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Reduced-intensity stem cell transplantation from an HLA-identical sibling donor in patients with myeloid malignancies

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Summary:

The purpose of this study was to evaluate the feasibility and efficacy of allogeneic hematopoietic stem cell transplantation with a reduced-intensity regimen (RIST) in patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). In all, 36 patients (median age 55 years) underwent RIST from an HLA-matched related donor between September 1999 and December 2002. The diagnoses included AML ($n=14$), leukemia evolving from MDS ($n=10$), and MDS (refractory anemia with excess blasts $n=6$, refractory anemia $n=6$). The RIST regimen consisted of purine analog (cladribine or fludarabine)/busulfan, with or without antithymocyte globulin. The regimen was well tolerated, and 34 patients achieved durable engraftment and most achieved remission after RIST. A total of 17 patients developed grade II–IV acute GVHD, and 27 developed chronic GVHD. Eight patients relapsed, and five of them received antithymocyte globulin (ATG) as part of the preparative regimen. A total of 12 patients died (four disease progression, six transplantation-related complications, and two others). Estimated 1-year disease-free survival (DFS) in low- and high-risk groups was 85 and 64%, respectively. We conclude that RIST can be performed safely in elderly patients with myeloid malignancies, and has therapeutic potential for those who fail conventional chemotherapy. In view of the significant association between GVHD or ATG and DFS, defined management of GVHD following RIST should become a major target of clinical research.

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Despite progress in the therapy of supportive care for acute myeloid leukemia (AML) over the past several decades, most patients relapse within the first 2 years of achieving complete remission (CR). Moreover, for the approximately 33% of patients who achieve a second remission, the median survival is only 18 weeks.[1] The median age of patients with AML is approximately 65 years, making it difficult to apply intensive chemotherapy to improve clinical outcome.

The natural history of myelodysplastic syndrome (MDS) ranges from a chronic course that may span years to very rapid leukemia progression. The morphological subtypes of MDS can generally be categorized into two risk groups based on outcomes.[2] The low-risk group includes refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS). The clinical course of low-risk MDS is usually protracted, with a median survival in excess of 60 months.[3] On the other hand, the high-risk group includes refractory anemia with excess blasts (RAEB-1 and RAEB-2)[4] and refractory cytopenia with multilineage dysplasia (RCMD), with or without ringed sideroblasts. The median survival is 33 months for RCMD, 18 months for RAEB-1, and 10 months for RAEB-2.[3] Few patients with high-risk MDS survive with cytotoxic chemotherapy alone.[5]

Clinical and laboratory evidence has been reported regarding a graft-versus-leukemia (GVL) effect following allogeneic hematopoietic stem cell transplantation (allo-HSCT),[6,7] which is now regarded as the treatment of choice for patients with advanced AML and MDS.[2] A small but significant proportion of patients with advanced AML or MDS can be cured with allo-HSCT.[8,9] Allo-HSCT should be offered to those who have an HLA-identical donor. However, the substantial toxicity and treatment-related mortality associated with a conventional transplantation regimen limit its application to young patients, and the feasibility of allo-HSCT has not been fully established in elderly patients with AML and MDS.

Recently, a new strategy for transplantation using a reduced-intensity (RIST) or nonmyeloablative preparative regimen has been developed to reduce regimen-related toxicity (RRT) while preserving an adequate antitumor effect associated with allo-HSCT.[10,11] Although preliminary

data appear to be attractive, suitable widely acceptable regimens and indications for this strategy remain to be established. In particular, little information is available concerning the use of RIST for the treatment of advanced myeloid malignancies.

Giralt *et al* reported the results of two feasibility studies on RIST for myeloid malignancies.[12,13] In the first study,[12] they treated 31 patients with AML and four patients with MDS. CR was achieved in 24 patients, with a 1-year overall survival (OS) of 47% and disease-free survival (DFS) of 34%. In the second study,[13] they treated 34 patients with AML and nine patients with MDS. Of the 43 patients with AML/MDS, 26 achieved CR. The 1-year OS and DFS were 39 and 35%, respectively. These pilot studies suggest that long-term disease control can be achieved by RIST in patients with advanced myeloid malignancies who are not candidates for conventional HSCT.

