludarabine and busulfan was associated with acceptable toxicities and a satisfactory antileukemia effect, regardless of the early chimerism status. Am. J. Hematol. 00:000–000, 2007. © 2007 Wiley-Liss, Inc.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment of choice for hematological malignancies. However, many centers limit HSCT to younger patients because of the threat of a higher risk of treatment-related toxicities including graft-versus-host disease (GvHD), nonrelapse mortality, and lower disease-free survival (DFS) in the older population, although the median age of onset of chronic myeloid leukemia (CML) is in the sixth decade of life, and the peak incidence of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) is in the seventh decade. To overcome this obstacle, allogeneic HSCT with a reduced-intensity (RIST) or nonmyeloablative conditioning regimen has recently been explored for patients who are ineligible to receive conventional myeloablative HSCT (CIST) due to age limits or comorbidities. Many studies suggested that RIST is a reasonable option for older patients or patients with comobidities with acceptable treatment-related complications or morbidity, while preserving adequate anti-tumor effects [1–12]. However, these studies mostly pursued different variables including disease types, stages [1,4–6, 8,12], donor type [1,2,5,10], graft source [1,2], conditioning regimens [4,5,7,9], and/or GvHD prophylaxis [1,4,9]. This

Contract grant sponsor: Health and Labour Sciences Research Grant from the Ministery of Health, Labour and Welfare.

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Received for publication 11 January 2007; Revised 5 March 2007; Accepted 30 March 2007

Am. J. Hematol. 00:000–000, 2007.

Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20977

TABLE I. Patient and Donor Characteristics

UPN	Patient age/sex	Donor age/sex	Stage and diagnosis	IPSS/cytogenetic risk in MDS patients	CD34+ cells (10^6)	Blood type patient/donor
1	60/M	46/F	MDS (RA)	Intermediate-2/Poor	3.92	A/A
2	61/M	54/F	MDS (RAEB)	Intermediate-2/Poor	5.84	O/O
3	67/M	60/F	AML (M4) in 2CR		2.74	B/O
4	60/M	55/F	AML (M2) in 2CR		4.58	B/B
5	63/M	60/M	CML in 2CP		12.59	O/O
6	54/M	59/F	MDS (RAEB)	High/Intermediate	5.4	O/A
7	52/M	55/F	AML (M2) in 1CR		6.77	A/A
8	61/M	54/M	AML (M1) in 1CR		3.29	B/A
9	58/F	64/F	CML in 1CP		2.9	A/AB
10	64/F	59/F	ALL (L2) in 1CR		5.54	A/A
11	55/M	44/M	AML (M1) in 1CR		3.13	A/A
12	55/F	51/F	CML in 1CP		4.94	A/O
13	52/F	42/M	AML (M4) in 1CR		3.59	A/A
14	59/M	64/M	MDS (RAEB)	Intermediate-2/intermediate	3.58	A/AB
15	59/M	56/M	MDS (RA)	Intermediate-1/Good	3.58	AB/A
16	53/F	55/F	MDS (RA)	Intermediate-2/Poor	2.2	O/O
17	55/F	68/M	AML (M3) in 2CR		2.63	A/A
18	54/M	50/M	MDS (RA)	Intermediate-1/Poor	3.74	O/B
19	51/M	44/F	AML (M1) in 1CR		4.86	AB/A
20	64/F	66/M	CML in 2CP		3.59	O/A
21	68/F	64/M	MDS (RAEB)	Intermediate-1/Good	3.56	B/B
22	53/M	44/M	MDS (RAEB)	High/Intermediate	7.2	B/B
23	60/F	53/M	AML (M2) in 1CR		2.83	A/B
24	59/M	62/M	AML (M4) in 2CR		5.47	A/O
25	51/F	47/F	MDS (RAEB)	Intermediate-2/Poor	5.93	A/A
26	59/M	62/F	MDS (RA)	Intermediate-2/Poor	4.02	B/O
27	59/M	48/M	AML (M2) in 2CR		4.94	B/A
28	56/M	62/F	MDS (RAEB-t)	High/Good	4.38	AB/A
29	53/F	62/F	AML (M2) in 1CR		3.06	O/O
30	54/F	63/M	AML (M2) in 1CR		6.47	A/O

M, male; F, female; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-t, refractory anemia with excess blasts in transformation; CR, complete remission; CP, chronic phase. All donors were HLA-matched siblings.

makes overall interpretation of studies difficult. Additionally, there has been no study to prospectively assess whether RIST consisting of 180 mg fludarabine plus 8 mg/kg busulfan without antithymocyte globulin actually produces less significant organ toxicities and treatment-related toxicities in an older patient population. Information regarding the impact of the speed and degree of lineage-specific donor chimerism on clinical outcomes after RIST in older patients has been limited [3,8,13–17]. Moreover, even studies evaluated with more homogeneous patient population, type of GvHD prophylaxis and/or tempo of withdrawal of immunosuppressive agents varied depending on transplant centers and a feasible prophylaxis regimen for acute GvHD has not been well evaluated in RIST, which is considered to require a sophisticated balance between GvHD and a graft-versus-leukemia (GvL) effect.

To address these points, we conducted a prospective randomized clinical trial to evaluate the safety and efficacy of RIST with fludarabine and oral busulfan in patients aged over 50 years and with appropriate GvHD prophylaxis. In this report, the results of an interim analysis, including clinical outcomes, complications, and chimerism kinetics, were compared with those previously published in the literature.

Patients and Methods

Patient eligibility and accrual

Eligible patients ranged in age from 50 to 69 years (median 58.5, range 51–68 years) and had a hematological malignancy, including AML or acute lymphoblastic leukemia (ALL) in 1st or 2nd complete remission (CR), CML in 1st or 2nd chronic phase (CP), and MDS. They were required to have an HLA-identical related donor. The study protocol was reviewed and approved by the institutional review boards of the participating transplantation centers (Appendix). Eligible patients and their donors gave written informed consent before enrollment. The enrollment criteria included a performance status (PS) of the Eastern Cooperative Oncology Group (ECOG) of less than two, a serum creatinine concentration of less than 2.0 mg/dl, a cardiac ejection fraction of more than 50%, arterial oxygen saturation without supplemental oxygen of more than 93%, liver function tests less than fourfold the upper limit of normal, total bilirubin less than 2.0 mg/dl, no active infection, and no previous allergy for drugs used for conditioning or GvHD prophylaxis. Donors were required to have a normal physical examination, and normal values in the serum chemistry and blood counts, and negative results of serologic testing for human immunodeficiency virus and hepatitis B. The patient and donor characteristics are shown in Table I. Those with AML/ALL in 1st CR, CML in 1st CP, or MDS in refractory anemia were defined as low risk, and the others were defined as high risk. All 12 patients with MDS except one (UPN 22) were transfusion dependent, and all those were grouped according to the International Prognostic Scoring System (IPSS) into intermediate or high risk at the time of transplantation: Intermediate-1, $n = 3$; intermediate-2, $n = 6$; high risk, $n = 3$. By IPSS criteria, 3 patients had good-risk, 3 had intermediate-risk, and 6 had poor-risk cytogenetics.

Donor selection and blood stem cell harvest

Related donors were selected based on compatibility of HLA-A, B and DRB1 by intermediate- or high-resolution DNA typing. After G-CSF treatment, apheresis procedures were performed daily until at least 2.0 $\times$ 10^6 CD34+ cells per kilogram of the recipient's body weight, up to three times, and all of the collected cells were cryopreserved until stem cell infusion.

