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.

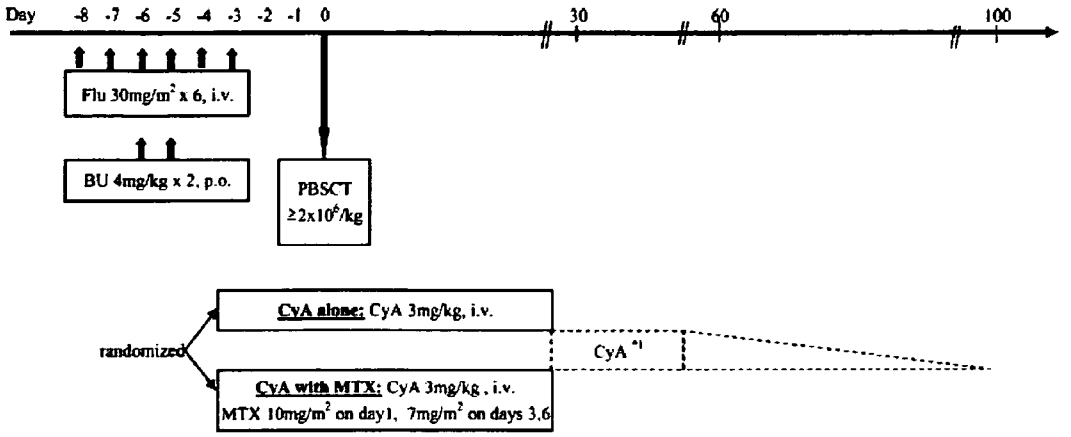

Figure 1. Treatment schedule. CyA; cyclosporine, MTX; methotrexate. *1: When acute GvHD was not observed, CyA was tapered by 10% a week starting at Day 28, and was eliminated by Day 100. When mixed chimerism was seen without active acute GvHD over Day 60, CyA was tapered and discontinued within 2 weeks. 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 $\geq$90] %, day 60:81 [41 to $\geq$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.

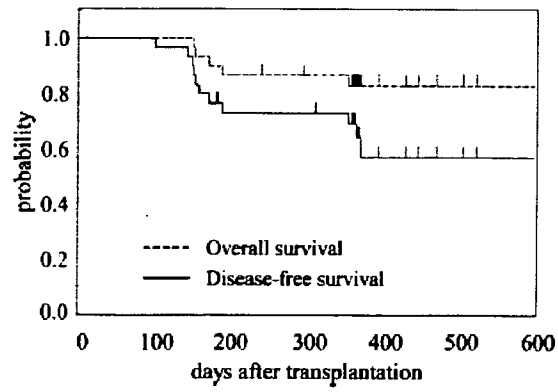

Figure 2. Kaplan–Meier product estimates of overall survival and disease-free survival.

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.

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Appendix

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bjh research paper

Chronic graft-*versus*-host disease after allogeneic bone marrow transplantation from an unrelated donor: incidence, risk factors and association with relapse. A report from the Japan Marrow Donor Program

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Summary

Chronic graft-*versus*-host disease (GVHD) remains the major cause of late morbidity and mortality after allogeneic stem cell transplantation. We retrospectively analysed 2937 patients who underwent bone marrow transplantation from an unrelated donor (UR-BMT) facilitated by the Japan Marrow Donor Program (JMDP) and survived beyond day 100 after transplantation. The cumulative incidence of chronic GVHD (limited + extensive) or extensive chronic GVHD at 5 years post-transplant was 45·8% and 28·2%, respectively. On multivariate analysis, seven variables predicting chronic GVHD were identified: recipient age over 20 years, donor age over 30 years, primary diagnosis of chronic myeloid leukaemia, human leucocyte antigen (HLA)-A or -B mismatch, total body irradiation-containing regimen, platelet count not having reached 50×10^9/l by day 100, and prior acute GVHD. Among 2609 patients with haematological malignancy, overall survival was significantly higher in patients with limited chronic GVHD but lower in patients with extensive chronic GVHD compared with those without chronic GVHD. The cumulative incidence of relapse among patients with limited or extensive chronic GVHD was significantly lower than that among patients without chronic GVHD. Our results suggest that limited chronic GVHD provides a survival benefit to patients with haematological malignancies by reducing the risk of relapse without increasing the risk of death from chronic GVHD.

Keywords: chronic graft-*versus*-host disease, unrelated bone marrow transplantation, Japan Marrow Donor Program, relapse, graft-*versus*-leukaemia effect.

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†A complete list of the centres that participated in the bone marrow transplantations facilitated by the Japan Marrow Donor Program (JMDP) appears in Appendix 1.