In September 1999, we initiated a feasibility study of RIST using a cladribine-based preparative regimen.[14] Cladribine was replaced by fludarabine in November 2000, when the former drug was no longer available because of the limitations in insurance coverage in Japan. This report summarizes our findings regarding the safety and efficacy of RIST for myeloid malignancies.

Patients and methods

Patient characteristics

From September 1999 to December 2002, 36 consecutive patients with AML or MDS underwent RIST from an HLA-identical related donor (34 siblings, and two daughters). The median age was 55 years (range 27–67 years). These patients were considered to be ineligible for myeloablative HSCT because of their age (ie, older than 50; $n=34$), hepatic impairment ($n=2$), or cardiac dysfunction ($n=3$) including one aortic insufficiency due to Ehlers–Danlos syndrome. One patient with AML evolving from MDS had myeloablative HSCT before enrollment in this study. Diagnosis at transplantation included AML ($n=24$) and MDS ($n=12$). Detailed information on each patient is shown in Table 1. Patients were classified into two risk groups according to disease status. Those with AML in first remission or MDS RA were defined as low risk, and the others were defined as high risk. Of the 24 patients with AML, 14 and 10 patients had *de novo* AML and AML evolving from MDS, respectively. At the time of enrollment, six of the 24 patients with AML were in first remission, seven in more than second remission, and the remaining 11 were not in remission. Six patients were categorized in the low-risk group and 18 in the high-risk group.

According to the WHO classification,[4] 12 patients with MDS were classified as RA ($n=6$), RAEB-1 ($n=3$), and RAEB-2 ($n=3$). Four of the six patients with RA had failed to respond to immunosuppressive therapy including cyclosporine ($n=3$) and antithymocyte globulin (ATG; $n=2$) prior to transplantation. The other patients were treated with supportive therapy alone. Among the six patients with RAEB-1 or RAEB-2, three received cytarabine-based induction chemotherapy, and two of them achieved CR. The other three patients received RIST without receiving any cytotoxic chemotherapy prior to transplantation. Of the 12 patients with MDS, eight and four patients were categorized in the low- and high-risk group, respectively. Donor eligibility required an HLA-matched donor determined by serologic typing for HLA A, B, and DR. All patients and donors gave their written informed consent in accordance with the requirements of the Institutional Review Board of the National Cancer Center Hospital.

Preparative regimens

From September 1999 to October 2000, eight patients received a cladribine-based preparative regimen consisting of cladribine (Leustatin; Ortho Biotech, Raritan, NJ, USA) 0.11 mg/kg on days −10 to −4 and busulfan 4 mg/kg on days −6 and −5.[14] Seven of the eight patients also received rabbit ATG (Thymoglobulin; Imtix-Sangstat, Lyons, France) 2.5 mg/kg for 2 or 4 consecutive days. From November 2000 to December 2002, 28 patients received a fludarabine-based regimen consisting of fludarabine 30 mg/m^2 on days −8 to −3 and busulfan 4 mg/kg on days −6 and −5.[11] One of these 28 patients received additional ATG as described above.

Mobilization and collection of peripheral blood stem cells

Donors were given subcutaneous G-CSF at 400 μg/m^2 daily for 3 consecutive days. Leukapheresis was performed on the 4th day, and subsequent days if necessary, using conventional techniques for peripheral blood stem cells collection. Cells were cryopreserved with the traditional controlled-rate method of cryopreservation. Stored cells were thawed in a 37°C water bath, and infused through a central or peripheral vein, without washing. The number of CD34+ cells infused on day 0 ranged from 1.50 to 6.55×10^6 cells/kg (median 2.85×10^6 cells/kg).