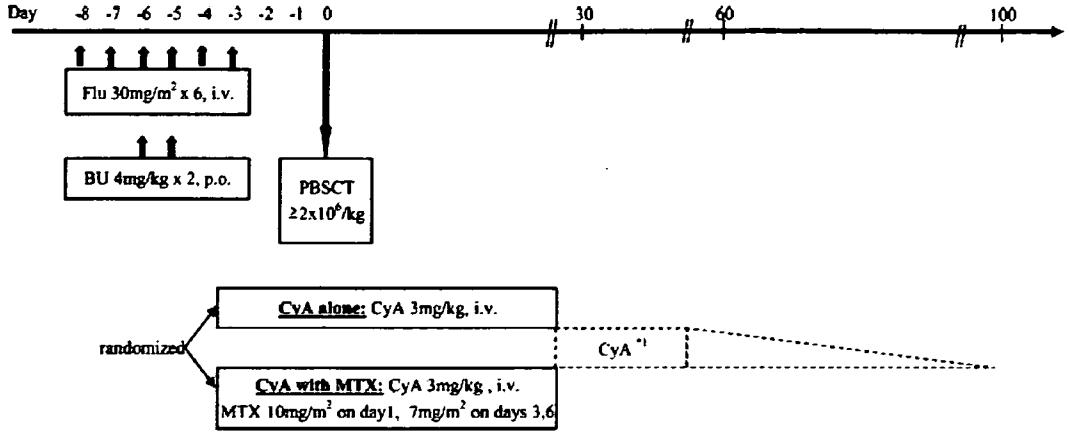

Figure 1. Treatment schedule. CyA; cyclosporine, MTX; methotrexate. *1: When acute GvHD was not observed, CyA was tapered by 10% a week starting at Day 28, and was eliminated by Day 100. When mixed chimerism was seen without active acute GvHD over Day 60, CyA was tapered and discontinued within 2 weeks. Patients who did not convert to complete chimerism after CyA withdrawal received donor lymphocyte infusion.

Treatment schedule

The treatment schedule is shown in Fig. 1. The conditioning regimen consisted of fludarabine (30 mg/m^2/day) infused over 30 min once a day on Days 8, 7, 6, 5, 4, and 3, and oral busulfan (4 mg/kg/day) on Days 6 and 5. To prevent seizures, the patients received oral valproate sodium, at a dose of 600 mg divided into 3 doses 2 days before busulfan administration, and this was continued until 24 hr after the last dose of busulfan.

Patients were randomized to receive either cyclosporine (CyA) alone or CyA plus short-term methotrexate (MTX) for GvHD prophylaxis. Randomization was performed by stratifying according to disease (AML, ALL, CML or MDS), transplant center, age (less than 60 years or more than or equal to 60 years), and sex (male or female). All patients received 3 mg/kg/day CyA by continuous iv infusion daily from Day 1 to maintain a therapeutic trough level of 250–400 ng/ml, and thereafter orally in an attempt to maintain a therapeutic trough level of 150–250 ng/ml. The patients who were assigned to CyA plus short-term MTX received a dose of 10 mg/m^2 iv MTX on Day +1, and 7 mg/m^2 on Days +3 and +6 after stem cell infusion. CyA was tapered starting at Day 28 in the absence of acute GvHD and was discontinued by Day 100 after transplantation. When a patient did not achieve complete donor chimerism by Day 60, CyA was tapered rapidly and discontinued within 2 weeks if clinically feasible, since anti-leukemic effect was presumed to occur after development of complete donor chimerism [14]. Cases of Grade II–IV acute GvHD were treated with 2 mg/kg/day of methylprednisolone in addition to CyA.

Supportive care

The following infection prophylaxis was recommended: prophylactic antibiotics (fluoroquinolones) were given during cytopenia, fluconazole (200 mg/day) was given at the start of conditioning and continued until the discontinuation of immunosuppressant, and oral acyclovir (1,000 mg/day) or iv acyclovir (750 mg/day) was given for prophylaxis of herpes simplex virus (HSV) and varicella zoster virus (VZV) from Day −7 to Day 35. Prophylaxis against *Pneumocystis carinii* was consisted of trimethoprim-sulfamethoxazole after neutrophil engraftment ($\geq 0.5 \times 10^9$ L^{-1}) and was continued until the discontinuation of immunosuppressant. During the first 100 days after transplantation, cytomegalovirus antigenemia assay with HRP-C7 or C10/C11 monoclonal antibody was performed weekly after neutrophil engraftment until Day 100 after transplantation. Pre-emptive therapy with ganciclovir was recommended upon the detection of positive antigenemia and was continued until it became negative. Patients were treated with G-CSF from Day +6 to neutrophil engraftment.

Chimerism analysis

Hematopoietic chimerism was evaluated with regard to peripheral T cell (CD3+) fraction by an analysis of DNA microsatellite polymorphisms by polymerase chain reaction (PCR) with D18S51, D20S471, and D22S684 fluorescence-labeled primers, which identified differences between patient and donor (on the basis of polymorphisms found in pretransplant patient/donor samples) using an BECKMAN COULTER CEQ8000 GENETIC ANALYSIS SYSTEM. T cell (CD3+) chimerism studies post HSCT were performed on Days 30, 60, 90, 120, and thereafter every other month through 1 year.

Assessment of response

Day 0 was defined as the day of stem cell infusion day. The day of neutrophil engraftment was defined as the first of two consecutive days on which the patient's absolute neutrophil count was above 0.5×10^9 L^{-1}. The day of platelet engraftment was defined as the first of seven consecutive days on which the platelet count was above 20×10^9 L^{-1} without platelet transfusion.

Regimen-related toxicity (RRT) was graded using the Seattle criteria [18] on the day before the initiation of conditioning regimens and at least 3 days a week until Day 20 after transplantation. All other observed adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0 (NCI-CTC ver. 2.0) until Day 100 after transplantation. Infectious diseases were diagnosed based on any positive blood culture or histologic evidence of tissue invasion.

To evaluate the general condition of patients associated with the toxicity profile, PS, and dietary oral intake were also reported at least three times a week during the initial hospitalization and once a week afterwards up to Day 100 post-transplant.

The diagnosis and grading of acute and chronic GvHD was made based on the date of onset (within or beyond 100 days) and clinical findings in conjunction with biopsy of the skin and digestive tract using the published criteria [19,20]. Patients who survived 100 days or longer were evaluable for the assessment of chronic GvHD.

Pharmacokinetic studies of fludarabine phosphate and busulfan

Blood sampling for pharmacokinetic studies was done on Day −5 to investigate the effect of concomitant busulfan administration on the pharmacokinetics of 2-fluoro-ara A (2F-ara-A), which is the major metabolite of fludarabine phosphate. Blood samples for determining the 2F-ara-A plasma level were collected at 0, 0.5, 1, 2, 5, and 23.5 hr after the 4th infusion of fludarabine. We also obtained blood samples for determining the busulfan plasma level at 0, 0.5, 1, 1.5, 2, 3, and 6 hr after the sixth administration of busulfan (1 mg/kg/dose for 8 times). Blood samples were taken in tubes containing heparin and erythro-9-(2-hydroxy-3-nonyl)adenine. Plasma was obtained by centrifugation, and then transported to the laboratory and were stored at −20°C until analysis. Plasma levels of 2F-ara-A and busulfan were determined using high-performance liquid chromatography with fluorescence and UV detection, respectively. The accuracy and precision of the assays for 2F-ara-A and busulfan were confirmed by measuring QC samples of both before this study. The maximum concentration of drug in plasma after drug administration (C_{max}, C_{peak}) and the time to reach

the maximum concentration following drug administration (T_{max}) were observed. The area under the plasma concentration-versus-time curve (AUC) for 2F-ara-A or busulfan was calculated by dividing the administered dose by the final plasma clearance estimate, whereas the plasma clearance was determined by modeling all plasma concentration versus time data. Terminal half-lives ($T_{1/2}$) were calculated from the primary parameters.

Statistical analysis

The primary endpoint of this study was to determine the percentage of patients who were alive at 100 days after transplantation with complete donor chimerism (over 90%) achieved by Day 90. Secondary endpoints included the time to engraftment of neutrophils and platelets, the incidence and severity of RRT, the incidence and severity of acute and chronic GvHD, the anti-leukemia effect, DFS, and overall survival (OS). A descriptive statistical analysis was performed to assess patient baseline characteristics and disease. Time to engraftment, complete chimerism, acute or chronic GvHD, OS, and DFS were calculated using the Kaplan–Meier method. OS was defined as the time between stem cell infusion to death from any cause. DFS was defined as the time between stem cell infusion to relapse and death from any cause, whichever occurred first. After 30 patients had been enrolled in the study, a data and safety monitoring committee undertook an interim analysis. This analysis, completed in October 2004, included data for the primary endpoint, i.e. survival at Day 100 and chimerism status at Day 90, and data on acute and chronic GvHD, survival, chimerism status, and anti-tumor effect through Day 180. Neither of the predefined criteria for stopping the study was met; however, a review of available safety data including incidence and severity of RRT and Day 100 mortality indicated that this conditioning regimen was adequately safe for older patients. According to the recommendation of the committee, we decided to continue the study and published an interim report when 30 patients were enrolled and evaluated without comparing the two different GvHD prophylaxis procedures. This report includes data on these 30 patients with all available follow-up data through December 2005, and does not include the results of a comparison of the two different GvHD prophylaxis procedures.