Haematopoietic stem cell transplantation (HSCT) has become established as one of the curative therapies for haematological malignancies and other haematological or immunologic disorders (Armitage, 1994). However, various late complications of HSCT rather than relapse decrease the quality of life of HSCT recipients (Socie *et al*, 1999; Kiss *et al*, 2002). Among late complications that may occur beyond 100 d post-transplant, chronic graft-*versus*-host disease (GVHD) affects approximately 30–70% of long-term survivors depending on the degree of human leucocyte antigen (HLA)-mismatch with the donor and the source of the stem cells, and remains a major cause of late morbidity and mortality post-transplantation (Atkinson *et al*, 1990; Sullivan *et al*, 1991; Vogelsang, 2001; Lee *et al*, 2002; Farag, 2004). Despite improvements in other areas of supportive care, little significant progress has been made in the management of chronic GVHD (Vogelsang, 2001). Patients with chronic GVHD have decreased performance status, impaired quality of life, and increased risk of mortality (Duell *et al*, 1997; Socie *et al*, 1999). In spite of its adverse effects, chronic GVHD is associated with a lower incidence of leukaemia relapse by a graft-*versus*-leukaemia (GVL) effect that is comparable or greater than that ascribed to acute GVHD (Weiden *et al*, 1981; Sullivan *et al*, 1989; Kataoka *et al*, 2004).

Bone marrow transplantation (BMT) from an unrelated volunteer donor (UR-BMT) has become established as an accepted treatment for patients in need of HSCT who do not have a HLA-matched sibling donor (Kernan *et al*, 1993; Hansen *et al*, 1998; Kodera *et al*, 1999; Davies *et al*, 2000). The incidence of chronic GVHD is assumed to be higher after UR-BMT than after transplants from an HLA-matched sibling donor. Previous studies have identified the incidence and risk factors for chronic GVHD after sibling transplant (Storb *et al*, 1983; Ringden *et al*, 1985; Atkinson *et al*, 1990; Remberger *et al*, 2002); however, there are no definite data available on the incidence and risk factors for chronic GVHD among patients who have undergone UR-BMT. The Japan Marrow Donor Program (JMDP) was established in December 1991. We previously analysed the data of 1298 patients who underwent UR-BMT facilitated by the JMDP between 1993 and 1998 to identify the effect of HLA matching on acute GVHD, chronic GVHD, engraftment, survival and relapse (Morishima *et al*, 2002). In that study, HLA-A and/or HLA-B allele mismatch and patient age were found to be significant risk factors for the occurrence of chronic GVHD. The current study extended the analysis to include the data of 2937 patients who underwent UR-BMT facilitated by the JMDP between January 1993 and June 2004 and survived for at least 100 d post-transplant to clarify the incidence and risk factors for chronic GVHD, and the effect of chronic GVHD on survival and relapse in UR-BMT recipients.

Patients and methods

Patients and transplant procedure

Between January 1993 and June 2004, 2937 Japanese patients who underwent UR-BMT through the JMDP, engrafted and survived for at least 100 d after UR-BMT were included in this analysis. We excluded patients who survived <100 d after UR-BMT to exclude the effect of early mortality. Because peripheral blood stem cell harvest has not been performed through the JMDP, all transplants were BMTs. Baseline characteristics and follow-up data were obtained using standard report forms designed by the JMDP. Follow-up reports were submitted at 100 d, 1 year, and annually thereafter post-transplantation. A final clinical survey of these patients was performed on 1 November 2004. The median follow-up time was 822 d (range, 100–4129 d). Informed consent was obtained from the patients and donors according to the Declaration of Helsinki.

The characteristics of the patients and donors are summarised in Table I. The median age of the patients was 27 years and the median age of the donors was 33 years. As much as 59·7% of the patients and 59·5% of the donors were male. The number of patients with a haematological malignancy was 2667 (90·8%). Transplantation was performed according to the protocol of each centre, and therefore the conditioning regimen and GVHD prophylaxis varied among patients. A conditioning regimen containing anti-thymocyte globulin (ATG) was used in 203 patients (6·9%), and a conditioning regimen containing total body irradiation (TBI) was used in 2329 patients (79·3%). Only 14 patients (0·5%) received T cell-depleted marrow.

HLA matching and typing

According to the donor selection criteria of the JMDP, patients received marrow transplants from serologically HLA-A, -B and -DR antigen completely matched or serologically 1 antigen mismatched donors. Genomic typing of HLA-A, -B and -DR antigens was also performed. 68·5% of the donors were fully HLA-matched by both serological and genomic typing.

Statistical analysis

The incidence of chronic GVHD was the primary endpoint of our study. Diagnosis of chronic GVHD and its clinical grading were performed according to the standard criteria at each institution (Atkinson, 1990). Chronic GVHD was graded as limited (localised skin or single organ involvement) or extensive (generalised skin or multiple organ involvement). The cumulative incidence of chronic GVHD was calculated from the time of transplantation. To evaluate potential risk factors for developing chronic GVHD, the time-dependent

Table I. Characteristics of the patients who underwent UR-BMT and donors.