GVHD

For GVHD prophylaxis, 30 patients received cyclosporine alone, 3 mg/kg, by continuous infusion starting on day −1. The other six patients received cyclosporine and short-term methotrexate at a dose of 10 mg/m^2 on day 1, and 7 mg/m^2 on days 3 and 6. Intravenous cyclosporine was switched to oral administration as soon as it became tolerable.

GVHD was diagnosed by clinical judgment as well as skin or digestive tract biopsies to support the clinical diagnosis. Acute and chronic GVHD were graded according to the consensus criteria.[15,16] When patients developed grade II–IV acute GVHD, our practice was to initiate 2 mg/kg/day methylprednisolone in addition to cyclosporine. Patients who survived 100 days or longer after transplantation were included in the analysis of chronic GVHD.

Supportive care

All of the patients were managed in reverse isolation in a laminar airflow-equipped room, and received routine antibacterial, antifungal, and antiviral prophylaxis, which

Table 1 Patient characteristics

Number	*Sex*	*Age*	*Diagnosis*	*Disease status*	*Chromosome*	*Blast in BM[a] (%)*	*Number of PB leukocytes[a] (/μl)*	*Blast in PB[a] (%)*	*Preparative regimen*	*Number of CD34-positive cells ($\times 10^{-6}$/kg)*	*GVHD prophylaxis*
(A) High-risk group											
1	M	49	MDS RAEB-1	Untreated	Not evaluable	Dry tap	1400	0.0	2CdA/BU/ATG	5.4	CSP + MTX
2	F	55	AML M4	Third remission	Not evaluable	<5.0%	3800	0.0	2CdA/BU/ATG	5.9	CSP + MTX
5	M	58	AML M4	Second remission	Normal	<5.0%	5000	0.0	2CdA/BU/ATG	2.0	CSP
7	M	53	AML M6	First relapse	46XY, t(9;13)	<5.0%	2900	0.0	2CdA/BU/ATG	1.6	CSP
8	M	56	MDS RAEB-2	Nonremission	+8	16.3	1500	0.0	2CdA/BU	2.8	CSP
9	M	55	MDS-AML	First relapse	−Y, der(1;7)	15.0	900	0.0	Flu/BU	2.5	CSP
10	M	65	MDS-AML	Second remission	t(2;3) + additional	<5.0%	1800	0.0	Flu/BU/ATG	2.1	CSP
12	F	45	AML M1	Second remission	Normal	<5.0%	3600	0.0	Flu/BU	6.6	CSP
13	F	51	MDS-AML	Refractory	ider(20), del(20)	41.7	400	4.0	Flu/BU	6.4	CSP
15	F	53	MDS-AML	Untreated	ins(1;7), +8, +5	81.7	900	40.0	Flu/BU	3.6	CSP
16	M	56	MDS-AML	Nonremission	der(1;7)	7.0	2500	1.0	Flu/BU	2.2	CSP
18	F	55	AML M2	First relapse	t(8;21)	86.0	3900	73.0	Flu/BU	3.7	CSP
19	M	65	AML M2	Second remission	Normal	<5.0%	3200	0.0	Flu/BU	4.2	CSP
21	M	67	AML M4	Second remission	Normal	<5.0%	5300	0.0	Flu/BU	1.9	CSP + MTX
22	M	60	AML M2	Second remission	inv(9)	<5.0%	4700	0.0	Flu/BU	2.7	CSP
23	M	57	AML M1	Second relapse	−Y	5.3	1800	0.0	Flu/BU	4.9	CSP
26	M	54	MDS RAEB-1	Untreated	add(6)(p21)	6.3	3700	1.0	Flu/Bu	2.5	CSP
27	M	54	MDS-AML	Refractory	del(9), +20	26.7	2100	77.0	Flu/Bu	1.5	CSP
29	F	55	AML M1	First relapse	NE	25.0	900	0.0	Flu/Bu	2.2	CSP
31	M	60	MDS-AML	Refractory	NE	34.0	2300	6.0	Flu/Bu	3.7	CSP
34	F	27	MDS-AML	Nonremission	Trisomy 8	7.0	2400	34.0	Flu/Bu	4.3	CSP
35	F	55	MDS RAEB-1	Refractory	Normal	6.5	1900	5.0	Flu/Bu	3.8	CSP
Median		55								3.2	
(B) Low-risk group											
3	M	57	MDS RA		Normal	<5.0%	1600	0.0	2CdA/BU/ATG	4.3	CSP
4	F	53	MDS-AML	First remission	Tetrasomy 8	<5.0%	2300	0.0	2CdA/BU/ATG	3.2	CSP
6	M	62	MDS RA		del(1)(p36)	<5.0%	4000	0.0	2CdA/BU/ATG	3.2	CSP
11	F	55	MDS RAEB-2	First remission	Normal	<5.0%	3100	0.0	Flu/BU	2.9	CSP
14	M	61	AML M0	First remission	−Y, del(17)	<5.0%	2300	0.0	Flu/BU	1.8	CSP
17	F	64	MDS RA		del(5)(q?), +21, del(13)(q?)	<5.0%	2600	0.0	Flu/BU	4.3	CSP
20	M	60	MDS RA		−7	<5.0%	1100	0.0	Flu/BU	4.1	CSP
24	F	59	AML M5	First remission	Normal	<5.0%	3400	0.0	Flu/BU	2.7	CSP
25	F	59	MDS-AML	First remission	+22, +8	<5.0%	500	0.0	Flu/Bu	2.1	CSP + MTX
28	M	52	AML M2	First remission	t(11;15)(p15, p15)	<5.0%	300	0.0	Flu/Bu	2.5	CSP + MTX
30	M	53	MDS RAEB-2	First remission	7 Monosomy, del(5q)	<5.0%	6000	0.0	Flu/Bu	1.7	CSP
32	F	45	MDS RA			<5.0%	900	0.0	Flu/Bu	4.4	CSP
33	F	52	AML M4	First remission	Normal	<5.0%	2600	0.0	Flu/Bu	2.7	CSP + MTX
36	M	59	MDS RA		Normal	<5.0%	2800	0.0	Flu/Bu	2.1	CSP
Median		58								2.8	