Results

Engraftment and chimerism analysis

The results are summarized in Table II. One and four patients were not evaluated for neutrophil and platelet engraftment, respectively, because they did not show a nadir. The remaining patients achieved sustained engraftment and none experienced graft failure. The median number of days to achieve a neutrophil count $\geq 0.5 \times 10^9\ L^{-1}$ was 13 (range, 10–25 days), and this was 18 (range, 11–24 days) for a platelet count $\geq 20 \times 10^9\ L^{-1}$ without transfusion. Full-donor (over 90%) T-cell (CD3+) chimerism was observed in 2 and 9 of the 30 patients on Day 30 and Day 60, respectively (median [range], Day 30:71 [40 to ≥90] %, day 60:81 [41 to ≥90] %). Twenty-two patients achieved full-donor chimerism, while the remaining eight patients had mixed chimerism ranging from 78% to 88% on Day 90. Among those with mixed chimerism on Day 90, five subsequently converted to full-donor chimerism without early CyA withdrawal because of the severe acute GvHD (n = 2: UPN 1 and 15) and/or donor lymphocyte infusion (DLI) by day 120 (n = 4) or day 180 (n = 1). One achieved full-donor chimerism on Day 120 after DLI since the patient did not respond to the discontinuation of immunosuppressive drugs, and two had persistent mixed chimerism without relapse through 180 days after transplantation (71% and 75% donor-type chimerism on Day 180). The diagnoses of two patients with persistent mixed chimerism through Day 180 were CML and MDS, and they had not received proceeding cytotoxic chemotherapy; the patient with CML (UPN 12) received immunomodulators, imatinib mesylate and hydroxyurea, and the patient with MDS (UPN 21) received low-dose cytarabine and aclarubicin in combination with granulocyte colony stimulating factor before RIST.

Regimen-related toxicities, complications, and general condition

The frequencies of Grade I–IV organ toxicities within 20 days after transplantation are listed in Table III. Although non-fatal toxicities including Grade I/II were seen in all 30 patients, all of the observed episodes were reversible and in no case required suspension of fludarabine. Stomatitis was the most frequently observed organ toxicity (57%, 17/30), with 47% of them (8/17) had Grade II events. None of the patients experienced veno-occlusive disease of the liver (VOD). Twenty patients had at least one episode of infectious complications within the first 100 days, with a total of 44 documented episodes (median, 2; range, 1–7 episodes) within the first 100 days after transplantation. These included proven bacterial infection (1 episode), suspected bacterial infection (1), suspected fungal infection (2), cytomegalovirus antigenemia (6), HSV infection (1), suspected viral infection (1), and uncertain causes (33). All infectious complications were recovered with or without appropriate antibiotic therapy.

The median PS for the first 28 days was 0 (range, 0–3). The worst PS of 2 (n = 5) or 3 (n = 2) within the first 28 days was experienced temporarily due to infection (n = 2), Grade III GvHD (n = 1), and nausea/vomiting (n = 4). Those (n = 6) observed from Day 29 to Day 100 were all caused by Grade II or III acute GvHD. A one-thirds reduction in dietary oral intake was temporarily seen in 20 and 11 patients within the first 28 days and from 29 days to 100 days post HSCT, respectively, which resulted from nausea/vomiting (n = 18) and treatment-related mucositis (n = 2) within Day 28, and Grade II–III acute GvHD (n = 9), prolonged infection with Grade II acute GvHD (n = 1) and gastroesophageal reflux disease (n = 1) between Day 29 and Day 100.

GvHD

Grade I–IV acute GvHD at 100 days was documented, respectively, in 5 (17%), 15 (50%), 3 (10%), and 0 (0%) patients. The median time to the occurrence of Grade II–IV acute GvHD was 74 days (range, 18–100 days). All 30 patients survived beyond Day 100 and were evaluated for chronic GvHD. Twenty-six of the 30 patients (87%) developed chronic GvHD (limited type in four cases and extensive type in 22 cases) with the onset at a median of 123 days after transplantation (range, 116–217 days).

Disease response, survival, and cause of death

No patient died within the first 100 days, and the median follow-up period was 555 days (149–1114 days) after transplantation. Twenty-nine of the 30 patients achieved CR within 100 days after transplantation, but two of them with MDS, who had poor-risk cytogenetics and were classified into intermediate-2, subsequently relapsed on Day 141 (UPN 26) and Day 156 (UPN 25). One was treated with DLI (UPN 25) and showed a temporary response, but died because of the disease progression on Day 401. The other patient (UPN 26) did not respond to DLI and died of progressive disease on Day 412. One patient (UPN 22) with MDS with high risk IPSS achieved full-donor chimerism on Day 90, but could not achieve CR on Day 98 and died with progressive disease on Day 306. This patient showed full-donor chimerism through Day 180. Five other patients died between 100 days and 1 year after transplantation (149, 151, 169, 187, and 354 days). In six patients who died within the first year, two patients were over 60 years and four patients were classified into high risk disease group. Causes of death included progressive disease of MDS with poor IPSS in 1, GvHD and/or its complications in 4, and recurrence of interstitial pneumonia in 1. In four patients, who died of GvHD and/or its complications, all had experienced

TABLE II. Summary of Clinical Outcomes

UPN	Chimerism analysis			Post transplant DLI (reason)	GvHD		Infection until day 100 (etiological agent)	Relapse	Outcome (Cause of death)	Follow up
	Day 90(%)	Day 120(%)	Day 180(%)		Acute	Chronic				
1	88.40	≥90	≥90		Gr II (S, G)	Extensive	–	–	Alive	1,114
2	85	≥90	≥90	Yes (d662, relapse)	Gr II (S)	Extensive	Yes (unknown)	Yes (d402)	Dead (recurrent disease and its complication)	652
3	≥90	≥90	≥90		–	Extensive	Yes (S. maltophilia, unknown)	–	Alive	735
4	≥90	≥90	D		Gr II (S)	–	–	–	Dead (IP)	169
5	≥90	≥90	≥90		–	Extensive	–	–	Alive	731
6	≥90	≥90	≥90		Gr II (L)	Extensive	–	–	Alive	716
7	≥90	≥90	≥90		Gr II (S, G)	Extensive	Yes (CMV antigenemia)	–	Dead (GvHD)	354
8	≥90	≥90	≥90		Gr III (S, G, L)	Extensive	Yes (bacteremia susp., fungal susp., CMV antigenemia, unknown)	–	Alive	431
9	≥90	≥90	≥90		–	Extensive	Yes (unknown)	–	Alive	592
10	≥90	≥90	≥90		Gr II (S, G)	Extensive	Yes (unknown)	–	Dead (GvHD)	757
11	≥90	≥90	≥90		Gr II (S)	Extensive	–	–	Alive	360
12	88	79	71		Gr III (S, L)	Extensive	Yes (unknown)	–	Alive	720
13	≥90	≥90	≥90		Gr I (S)	Extensive	Yes (HSV, unknown)	–	Dead (GvHD)	517
14	≥90	≥90	≥90		Gr II (S, G, L)	Limited	Yes (fungal susp., unknown)	–	Dead (GvHD and its complication)	187
15	85	88	≥90		Gr III (S, G)	–	–	–	Alive	702
16	84	88	≥90		–	Limited [a]	Yes (CMV antigenemia)	–	Alive	642
17	80	≥90	D	Yes (d98, mixed chimerism)	Gr II (S, G)[b]	–	–	–	Dead (GvHD and its complication)	149
18	≥90	88	≥90		Gr II (S)	Extensive	Yes (CMV antigenemia)	–	Alive	729
19	≥90	≥90	≥90		Gr I (S)	Limited	–	–	Alive	737
20	≥90	≥90	≥90		Gr II (G)	–	Yes (CMV antigenemia)	Yes (d147)[c]	Alive	688
21	78	77	75		Gr II (S, L)	Extensive	–	Yes (d364)	Dead (BOOP)	593
22	≥90	≥90	≥90		Gr II (S, G)	Extensive	Yes (unknown)	Yes (d98)	Dead (progressive disease)	306
23	≥90	≥90	D		–	Extensive	Yes (unknown)	–	Dead (GvHD and its complication)	151
24	≥90	≥90	≥90		Gr II (G)	Extensive	–	Yes (>d365)[d]	Dead (recurrent disease)	825
25	≥90	87	≥90	Yes (d186, d238, relapse)	Gr II (S)	Extensive	Yes (CMV antigenemia)	Yes (d156)	Dead (recurrent disease)	401
26	≥90	≥90	≥90	Yes (d204, relapse)	Gr I (S)	Extensive	Yes (unknown)	Yes (d141)	Dead (recurrent disease and its complication)	412
27	84	≥90	≥90		–	Extensive	Yes (unknown)		Alive	371
28	≥90	≥90	≥90		Gr II (S)	Extensive	Yes (viral susp., unknown)	–	Alive	365
29	≥90	≥90	≥90		Gr I (S)	Extensive	Yes (unknown)	–	Alive	366
30	≥90	≥90	≥90		Gr I (S)	Limited	Yes (unknown)	Yes (d370)	Alive	370