Number of patients	2937
Median age of patients, years (range)	27 (0–67)
Patient sex (male/female), *n*	1753/1184
Diagnosis, *n*	
Haematological malignancy	
AML	793
ALL	768
CML	604
MDS	285
NHL	168
Others	49
Non-malignant disease	
AA	191
Hereditary disorders	68
Conditioning, *n*	
ATG	203
TBI	2329
GVHD prophylaxis, *n*	
CsA + MTX	1545
FK506 + MTX	1118
Others	274
Median age of donors, years (range)	33 (20–52)
Donor sex (male/female), *n*	1748/1189
Sex (recipient/donor), *n*	
Male/male	1151
Male/female	602
Female/female	587
Female/male	597
HLA disparity, *n*	
Full match	2012
Class I one locus or one allele mismatch	286
Class II one locus or one allele mismatch	473
Others	166
Blood-type disparity, *n*	
Match	1535
Major mismatch	677
Minor mismatch	616
Major–minor mismatch	72
Bone marrow treatment, *n*	
No	1529
Yes	
Removal of red blood cells	764
Removal of plasma	750
T cell depletion	14
Time from diagnosis to BMT, months	
<13	1180
13–24	865
≥25	865
Median time from BMT to WBC = $1{\cdot}0 \times 10^9$/l, d (range)	17 (1–99)
Platelet count = 50×10^9/l by day 100 from BMT, *n*	
Yes	2714
No	223
Prior acute GVHD, *n*	
No	884
Grade I	915
Grade II	793
Grade III	281
Grade IV	64

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia, CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; AA aplastic anaemia; ATG, anti-thymocyte globulin; TBI, total body irradiation; GVHD, graft-*versus*-host disease; CsA, ciclosporin A; MTX, methotrexate; FK506, tacrolimus; HLA, human leucocyte antigen; BMT, bone marrow transplantation; WBC, white blood cell count.

Cox proportional hazard regression model was used for univariate and multivariate analyses (Cox, 1972). Factors with a *P*-value of 0·2 or less in the univariate analysis were included in the multivariable analysis. Factors that remained significant were retained in the final model.

Patients were also analysed for overall survival (OS) and relapse. To illustrate the effect of chronic GVHD on relapse and survival, semi-landmark plots were constructed (Baron *et al*, 2005). In patients who developed chronic GVHD, the post-transplant day of development of chronic GVHD was defined as the landmark day; in patients who did not develop chronic GVHD, post-transplant day 112, which was the median day of occurrence of chronic GVHD, was defined as the landmark day. OS was calculated from the landmark day to death from any cause or date of last contact. Relapse was defined on the basis of evidence of the respective malignancy and its cumulative incidence was plotted as a function of time since the landmark day.

Survival analyses were performed by the Kaplan–Meier method (Kaplan & Meier, 1958) and the log-rank test was used for univariate comparisons. The cumulative incidences of chronic GVHD and relapse were calculated using the Gray method, considering death without chronic GVHD or death without relapse, respectively, as the competing risk (Gray, 1988). For most of the statistical analyses, the Statistical Package for the Social Sciences (SPSS) software version 11 (SPSS Inc., Chicago, IL, USA) was used. Analyses of cumulative incidences were carried out with package 'cmprsk' of the R statistical software 2.1.0 (the R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org). All *P*-values were two-sided and differences were considered to be statistically significant when $P < 0{\cdot}05$. Differences with *P*-values > 0·10 are reported as not significant (NS), whereas differences with *P*-values between 0·05 and 0·1 are reported in detail.

Results

Incidence and severity of chronic GVHD

Among the 2937 patients, 1267 (43·1%) developed chronic GVHD, of whom 268 patients (21·2%) had *de novo* onset of

chronic GVHD. The median time to onset of chronic GVHD was 112 d following transplant. The 5-year cumulative incidence of chronic GVHD was 45·8%, and that of extensive chronic GVHD was 28·2% (Fig 1A). Fig 1B shows the cumulative incidences of chronic GVHD according to the primary diagnosis.

Risk factors for developing chronic GVHD

Multivariate analysis for risk factors for the development of chronic GVHD included the 2909 patients in whom data on the variables with $P \leq 0{\cdot}2$ in the univariate analysis were available (Table II). Recipient age ≥20 years, donor age ≥30 years, primary diagnosis of chronic myeloid leukaemia (CML), HLA-A or -B mismatch by serological or genomic typing, total body irradiation (TBI)-containing regimen, platelet count $<50 \times 10^9$/l by day 100, and prior acute GVHD remained in the optimal model on multivariate analysis and increased the risk of chronic GVHD significantly. Aplastic anaemia (AA) and hereditary disorders were significantly associated with a low incidence of chronic GVHD.

When the patients were divided by age decade, the incidence of chronic GVHD was significantly lower in recipient groups aged <10 years and 10–19 years; however, among recipients aged ≥20 years, there were no differences in the incidence of chronic GVHD (Fig 2). When the donors were divided by age decade, the cumulative incidence of chronic GVHD was significantly lower among patients transplanted from donors aged 20–29 years than among patients transplanted from donors aged ≥30 years ($P = 0{\cdot}005$, method of Gray). No differences in the incidence of chronic GVHD were found among patients transplanted from donors aged ≥30 years.