MDS = myelodysplastic syndromes; AML = acute myeloblastic leukemia; MDS-AML = leukemia evolving from MDS; 2CdA = cladribine; BU = busulfan; ATG = anti-thymocyte globulin; CSP = cyclosporin; MTX = methotrexate; BM = bone marrow; PB = peripheral blood; INT = intermediate.
[a]These counts were collected prior to preparative regimen.

included trimethoprim/sulfamethoxazole or pentamidine, fluoroquinolone, and fluconazole for the prophylaxis of pneumocystis carinii, bacterial, and fungal infection, respectively: five patients with prior histories of pulmonary aspergillus infections received amphotericin B. Prophylaxis of herpes virus infection using acyclovir has been reported previously.[17] Neutropenic fever was managed as reported previously.[18] All patients were monitored weekly by CMV pp65 antigenemia. If the antigenemia assays were positive, pre-emptive therapy with ganciclovir was initiated.[19]

Chimerism analysis

Chimerism of CD3-positive cells in peripheral blood was assessed every 30 days. Variable number of tandem repeats (VNTR) PCR was used to document engraftment or residual host-type cells, with residual host or donor cells detected at a sensitivity of 5%.[20] The values are given as a percentage of donor signals.

Engraftment

G-CSF was administered intravenously at 5 μg/kg/day from day 6 of transplant until the patient's absolute neutrophil count became at least 0.5×10^9/l for 2 consecutive days. Engraftment was defined as a white blood cell count (WBC) of $>1.0 \times 10^9$/l with an absolute neutrophil count (ANC) of $>0.5 \times 10^9$/l for 3 consecutive days, and platelet counts of $>20 \times 10^9$/l for 7 days without receiving transfusion. Graft rejection was defined as peripheral blood aplasia and marrow hypoplasia >21 days after transplantation, with no evidence of donor markers as revealed by cytogenetic and molecular techniques.