ND, not done; D, dead; DLI, donor lymphocyte infusion; Gr, grade; GvHD, graft-versus-host disease; GvHD site codes, S-skin, G-gut, L-liver; CMV, cytomegalovirus; susp., suspected; unknown, no microbiological evidence despite symptoms; IP, interstitial pneumonia.

[a]This patient developed a GvHD starting on day 112 after receiving DLI for mixed chimerism.

[b]This patient developed gut GvHD starting on day 92.

[c]CNS relapse without hematological relapse.

[d]This patient relapsed after day 365, but the exact date of relapse is unknown.

TABLE III. Regimen-Related Toxicities Within 20 Days After HSCT According to the Seattle Criteria in 30 Patients

	Grade			
Toxicity	1	2	3	4
Heart	1	0	0	0
Bladder	0	1	0	0
Kidney	5	1	0	0
Lung	2	0	0	0
Liver	8	0	0	0
CNS	1	0	0	0
Stomatitis	9	8	0	0
GI toxicity	4	1	0	0

HSCT, hematopoietic stem cell transplantation; CNS, central nervous system; GI, gastro-intestinal.

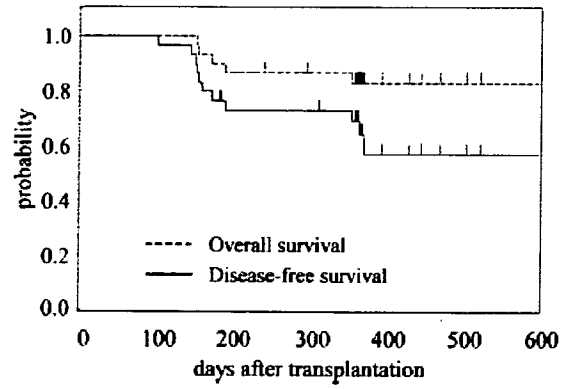

Figure 2. Kaplan–Meier product estimates of overall survival and disease-free survival.

gut GvHD, three of those developed extensive chronic GvHD and all were treated with corticosteroid.

The Kaplan-Meier estimated probability of OS and DFS at 1 year was, respectively, 83% and 62% (Fig. 2). Both patients age (≤55 years versus >55 years) and CD34+ cell dose (>5.0 $\times 10^6$ kg^{-1} versus ≤5.0 $\times 10^6$ kg^{-1}) were not associated with better outcomes by a stratified analysis (data not shown).

Pharmacokinetic results for fludarabine and busulfan

2F-ara-A and busulfan PK parameters were calculated from data obtained from blood samples from six consenting patients (UPN 1, 3–7). After the start of the 4th infusion of fludarabine phosphate (30 mg/m^2/dose), the maximum plasma level of 2F-ara-A was 3.12 ± 1.08 nmol/ml, with a subsequent decline to $T_{1/2}$ of 8.59 ± 1.57 h. The AUC (0–24 hr) and CL were 17.7 ± 2.82 nmol hr/ml and 78.9 ± 13.1 ml/min/m^2, respectively. After the 6th administration of busulfan (1 mg/kg/dose for eight times), the maximum plasma level of busulfan was 1.37 ± 0.34 nmol/ml, with a subsequent decline to a $T_{1/2}$ of 2.88 ± 0.65 hr. The AUC (0–6 hr) and CL were 4.85 ± 1.07 nmol hr/ml and 3.60 ± 0.88 ml/min/m^2, respectively. Since these parameters are similar to those in a previous study with the repeated administration of fludarabine phosphate alone at 15, 20, and 25 mg/m^2/dose (data not shown), combination with busulfan seemed to have no effect on the pharmacokinetics of 2F-ara-A. The steady-state plasma level of busulfan (808 ± 178 ng/ml) was observed to remain within a therapeutic level (600–900 ng/ml) in adults [21].

Discussion

In this prospective study, we showed that a combination of fludarabine (180 mg/m^2) and oral busulfan (8 mg/kg), despite the omission of antithymocyte globulin from the original regimen by Slavin et al. [6], can be successfully used to help prepare patients older than 50 years with hematological malignancies for HSCT from an HLA-matched related donor: All patients achieved sustained engraftment without graft failure, only an insignificant occurrence of RRT and treatment-related complications were seen, and PS and dietary intake were well maintained, which agrees with published observational studies on RIST with fludarabine and busulfan [16,22,23].

The rapid induction of complete donor-type chimerism was considered as an essential part of the RIST procedure. Although all of our patients rapidly developed conventional neutrophil and platelet engraftment, two of the 30 patients without preceding cytotoxic chemotherapy remained in mixed T-cell chimerism during the first 6 months after transplantation. A more rapid induction of T-cell chimerism has been observed in other studies of RIST in patients who had been previously treated with chemotherapy for diseases other than CML or MDS [24]. Although a close association between the occurrence of acute GvHD and the induction of higher levels of donor T-cell chimerism has been reported [14], in our experience over 50% of patients did not achieve complete chimerism at the onset of acute GvHD, demonstrating that mixed chimerism status did not provide absolute protection from GvHD, which is in agreement with data published by Baron et al. [15]. We speculate that differences in the conditioning regimen and GvHD prophylaxis may result in different observations.

While our less intensive regimen was associated with less toxicity, this strategy will only work if modifications to the conditioning regimen intensity that allow early clinical benefits do not also lead to reduced induction of GvL effect or other complications that increase relapse rate or result in worse survival in later time period [25]. A recent observational study from European Group of Blood and Marrow Transplantation Registry compared treatment-related mortality (TRM) and other outcomes between 315 RIST recipients and 407 CIST recipients, who were over 50 years and transplanted from a HLA matched sibling donor [26], and suggested that lower TRM but higher relapse rate were seen in RIST recipients. Given the fact that all three patients, who relapsed within 6 months after transplantation, were MDS with poor prognostic factors, the incidence of relapse in our study seems to be no higher than that in published data for CIST [27–30]. Taussig et al. evaluated the feasibility and safety of the fludarabine based RIST regimen in 16 patients with standard risk diseases [31]. In this study, TRM rate within 100 days was 0%, however, OS and DFS at 1 year read from Fig. 2 were 69% and 56%, respectively, where most of the patients included in this study had early stage diseases and over 30% of patients were aged less than 50 years. Despite the older patient population, our data showing no treatment-related mortality (TRM) within the first 100 days after transplantation and OS and DFS at 1 year of 83% and 62%, respectively, was encouraging.