Prior acute GVHD was the strongest risk factor for chronic GVHD (Table II and Fig 1C). Among patients with no history of acute GVHD ($n = 870$), risk factors for chronic GVHD on multivariate analysis were recipient age ≥20 years {hazard ratio (HR) = 1·45 [95%confidence interval (95% CI), 1·06–1·98], $P = 0{\cdot}019$}, donor age ≥30 years [HR = 1·54 (95%CI, 1·19–2·00), $P = 0{\cdot}001$] and one locus mismatch or one allele mismatch at HLA-A/-B loci [*versus* full match, HR = 1·50 (95% CI, 1·02–2·20) $P = 0{\cdot}039$]. Among patients with a history of grade II–IV acute GVHD ($n = 1107$), platelet count $<50 \times 10^9$/l by day 100 [HR = 1·30 (95%CI, 1·00–1·67), $P = 0{\cdot}048$] was the only risk factor on multivariate analysis.

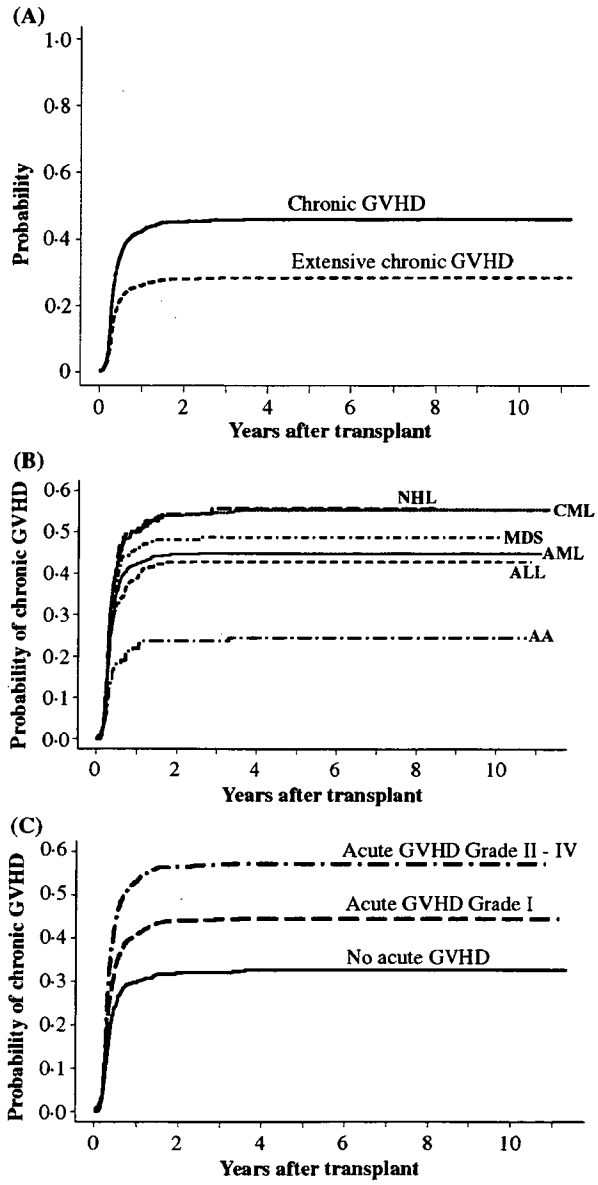

Fig 1. Cumulative incidence of chronic GVHD after UR-BMT. (A) Cumulative incidences of chronic GVHD (limited + extensive) and extensive chronic GVHD. The 5-year cumulative incidence of chronic GVHD was 45·8% (95% CI, 43·9–47·7%) and that of extensive chronic GVHD was 28·2% (95% CI, 26·5–29·9). Competing risks were death without chronic GVHD and death without chronic extensive GVHD (19·3% and 24·4%, respectively). (B) Cumulative incidences of chronic GVHD according to the primary diagnosis. The 5-year cumulative incidence and competing risk were 44·7% and 25·4% among patients with acute myeloid leukaemia (AML, solid line), 42·9% and 25·7% among patients with acute lymphoblastic leukaemia (ALL, dashed line), 49·0% and 16·1% among patients with myelodysplastic syndrome (MDS, dot-dash line), 55·3% and 12·8% among patients with chronic myeloid leukaemia (CML, dotted line), 55·7% and 11·5% among patients with non-Hodgkin lymphoma (NHL, long-dash line), and 24·4% and 6·1% among patients with aplastic anaemia (AA, dot-long dash line), respectively. (C) Cumulative incidences of chronic GVHD according to the severity of prior acute GVHD. The 5-year cumulative incidence was 32·4% among patients without a history of acute GVHD (solid line), 44·4% among patients with a history of grade I acute GVHD (dashed line), and 57·3% among patients with a history of grades II–IV acute GVHD (dot-dashed line). Competing risks were 20·0% without prior acute GVHD, 20·2% for grade I, and 17·9% for grades II–IV.

Table II. Univariate and multivariate analyses of risk factors for the development of chronic GVHD.