In a previous report, we suggested that the development of GvHD is not essential for the control of low-risk myeloid malignancies, and that GvHD and infection, rather than relapse, are more important problems to be addressed in these patients [25]. Although our data showed favorable outcomes, six patients with four low risk disease and three patients aged less than 55 years died of GvHD or its complication within the first year should be interpreted with care. The incidence of Grade II–IV acute GvHD in this

study was somewhat higher than that in published literature and our own observational data with elder patients and high risk diseases [25]. However, Grade III–IV acute GvHD was infrequent and none died from acute GvHD. The incidence of chronic GvHD was higher than that in our previous experience (56%) [32] or in other reports [31,33] even after considering inevitable differences in the ethnicity, GvHD prophylaxis and matching practice of HLA, or disease risk. G-CSF mobilized peripheral blood stem cells may have been associated with an increased incidence of GvHD, particularly in its chronic form [34,35]. Conditioning regimen excluded antithymocyte globulin was also a possible explanation of this finding [23]. Most importantly, patients undergoing RIST are usually older than those undergoing CIST, which leads to a higher risk for GvHD [36,37]. Early CyA withdrawal regulation to get speedy achievement of complete donor chimerism after RIST in our protocol might have influenced the increased incidence of Grade II–IV acute GvHD, which might have affected the rate of chronic GvHD [33,35,38,39]. Although severe GvHD will be unavoidable for some patients including MDS with poor prognostic factors [40,41], the balance between GvHD and GvL is a significant concern in RIST and we should seriously evaluate the type and tapering speed of immunosuppressive agents after RIST. Current findings suggested GvHD control might be improved simply by extending the duration of CyA administration. Additionally, we noticed that the clinical features of GvHD are different in RIST than in CIST, i.e. a syndrome compatible with acute GvHD occurs well after Day 100. Hence, the current grading system for GvHD, which was developed on the basis of experience in ablative settings, may not be an optimal tool for assessing GvHD after RIST. We observed a late onset of acute GvHD and an early onset of chronic GvHD, and therefore believe that a significant number of late-onset acute GvHD may have been judged as chronic GvHD in this study simply because the onset of GvHD was over 100 days after transplantation. Our results support the current proposition by Mielcarek and Storb concerning the abandonment of the traditional Day 100 cutoff for separating acute from chronic GvHD [35].

In this prospective study, we confirmed the short-term safety and efficacy of our RIST procedure for hematological malignancies in the elderly. Long-term follow-up of patients to evaluate disease control and the consequence of therapy is mandatory, and the development of optimal GvHD prophylaxis, with the use of novel assessment criteria, will be of primary importance for the wider application of the RIST procedure. RIST may also be beneficial in young patients, since organ damage, including infertility, might be milder and less frequent in RIST than in CIST, which should be confirmed by further prospective clinical trials. Although the number of patients studied was limited, the analysis of fludarabine pharmacokinetics has for the first time provided reliable information on the interaction of key drugs, and we found no evidence to suggest that synergic or specific toxicities were associated with increased exposure to the concomitant use of busulfan, or vice versa. This information should be useful in future studies in which different drugs are combined with fludarabine.

Acknowledgments

This study was supported solely by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare, with fludarabine phosphate kindly supported by Nihon Schering K. K. We thank the medical and nursing staff of all of the transplant centers for their important contributions. We are indebted to the members of the Japan Clinical Research Support Unit for data management. We are also grateful to the members of the University of Tokyo, the Statcom, and the EPS for the statistical analyses.

Appendix

The following institutions contributed data to this study: National Cancer Center Hospital, Kanazawa University Hospital, Toranomon Hospital, Imamura Bun-in Hospital, Ehime Prefectural Central Hospital, Ishikawa Prefectural Central Hospital, Osaka City University Hospital, and Toyama Prefectural Central Hospital.

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[redacted]

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.

Clinical Lymphoma & Myeloma, Vol. 7, No. 7, 475-479, 2007

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 γδ T-cell LGLL of cord donor origin after a second cord blood transplantation for acute myeloid leukemia.

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Submitted: Sept 5, 2006; Revised: Dec 29, 2006; Accepted: Jan 18, 2007

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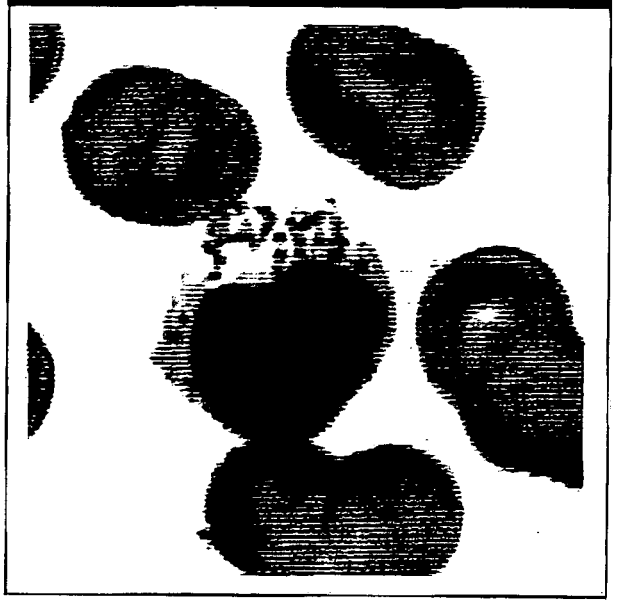
Case Report

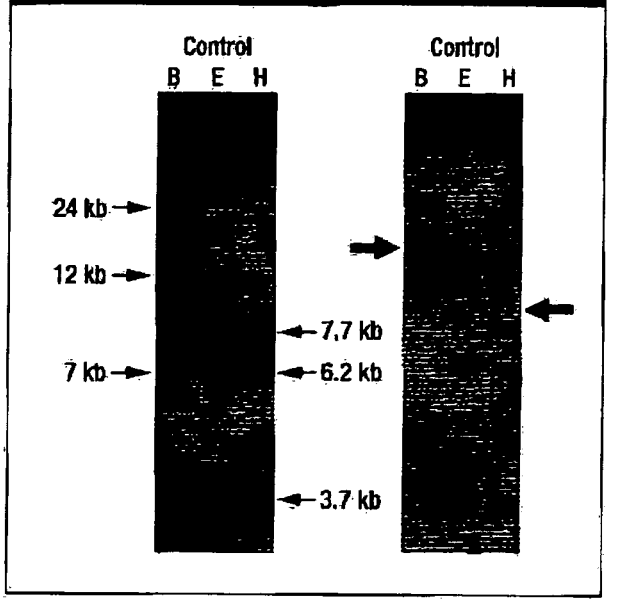
A 58-year-old Japanese man with acute myeloid leukemia (French-American-British classification; M2) in second complete remission received allogeneic HSCT from an unrelated female cord blood donor. The conditioning regimen consisted of total body irradiation of 12 Gy in 6 fractions from day −6 to −4, 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.

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 ×1000.

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



DNA from BM of this patient was hybridized with a TCR Cβ1 probe.
Arrows indicate rearranged bands.
Abbreviations: B = Bam H1; E = Eco R; H = Hind III

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/μL (10.3 × 10^9/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 × 10^3/μL (1.92 × 10^9/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)–γδ, but negative for CD4 and TCR-αβ. 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).

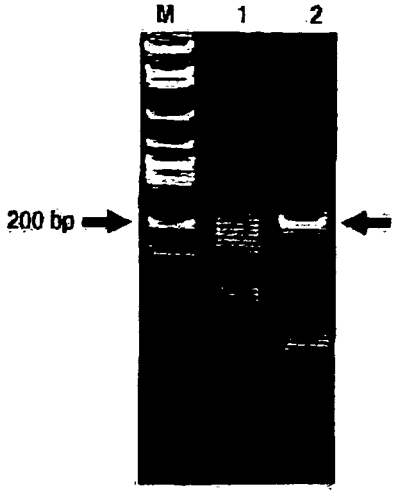
Abdominal paracentesis was performed with milky chylous fluid, and a flow cytometry examination showed results similar to those in the peripheral blood. Multiprimer-based polymerase chain reaction (PCR) analysis of ascitic cells also showed clonal rearrangement of the TCR-δ chain, as shown in Figure 3. The primer sets were used in the following locations: Vδ1, 5'-AAA GTG GTC GCT ATT CTG TC-3'; Vδ2A, 5'-GCA CCA TCA GAG AGA GAT GA-3'; Jδ, 5'- TGG TTC CAC AGT CAC ACG GG-3'; Dδ3B, 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 Vδ1 and Jδ, 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 γδ 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 AmpFlSTR 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 γδ T-LGL cells were exclusively of second cord blood transplantation donor origin.

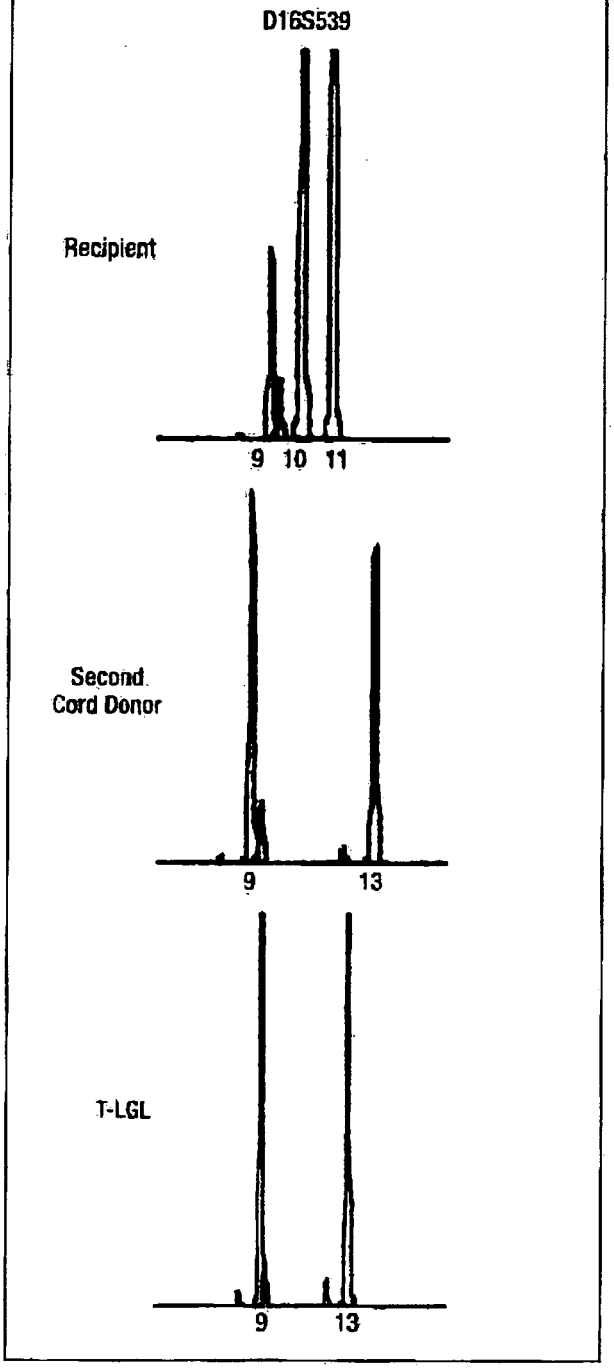
Serologic examination showed no evidence of viral infection. Real-time PCR analysis revealed a high load of EBV (7.9 × 10^3 copies/10^6 cells). However, in situ hybridization studies of BM cells did not reveal EBV-encoded small RNA, and Southern blot analysis of BM cells also showed no band for

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

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).

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 compatible with T-cell LGLL.

Most cases of PTLD, 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 PTLD after cord blood transplantation,[7-10] the incidence of PTLD of T-cell origin has been reported to be only 4%-14% with a less frequent association with EBV.[6,11]

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

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	7/Male	Sibling*	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[7]	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	5/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)	851
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[7]	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 PTLD as γδ T-cell LGL of donor origin after a second cord blood transplantation. The occurrence of T-cell PTLD 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.

Acknowledgements

We thank Atsuya Nakano and Yukiko Sado, Clinical Laboratory Division, National Cancer Center Hospital, for reviewing and interpreting the cytologic and immunophenotypic findings.

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Abdominal Pain and Syndrome of Inappropriate Antidiuretic Hormone Secretion as a Manifestation of Visceral Varicella Zoster Virus Infection in a Patient With Non-Hodgkin's Lymphoma

To the Editor: Lesions of varicella zoster virus (VZV) disease are usually limited to a few dermatomes. However, in immunocompromised patients, disseminated cutaneous and visceral involvement occurs. We report here a rare case of such a disseminated disease with manifestation of severe abdominal pain and syndrome of inappropriate anti-diuretic hormone (SIADH), which occurred 2 months after completion of conventional chemoradiotherapy for non-Hodgkin's lymphoma (NHL).

A 65-year-old woman was diagnosed as having diffuse large B-cell lymphoma of stomach origin. The clinical stage was III by the Lugano classification, and the International Prognostic Index score was low. HIV test was negative. She received chemotherapy consisting of three cycles of cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP), followed by radiation at a total of 40.5 Gray to the involved field, and a complete response was obtained. Two months later, she was re-admitted to our hospital because of severe abdominal pain lasting 2 days.

Her vital signs and physical examination were normal. Laboratory examination revealed prominent hyponatremia (Na 122 mmol/l, normal 138–146) and mild liver injury (GOT 74 U/L, normal 13–33; and GPT 63 U/L, normal 6–27). The serum osmolarity was 262 mOsm/kg, and urine osmolarity 532 mOsm/kg, which was consistent with SIADH. The pituitary size and intensity was normal but the occipital lobe of the cerebrum showed a high intensity on brain magnetic resonance imaging. In spite of fluid restriction, hyponatremia and her pain deteriorated. On the sixth hospital day, a subtle vesicular skin lesion on her abdominal wall was observed. We reasoned that her complaint might be attributed to the visceral involvement of VZV extending to the peritoneum, liver, brain, and skin. Upon starting treatment with acyclovir at 1,500 mg/day, her abdominal pain and hyponatremia improved, and she was discharged on the 14th hospital day. Polymerase chain reaction (PCR) for VZV of her peripheral blood and cerebrospinal fluid taken before acyclovir therapy was later found to be positive. The number of CD4-positive lymphocytes was 191/μL, and this low level has been maintained for as long as 1 year. The complete remission of NHL was also maintained throughout the episodes.

The occurrence of disseminated VZV including visceral involvement has been limited to immunocompromised patients; after stem cell transplantation (SCT), ~17–50% of cases develop VZV infection [1,2], and, among them, visceral infection is rare (3.6% [2]). Especially, there are only a few VZV infection cases after SCT consisting of SIADH [3]. And only one case has been reported which developed along with severe abdominal pain and SIADH after conventional chemotherapy [4].

Storek et al. reported that the CD4-positive lymphocyte count after allogeneic SCT was inversely correlated with the infection score [5]. We suppose that her low CD4 count might have contributed to the visceral VZV infection. The reason why she showed such a low CD4 cell count is currently unknown.

It should be noted that this rare manifestation could occur even after conventional chemotherapy in NHL patients. Importance of recognition of this manifestation should be stressed because it is critical for the prompt diagnosis of the disease and its successful treatment.

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Published online 28 November 2006 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20827

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International Journal of
HEMATOLOGY

Unrelated-Donor Bone Marrow Transplantation with a Conditioning Regimen Including Fludarabine, Busulfan, and 4 Gy Total Body Irradiation

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Received October 10, 2006; received in revised form December 26, 2006; accepted January 9, 2007

Abstract

We investigated the feasibility of reduced-intensity conditioning with 4 Gy total body irradiation, fludarabine (30 mg/m^2 for 6 days), and busulfan (4 mg/kg for 2 days) for bone marrow transplantation from a serologically HLA-matched unrelated donor. Seventeen adult patients (median age, 55 years; range, 27-67 years) with various hematologic malignancies (6 in remission, 11 not in remission) were treated. Successful engraftment was achieved in all patients at a median of day 18 (range, day 14-35) after transplantation, although subsequent secondary graft failure was observed in 2 patients. The cumulative incidence of acute graft-versus-host disease (GVHD) of grades II to IV at day 100 was 48%. With a median follow-up of 286 days (range, 56-687 days), the rates of 1-year overall survival, 100-day nonrelapse mortality, and 1-year nonrelapse mortality were 41%, 14%, and 46%, respectively. Eleven patients died, and the causes of death were relapse (n = 4), pulmonary complications (n = 4), acute GVHD (n = 2), and sepsis (n = 1). The remaining 6 patients (at transplantation, 2 were in remission, and 4 were not in remission) are currently still in remission. These results suggest that this regimen reduces the risk of graft failure, but further studies are needed to ameliorate transplantation-related toxicities, primarily GVHD and/or pulmonary complications.