	Univariate analysis			Multivariate analysis (n = 2909)		
Factor	n	HR (95% CI)	P-value	n	HR (95% CI)	P-value
Recipient age						
0–19 years	972	1·0		961	1·0	
≥20 years	1965	1·41 (1·24–1·59)	<0·0001	1948	1·19 (1·04–1·36)	0·013
Recipient sex						
Female	1184	1·0				
Male	1753	1·11 (0·99–1·25)	0·07			NS
Donor age						
20–29 years	1007	1·0		994	1·0	
≥30	1930	1·28 (1·14–1·45)	<0·0001	1915	1·20 (1·07–1·36)	0·003
Sex matching						
Match	1738	1·0		1721	1·0	
Female to male	602	1·01 (0·87–1·16)	0·94	595	1·05 (0·91–1·22)	NS
Male to female	597	0·89 (0·77–1·03)	0·11	593	0·85 (0·74–0·99)	0·03
Diagnosis						
AML	793	1·0		787	1·0	
ALL	768	0·92 (0·79–1·08)	0·31	764	0·89 (0·76–1·04)	NS
MDS	285	1·13 (0·92–1·38)	0·25	283	1·11 (0·90–1·36)	NS
CML	604	1·27 (1·09–1·48)	0·002	602	1·19 (1·02–1·39)	0·03
NHL	168	1·32 (1·04–1·67)	0·02	166	1·18 (0·93–1·50)	NS
AA	191	0·43 (0·32–0·60)	<0·0001	190	0·51 (0·37–0·71)	0·0001
Other haematological malignancies	49	1·05 (0·67–1·65)	0·83	49	0·94 (0·60–1·48)	NS
Hereditary disorders	68	0·47 (0·29–0·77)	0·003	68	0·56 (0·34–0·93)	0·02
Time from diagnosis to BMT						
<13 months	1180	1·0				
13–24 months	865	1·05 (0·92–1·20)	0·45			
≥25 months	865	0·98 (0·85–1·12)	0·71			
Blood type disparity						
Match	1535	1·0				
Major mismatch	677	1·03 (0·89–1·18)	0·73			
Minor mismatch	616	1·08 (0·94–1·24)	0·30			
Major minor mismatch	72	1·12 (0·79–1·58)	0·54			
HLA disparity						
Full match	2012	1·0		1991	1·0	
Class I one mismatch	286	1·26 (1·05–1·51)	0·01	285	1·26 (1·05–1·52)	0·01
Class II one mismatch	473	1·03 (0·88–1·20)	0·73	468	0·90 (0·77–1·05)	NS
≥2 mismatches	166	1·31 (1·05–1·64)	0·02	165	1·14 (0·91–1·43)	NS
Preparative regimen TBI for conditioning						
Non-TBI regimen	608	1·0		601	1·0	
TBI-based regimen	2329	1·23 (1·06–1·42)	0·005	2308	1·16 (1·00–1·35)	0·04
ATG for conditioning						
No	2718	1·0				
Yes	203	0·58 (0·44–0·75)	0·0001			NS
GVHD prophylaxis						
CsA + MTX	1545	1·0				
FK506 + MTX	1118	1·00 (0·88–1·19)	0·93			
Treatment of bone marrow						
No	1529	1·0				
Yes	1384	1·06 (0·95–1·19)	0·29			
Platelet recovery (50×10^9/l or more by 100 d from BMT)						
Yes	2714	1·0		2688	1·0	
No	223	1·33 (1·10–1·61)	0·003	221	1·34 (1·10–1·63)	0·004

Table II. *Continued*

Factor	Univariate analysis			Multivariate analysis (n = 2909)		
	n	HR (95% CI)	P-value	n	HR (95% CI)	P-value
Days from BMT to WBC recovery						
<Day 18	1622	1·0				
≥Day18	1314	0·90 (0·80–1·00)	0·049			NS
Prior acute GVHD						
No	884	1·0		869	1·0	
Grade I	915	1·54 (1·31–1·80)	<0·0001	911	1·47 (1·25–1·72)	<0·0001
Grade II–IV	1138	2·28 (1·98–2·64)	<0·0001	1129	2·08 (1·80–2·42)	<0·0001

CI, confidence interval; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; AA aplastic anaemia; ATG, antithymocyte globulin; TBI, total body irradiation; GVHD, graft-*versus*-host disease; CsA, ciclosporin A; MTX, methotrexate; FK506, tacrolimus; HLA, human leucocyte antigen; BMT, bone marrow transplantation; WBC recovery, the first of three consecutive days with a persistent white blood cell count >1·0 × 10^9/l; HR, hazard ratio.

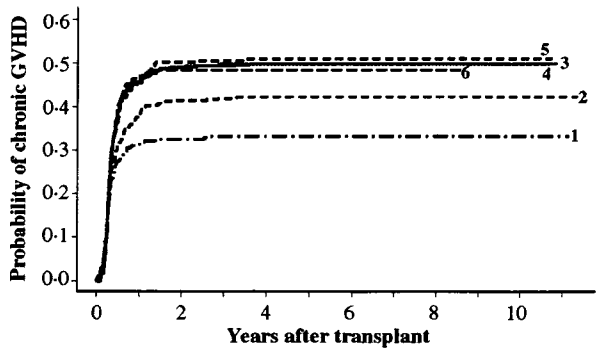

Fig 2. Cumulative incidence of chronic GVHD according to recipient's age decade. The competing risk was death without chronic GVHD. The 5-year cumulative incidence and competing risk were: 32·9% and 14·1% among patients aged 0–9 years (line 1; dot-dash line), 42·1% and 18·8% among those aged 10–19 years (line 2; dash line), 49·1% and 15·1% among those aged 20–29 years (line 3; solid line), 49·4% and 23·1% among those aged 30–39 years (line 4; dotted line), 51·0% and 23·2% among those aged 40–49 years (line 5; dash line), and 48·3% and 25·6% among those aged >50 years (line 6; long-dash line), respectively.