Int J Hematol. 2007;85:256-263. doi: 10.1532/IJH97.06199

Key words: Unrelated donor bone marrow transplantation; Fludarabine; Busulfan, TBI

1. Introduction

Although allogeneic hematopoietic stem cell transplantation (HSCT) is a possible curative approach for patients with various hematologic malignancies, only 30% to 40% of patients in Japan have an appropriate family donor available [1]. Hence, the application of unrelated-donor transplantation using bone marrow or cord blood cells has been expanding. Another area of current interest is the application of reduced-intensity conditioning regimens, mostly incorporating fludarabine as a primary agent, because conventional allogeneic HSCT using a conditioning regimen with high doses of systemic chemotherapy/radiation is associated with significant toxicities. In contrast, HSCT with a reduced-intensity conditioning regimen allows older patients and those who have contraindicating comorbidities to undergo HSCT [2-7].

Nevertheless, special consideration should be paid to developing reduced-intensity conditioning protocols for the unrelated-donor HSCT setting, because the incidences of both graft rejection and graft-versus-host disease (GVHD) are greater than in related-donor transplantation. In addition, the intensity of the reduced-intensity conditioning regimen influences transplantation-related toxicities and the relapse rate, and the stem cell source (ie, peripheral blood stem cells or bone marrow cells) influences engraftment [8]. Accordingly, several reduced-intensity conditioning protocols have been tested to address a variety of problems [8-17]. In this study, we investigated the feasibility of bone marrow transplantation (BMT) from a serologically HLA-matched unrelated donor with a regimen containing

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4 Gy of total body irradiation (TBI), fludarabine (Flu), and busulfan (BU).

2. Patients and Methods

2.1. Patients and Donors

The data for adult patients with hematologic malignancies who underwent unrelated-donor BMT through the Japan Marrow Donor Program between June 2002 and December 2003 at the National Cancer Center Hospital were analyzed retrospectively. This protocol was approved by the Ethics Committee, and written informed consent was obtained from each patient. The patients who were enrolled in this study were ineligible for conventional allogeneic HSCT because of age (older than 50 years) and/or concomitant diseases or preceding intensive therapies, such as autologous HSCT or multiple chemotherapies. Donor-recipient pairs were selected on the basis of serologic matching for HLA-A and HLA-B and molecular matching for HLA-DRB1. HLA allele typing was performed by intermediate-resolution polymerase chain reaction (PCR) analysis. The stem cell source, which was determined by the Japan Marrow Donor Program donor center, was bone marrow in all cases.

2.2. Treatment Plan and Evaluations

The conditioning regimen consisted of 30 mg/m^2 Flu intravenously daily for 6 days (day –8 to day –3), 4 mg/kg BU orally daily for 2 days (days –6 and –5, without BU dose adjustment), and 4 Gy TBI without lung shielding (day –9 or day –1, single dose or 2 divided doses). Non–T-cell–depleted bone marrow was infused on day 0. The time of neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^9$/L, and the time of platelet engraftment was defined as the first of 7 consecutive days with a platelet count $\geq 20 \times 10^9$/L without transfusion support. Granulocyte colony-stimulating factor (G-CSF) was administered at 300 μg/m^2 from day 6 and continued until neutrophil engraftment. The degree of donor chimerism among peripheral blood mononucleated cells was evaluated by PCR analysis of short tandem repeat polymorphisms with fluorescently labeled primers. Secondary graft failure was defined as cytopenia with an absolute neutrophil count $< 0.1 \times 10^9$/L or decreasing chimerism not associated with relapsing disease in patients who had recovered in the early posttransplantation period.

GVHD prophylaxis consisted of cyclosporin A (CsA) from day –1 (daily administration of 3 mg/kg by continuous intravenous infusion or 6 mg/kg orally in 2 divided doses) and methotrexate (10 mg/m^2 intravenously on day 1 and 7 mg/m^2 on days 3, 6, and 11). The CsA dosage was adjusted according to the patient's renal function and to maintain therapeutic levels (250-350 ng/mL) with continuous infusion or trough levels (150-250 ng/mL) with oral administration. In patients without GVHD, CsA was tapered from day 100 over a 3- to 6-month period. Standard criteria were used to grade acute and chronic GVHD [18,19]. Chronic GVHD was evaluated in patients who survived at least 100 days and was classified as limited or extensive. Patients who developed acute GVHD ≥grade II were treated with methylprednisolone at 1 to 2 mg/kg per day.

2.3. Supportive Care

Antimicrobial prophylaxis consisted of ciprofloxacin, fluconazole, acyclovir, and trimethoprim/sulfamethoxazole according to our institutional protocol. All patients were nursed in a room equipped with high-efficiency air filtration of particulates. Monitoring for cytomegalovirus (CMV) antigenemia was performed once a week after neutrophil engraftment by means of the horseradish peroxidase–C7 method. Patients positive for CMV antigenemia were started preemptively on ganciclovir therapy.

2.4. Statistical Analysis

Overall survival was calculated from the time of transplantation until death from any cause. Progression-free survival was measured from transplantation until disease progression or death from any cause. Nonrelapse death was defined as death due to any cause other than relapse. Survival curves for overall survival and progression-free survival were estimated by the Kaplan-Meier method.

3. Results

3.1. Patients

The median age of the 17 patients was 55 years (range, 27-67 years; Table 1). The diagnoses were acute myeloid leukemia (AML) (n = 7), myelodysplastic syndrome (MDS) (n = 4), chronic myelogenous leukemia (n = 1), non-Hodgkin's lymphoma (n = 4), and multiple myeloma (n = 1). Six patients were in remission at transplantation, and the remaining 11 were not in remission. Three patients with MDS or AML following MDS underwent unrelated-donor BMT as a primary treatment. Seven donor-recipient pairs were fully matched for HLA-A, HLA-B, and HLA-DRB1 at the allele level, 4 donor-recipient pairs had an allele-level mismatch at the HLA-A locus, and 5 pairs had an allele-level mismatch at the HLA-DRB1 locus. One patient was mismatched with the donor at 3 HLA alleles.

3.2. Engraftment and Chimerism

The median number of infused nucleated cells was 2.7×10^8/kg (range, $0.65\text{-}5.5 \times 10^8$/kg). All patients achieved neutrophil recovery, but 5 patients did not become independent of platelet transfusion during their follow-up period (Table 2). The median times until neutrophil and platelet recoveries were 18 days (range, 14-35 days) and 26 days (range, 15-112 days), respectively (Figure 1). Late graft failure was observed in 2 patients, one of whom had secondary graft failure due to myelosuppression caused by ganciclovir treatment for CMV colitis. In this patient, donor chimerism was not assessed after day 30 when complete donor chimerism was confirmed. In the other case, donor chimerism decreased from 89% on day 30 to 33% on day 60, despite the tapering of CsA from day 30. Chimerism was

Table 1.
Patient and Disease Characteristics*

Patient No.	Age, y/Sex	Disease	Status	Time from Dx to HSCT, mo	HLA Allelic Mismatch	GVH Vector	HVG Vector	Contraindications to Conventional HSCT	Pretransplantation Comorbidities
1	55/F	AML	CR3	117				Age	No
2	52/F	AML	Primary Ref	13	DRB1	1	1	Age + comorbidity	Pneumonia
3	57/F	AML	Rel2	28				Age	Atrial fibrillation
4	55/M	MDS	Primary Ref	3				Age	Atrial fibrillation
5	57/M	MDS	CR1	8				Age	No
6	59/M	CML	CP2	8				Age	No
7	55/M	PTCL	PR	16	DRB1	1	1	Age	Gastric ulcer
8	58/M	AML	Untreated	10	DRB1	1	1	Age	Bronchial asthma, FEV_1 75%
9	59/M	AML	Untreated	33	DRB1	1	1	Age	Bilirubin 1.5 mg/dL
10	52/M	AML	CR1	11	A	1	1	Age	FEV_1 67%
11	57/M	MDS	CR1	13				Age	Prior gastric cancer
12	61/M	AML	CR2	58	A, both DRB1	3	3	Age	No
13	67/F	FL	Primary Ref	58	, A	1	1	Age + comorbidity	Dyspnea requiring oxygen
14	27/M	DLBCL	Rel3	38	A	1	0	Prior autologous HSCT	No
15	48/F	MM	Primary Ref	80				Comorbidity	Ventricular septal defect
16	52/F	MDS	Untreated	130	A	1	1	Age	No
17	49/M	FL	Rel1	28	DRB1	1	1	Prior multiple chemotherapies	No

*Dx indicates diagnosis; HSCT, hematopoietic stem cell transplantation; GVH, graft-versus-host; HVG, host-versus-graft; AML, acute myeloid leukemia; CR3, third complete remission; Ref, refractory; Rel2, second relapse; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; CP2, second chronic phase; PTCL, peripheral T-cell lymphoma; PR, partial remission; FEV_1, forced expiratory volume in 1 second; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; MM, multiple myeloma.

evaluated by analysis of short tandem repeats in 14 patients, and complete donor chimerism was confirmed in 12 of these patients. One patient who relapsed on day 32 had exhibited 54% donor chimerism on day 30. In the remaining 3 patients who relapsed after transplantation, complete donor chimerism had been achieved by day 30. In the patient who relapsed on day 78, donor chimerism decreased from 100% on day 30 to 64% on day 60. Mixed chimerism was not confirmed in the other 2 patients before disease progression or relapse. The patients without graft failure or relapse did not have mixed chimerism during their follow-up periods.