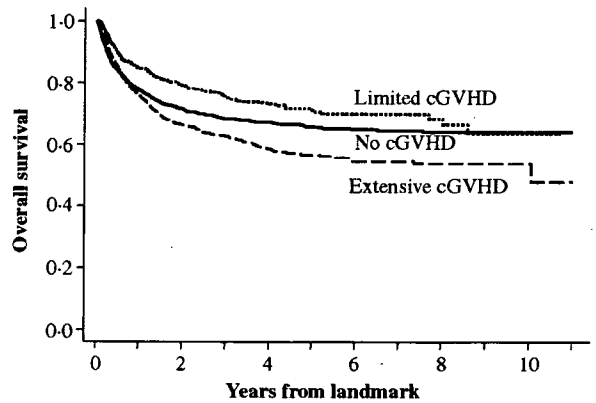

Fig 3. Overall survival according to chronic GVHD grading. The OS of all patients who survived beyond 100 d post-transplant according to chronic GVHD grade (n = 2877), is shown. The 5-year OS rate was 71·1% (95% CI, 66·4–75·8) among those with limited chronic GVHD (n = 489), 56·4% (95% CI, 52·3–60·5) among those with extensive chronic GVHD (n = 771), and 65·9% (95% CI, 63·2–68·5) among patients who did not develop chronic GVHD (n = 1617). The landmark day was the day of onset of chronic GVHD for patients with chronic GVHD, and it was day 112 from transplant, which was the median day of the onset of chronic GVHD, for patients without chronic GVHD. No chronic GVHD, solid line; limited chronic GVHD, dotted line; extensive chronic GVHD, dashed line.

Influence of chronic GVHD on OS and relapse

We analysed how chronic GVHD affects the prognosis after UR-BMT among 2877 patients (Fig 3). Patients with limited chronic GVHD had significantly better prognosis than patients with extensive chronic GVHD (log-rank test, P < 0·0001) or patients without GVHD (P = 0·009), whereas patients with extensive chronic GVHD had significantly poorer prognosis (*versus* without chronic GVHD, P = 0·003). The same tendencies were observed among 2609 patients with a haematological malignancy. On multivariate analysis using the Cox proportional hazard model with chronic GVHD as a time-dependent covariate, patients with extensive chronic GVHD had significantly increased mortality and patients with limited chronic GVHD had a survival advantage compared with those without chronic GVHD (Table III). However, patients with chronic GVHD had a lower cumulative incidence of relapse than patients without chronic GVHD (*versus* limited chronic GVHD, P = 0·049; *versus* extensive chronic GVHD, P = 0·009). There was no difference in relapse rate between patients with limited chronic GVHD and those with extensive chronic GVHD. The 5-year probability of relapse was 15·8% (95% CI, 12·1–19·5) among patients with limited chronic GVHD, 15·3% (95% CI, 12·3–18·4) among patients with extensive chronic GVHD, and 21·0% (95% CI, 18·5–23·6) among patients without chronic GVHD.

Table III. Multivariate analysis of prognostic factors in patients with haematological malignancies.

Factor	HR (95% CI)	*P*-value
Recipient age		
≥20 years	1·54 (1·30–1·83)	<0·0001
Donor age		
≥40 years	1·18 (1·18–1·38)	0·04
Diagnosis		
CML (*versus* AML)	0·67 (0·55–0·82)	0·0001
HLA disparity		
Class I one mismatch (*versus* full-match)	1·58 (1·27–1·97)	0·0001
≥2 mismatches (*versus* full-match)	1·52 (1·14–2·01)	0·0038
Platelet recovery (≥50 × 10^9/l by day 100 from BMT)		
No	1·58 (1·24–2·01)	0·0002
Prior acute GVHD		
Grade II–IV (*versus* No prior acute GVHD)	1·60 (1·31–1·95)	<0·0001
Relapse		
Yes	11·62 (10·06–13·41)	<0·0001
Secondary malignancies		
Yes	6·23 (3·28–11·83)	<0·0001
Chronic GVHD		
Limited (*versus* No)	0·67 (0·54–0·83)	0·0003
Extensive (*versus* No)	1·21 (1·03–1·43)	0·02

CI, confidence interval; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; HR, hazard ratio.

Discussion

In the present study, the 5-year cumulative incidence of chronic GVHD was 45·8% and that of extensive chronic GVHD was 28·2%. These cumulative incidences, especially the cumulative incidence of extensive chronic GVHD, are slightly lower than those of the data of the National Marrow Donor Program (NMDP) (Kollman *et al*, 2001) and other previous reports (Sullivan, 1999). Notably, nearly 100% of the recipient and donor pairs in the present study were composed of a single ethnic population of Japanese people. Recently, Oh *et al* (2005) reported that Japanese and Scandinavian people had significantly lower incidences of acute GVHD than American and Irish people in HLA-identical sibling BMT. Because Japanese people have been geographically isolated for a long period of time historically, Japanese people are genetically more similar than people of the USA. or Western countries and it is unclear whether our results apply to other more diverse genetic groups.