3.3. Regimen-Related Toxicities and Infections

Regimen-related toxicity was graded according to the National Cancer Institute Common Toxicity Criteria, version 2.0, and maximum toxicities are shown in Table 3. Fifteen of the 17 patients had grade III oral/pharyngeal mucositis that required morphine as an analgesic. Reversible elevation (grades III-IV) in transaminase and bilirubin levels occurred in 35% and 12% of the cases, respectively. No veno-occlusive disease was observed. Four patients developed transient grade III hyponatremia within 28 days after transplantation. Four patients developed transient pulmonary infiltration or congestive heart failure due to hypercytokinemia at engraftment, and 2 of these patients developed grade II acute GVHD after engraftment. No histologic findings of acute GVHD were seen in the other 2 patients. One patient developed reversible paroxysmal supraventricular tachycardia. One patient developed bloody diarrhea and abdominal pain even after improvement of acute GVHD of the skin, and we diagnosed intestinal thrombotic microangiopathy from the results of a gut biopsy. This patient was successfully managed by diminishing immunosuppressive treatment. Four patients who had blood cultures positive for bacterial infection (*Pseudomonas aeruginosa*, *Acinetobacter lwoffii*, *Corynebacterium* sp, and *Staphylococcus* sp) within 28 days after transplantation were successfully treated with antibiotics. Invasive aspergillosis was encountered in 2 patients (1 proven and 1 possible case). In the proven case, the patient had bronchiolitis obliterans, which was the ultimate cause of death. Of the 17 patients, CMV antigenemia was detected in 12 patients, 2 of whom had CMV colitis.

3.4. Graft-versus-Host Disease

Acute GVHD of grades II to IV was diagnosed in 8 patients (48%; 95% confidence interval [CI], 36%-59%); the GVHD was grade II in 3 patients and grade IV in 5. The median time to the onset of acute GVHD was 32 days (range, 20-81 days) after transplantation (Figure 2A). Two of 4 patients who skipped methotrexate treatment on day 11 because of severe mucositis developed grade IV acute GVHD. Two of the 5 patients with grade IV acute GVHD subsequently died. One of these patients had acute GVHD after the withdrawal of CsA treatment at the time of leukemia relapse, and the other patient had received bone

Table 2.
Transplantation Outcomes*

Patient No.	Time to ANC >0.5 × 10^9/L, d	Time to Platelets >20 × 10^9/L, d	Acute GVHD						Chronic GVHD (Involved Organs)	Follow-up, d	Current Disease Status	Cause of Death
			Grade	Skin	Liver	Gut	mPSL, mg/kg	Response				
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.

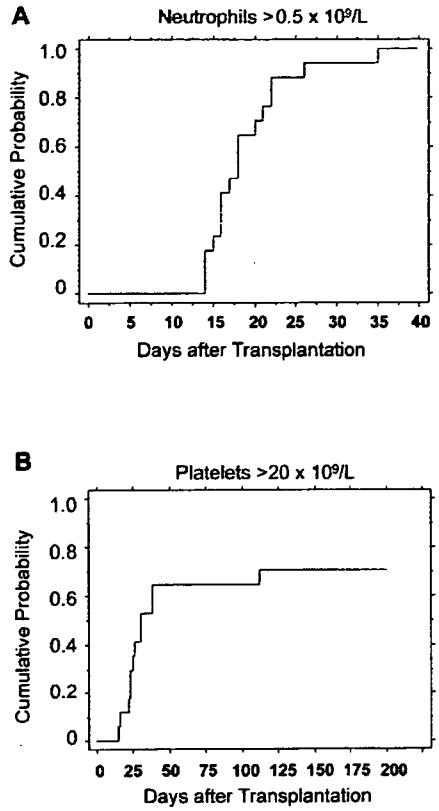

Figure 1. Engraftment after unrelated-donor bone marrow transplantation following reduced-intensity conditioning expressed as the cumulative probability of a neutrophil count >0.5 × 10^9/L (A) and a platelet count >20 × 10^9/L (B). All patients achieved neutrophil recovery, but 5 patients did not achieve 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 pneumonitis (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.

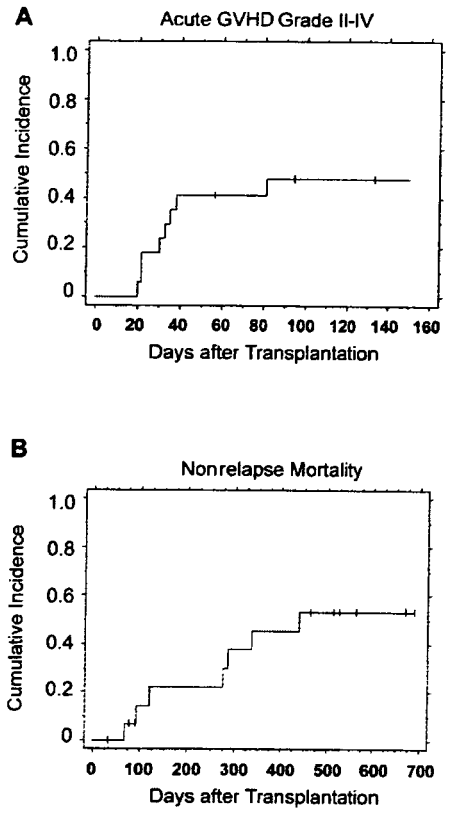

Figure 2. Cumulative incidence of acute GVHD (grades II-IV) (A) and nonrelapse mortality (B) after unrelated bone marrow transplantation following reduced-intensity conditioning. Acute GVHD (grades II-IV) was diagnosed in 8 patients (48%) (grade II in 3 patients and grade IV in 5) at a median of day 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/m^2) 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/m^2 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

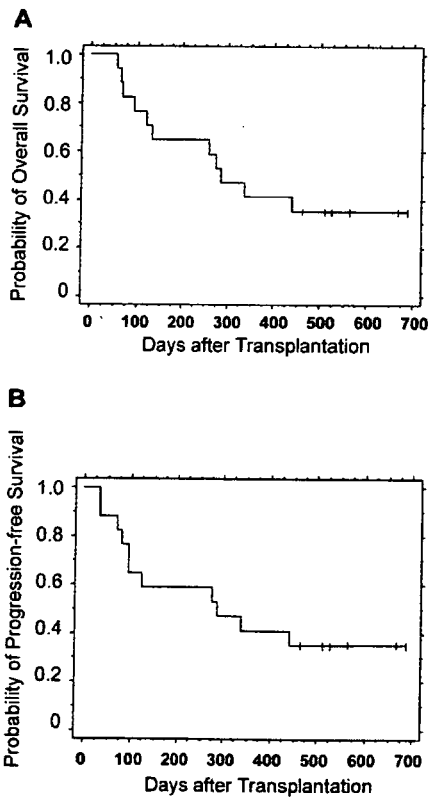

Figure 3. Kaplan-Meier actuarial probability of overall survival (OS) (A) and progression-free survival (PFS) (B) after unrelated-donor bone marrow transplantation following reduced-intensity conditioning. The median follow-up was 286 days (range, 56-687 days). The 1-year OS and PFS rates were both 41%.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