Our previous study revealed two significant risk factors for chronic GVHD by multivariate analysis: HLA-A/-B allele mismatch and patient age (Morishima *et al*, 2002). In the current extended analysis, seven risk factors were found to be significant for the development of chronic GVHD on multivariate analysis.

Zecca *et al* (2002) reported that the incidence of chronic GVHD in children after HSCT was 27%, which was assumed to be lower than that in adult recipients. In the current analysis, the incidence of chronic GVHD among patients <20 years of age was significantly lower than that among patients over 20 years of age. However, there was no significant difference in the incidence of chronic GVHD when adult patients over 20 years of age were grouped by age decade, although the OS rate was significantly lower in older adults than in younger adults, probably because of an increased incidence of death from other causes rather than chronic GVHD.

Donor age ≥30 years was a significant risk factor for the development of chronic GVHD and it also tended to decrease the survival rate. Kollman *et al* (2001) also reported that younger donor was a significant predictor of lack of development of chronic GVHD. Although the reason for this is not well understood, our findings suggest that donors of younger age may be preferable when selecting from comparably HLA-matched volunteer donors.

In our previous study (Morishima *et al*, 2002), HLA-C allele mismatch also tended to increase the incidence of chronic GVHD, while HLA-DR/-DQ mismatch showed no effect. Petersdorf *et al* (2004) showed that a single HLA-C mismatch conferred increased risk of mortality compared with matches. Greinix *et al* (2005) also showed that HLA class I mismatch, as detected by high-resolution typing, had a significant impact on the development of chronic GVHD and survival of UR-BMT recipients. The present study returned the same result as that in the previous report, although the effect of HLA-C was not analysed.

Previous analysis of risk factors for chronic GVHD after HLA-identical sibling BMT (Atkinson *et al*, 1990) revealed that the strongest risk factor for chronic GVHD was the existence of prior acute GVHD. In that report, several risk factors including recipient age >20 years predicted a higher risk of chronic GVHD in patients with a history of grade I acute GVHD or without a history of acute GVHD; however, among patients with a history of moderate to severe acute GVHD, no other risk factor predicted the development of chronic GVHD. In our study, recipient age and donor age were important risk factors for *de novo* onset of chronic GVHD, whereas in patients with a history of moderate to severe acute GVHD, patient age and donor age were not risk factors for chronic GVHD. These results are similar to the results of the other report (Atkinson *et al*, 1990). Remberger *et al* (2002) revealed that CML was a risk factor for chronic GVHD. We also identified that the incidence of chronic GVHD among patients with CML was significantly higher than that among patients with acute myeloid leukaemia (AML).

Whether the primary disease was a haematological malignancy or not significantly affected the development of chronic GVHD. In our previous study, among patients with AA who underwent UR-BMT, the incidence of chronic GVHD was

30% (Kojima *et al*, 2002), and it was 24·4% in the present extended analysis. Moreover, we found that the incidence of chronic GVHD among patients with hereditary disorders was significantly low in multivariate analysis. This finding might be due to the difference in treatment strategies for patients with haematological malignancy and those with AA. Immunosuppressive agents might be stopped or decreased earlier in patients with haematological malignancy than in those with non-malignant disease in order to induce the GVL effect.

Limited chronic GVHD had a significant impact on increasing patient survival, whereas patients with extensive chronic GVHD had a poor prognosis. In patients with a haematological malignancy, we found no significant difference in relapse rates between patients with limited chronic GVHD and those with extensive chronic GVHD, indicating that extensive chronic GVHD does not provide a strong GVL effect compared with limited chronic GVHD.

We used the grading system of limited and extensive chronic GVHD, which was originally proposed in 1980 based on the clinicopathological findings in 20 patients (Shulman *et al*, 1980). However, this grading system has several limitations. Akpek *et al* (2001, 2003) proposed a new prognostic model by analysing GVHD-specific survival and suggested that three factors, i.e. skin involvement, platelet count and progressive-type onset, significantly influence the survival of patients who developed chronic GVHD. However, a recent Japanese report showed that Japanese patients could not be accurately classified when these proposed prognostic models were used because the manifestation of chronic GVHD differed between Japanese and Western ethnic populations (Atsuta *et al*, 2006). We have started to collect more detailed information on Japanese patients with chronic GVHD, such as organ involvement, treatment strategy, and treatment outcome, to establish prognostic models.

In conclusion, this large-scale study demonstrated the incidence of chronic GVHD after UR-BMT in a single Japanese ethnic population and provides strong evidence for seven risk factors for chronic GVHD after UR-BMT. This study also suggests that limited chronic GVHD provides a survival benefit to patients with a haematological malignancy by reducing the risk of relapse without increasing the risk of death from chronic GVHD. Extended intervention and clinical trials are necessary to overcome extensive chronic GVHD.

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Appendix 1

The following centres participated in the bone marrow transplantations facilitated by the JMDP: Asahikawa Medical College Hospital, Asahikawa Red Cross Hospital, Sapporo Medical University Hospital, Sapporo Hokuyu Hospital, Hokkaido University Hospital, Asahikawa City Hospital, Hakodate City Hospital, Hirosaki University Hospital, Aomori Prefectural Central Hospital, Akita University Hospital, Iwate Medical University Hospital, Miyagi Cancer Centre, Tohoku University Hospital, Yamagata University Hospital, Fukushima Medical University Hospital, Ibaraki Children's Hospital, Tsukuba University Hospital, Tsuchiura Kyodo General Hospital, Jichi Medical School Hospital, Dokkyo Medical University Hospital, Saiseikai Maebashi Hospital, Gunma University Hospital, Saitama Medical University Hospital, Saitama Cancer Centre Hospital, Saitama Children's Medical Centre, Fukaya Red Cross Hospital, National Defense Medical College Hospital, Kameda General Hospital, Matsudo Municipal Hospital, Chiba Children's Hospital, Chiba Aoba Municipal Hospital, Chiba University Hospital, Jikei University Kashiwa Hospital, Keio University Hospital, Toranomon Hospital, National Cancer Centre Central Hospital, International Medical Centre of Japan, National Centre for Child Health and Development, Juntendo University Hospital, Showa University Hospital, Teikyo University Hospital, Tokyo Medical and Dental University Hospital, Tokyo Medical College Hospital, Jikei University Hospital, Tokyo Women's Medical University Hospital, Research Hospital of the Institute of Medical Science-the University of Tokyo, The University of Tokyo Hospital, Tokyo Metropolitan Komagome Hospital, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo Metropolitan Hospital of Fuchu, Toho University Omori Medical Centre, National Hospital Organisation Tokyo Medical Centre, Nippon Medical School Hospital, Japanese Red Cross Medical Centre, Nihon University Itabashi Hospital, Yokohama City University Medical Centre, Yokohama City University Hospital, Kanagawa Cancer Centre, Kanagawa Children's Medical Centre, St. Marianna University School of Medicine Hospital, Tokai University Hospital, Niigata University Medical & Dental Hospital, Nagaoka Red Cross Hospital, Niigata Cancer Centre Hospital, University of Yamanashi Hospital, Saku Central Hospital, Shinshu University Hospital, Nagano Children's Hospital, Nagano Red Cross Hospital, Toyama Prefectural Central Hospital, Kanazawa Medical University Hospital, Kanazawa University Hospital, Ishikawa Prefectual Central Hospital, University of Fukui Hospital, Hamamatsu Medical Centre, Seirei Hamamatsu General Hospital, Shizuoka Children's Hospital, Shizuoka General Hospital, Shizuoka Red Cross Hospital, Hamamatsu University School of Medicine Hospital, Aichi Medical School Hospital, Aichi Cancer Centre Hospital, Anjo Kousei Hospital, Showa Hospital, National Hospital Organisation Nagoya Medical Centre, Fujita Health University Hospital, Nagoya City University Hospital, Nagoya University Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Nagoya Ekisaikai Hospital, Meitetsu Hospital, Mie University Hospital, Yamada Red Cross Hospital, Suzuka Kaisei Hospital, Suzuka General Hospital, Shiga University of Medical Science Hospital, Kyoto Katsura Hospital, Kyoto City Hospital, Kyoto University Hospital, Kyoto First Red Cross Hospital, Kyoto Prefectural University of Medicine Hospital, Social Insurance Kyoto Hospital, Rinku General Medical Centre, Kansai Medical University Hospital, Kinki University Hospital, Matsushita Memorial Hospital, Osaka Medical College Hospital, Osaka City University Hospital, Osaka Red Cross Hospital, Osaka University Hospital, Osaka Medical Centre for Cancer and Cardiovascular Diseases, Osaka Medical Centre and Research Institute for Maternal and Child Health, Kobe City General Hospital, Kobe University Hospital, Hyogo College of Medicine Hospital, Hyogo Children's Hospital, Hyogo Medical Centre for Adults, Tenri Hospital, Nara Medical University Hospital, Wakayama Medical University Hospital, Tottori Prefectural Central Hospital, Tottori University Hospital, Shimane Prefectural Central Hospital, Okayama University Hospital, National Hospital Organisation Okayama Medical Centre, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima University Hospital, National Hospital Organisation Kure Medical Centre, Kurashiki Central Hospital, Yamaguchi University Hospital, Tokushima University Hospital, Kagawa University Hospital, Ehime Prefectural Central Hospital, Ehime University Hospital, Matsuyama Red Cross Hospital, Kochi Medical School Hospital, Kurume University Hospital, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Centre, University of Occupational and Environmental Health Hospital, Kokura Memorial Hospital, St Mary's Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, National Hospital Organisation Kumamoto Medical Centre, Oita Prefectural Hospital, Oita University Hospital, Miyazaki Prefectural Hospital, Imamura Hospital, and Kagoshima University Hospital.

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

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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.

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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