Regimen-related toxicities

All nonhematological organ dysfunctions from day 0 to day 28 were considered to be RRT, and were graded according to Bearman *et al*'s criteria.[21] Pulmonary dysfunction was additionally evaluated on day 100 after RIST. Transplantation-related mortality (TRM) was defined as death directly caused by the transplantation procedure.

End points and statistical analysis

The primary end points of this study included durable hematopoietic engraftment and TRM. Secondary end points included RRT, the incidence and severity of GVHD, DFS, and OS. Responses to transplantation were defined according to the recommendations of the international working group.[22] An analysis of acute GVHD was performed for patients who achieved initial engraftment. An analysis of chronic GVHD was performed for those who survived 100 days or longer. The probabilities of OS and DFS were calculated as a function of time using the Kaplan–Meier method from the date of stem cell infusion (day 0). DFS was also calculated from the day of transplantation because all of the recipients were in CR when they achieved engraftment. Disease was defined as the day of relapse or death. Surviving patients were censored on the last day of follow-up. These data were calculated as of April 20, 2003.

Results

Engraftment and chimerism

While one patient had full autologous reconstitution with blasts before achieving the primary endpoints, the other 35 patients achieved engraftment. The median time for ANC to reach 0.5×10^9/l was 13 days.[4–18] Platelet counts did not decrease below 20×10^9/l in one patient. In the remaining 34 patients, the median time for platelet recovery to increase above 20×10^9/l without platelets transfusion was 18 days.[10–24] One patient (patient 6) developed late graft failure due to septicemia, and finally died of multiple organ failure on day 78. Overall, graft failure developed in two of the 28 patients.

A total of 32 patients were evaluated with chimerism analysis using cytogenetic or molecular techniques. The median percentage of donor CD3-positive cells at the first examination (30 days after transplant) was 86% (range 40–100). In all, 15 patients achieved complete donor chimerism, and 17 patients had mixed chimerism at day 30. Of these 17 patients, 14 finally converted into complete chimerism by day 120 with a reduction in the dose of cyclosporine or observation. The other three patients showed prolonged mixed chimerism, one after late graft failure.

Regimen-related toxicities

RRTs are summarized in Table 2. The RIST regimens were tolerated well, and most toxicity was temporary and reversible. Liver toxicity was the most common complication following RIST. In all, 21 patients (58%) developed hepatic toxicity within 28 days of transplant. Neither grade 3–4 hepatic toxicity nor veno-occlusive disease of the liver was seen in any of the patients.

One patient developed congestive heart failure on day 14, corresponding to grade 3 cardiac toxicity (patient 6). Since this patient's cardiac function had been impaired before transplant, the relationship between the preparative regimen and development of heart failure was unclear. The patient developed grade 3 toxicities of the liver and the central nervous system, resulting in TRM on day 78.

Table 2 Regimen-related toxicity

	Grade			
	1	*2*	*3*	*4*
Mucosa	11	3	0	0
Liver	11	9	0	0
Lung	3	0	1	0
Kidney	10	1	0	0
Heart	4	1	1	0
CNS	0	0	1	0
Gastrointestinal	1	0	0	0
Bladder	0	0	0	0

The incidence of TRM within 100 days of transplant was 2.7%.

Infectious complications

Neutropenic fever requiring the empiric use of antibiotics occurred in 11 patients (30%). Bacterial bloodstream infection was documented in 10 patients (27%). Five patients developed bacteremia before and five after engraftment.

CMV antigenemia developed in 13 patients (36%). All of the patients responded to pre-emptive use of ganciclovir without developing symptomatic CMV disease.

Concerning fungal infection, two of the 36 evaluable patients developed invasive aspergillosis during the treatment of chronic GVHD, and one developed *Candida glabrata* infection. Six patients, who had had a history of invasive fungal infection, including invasive pulmonary aspergillosis ($n=5$) and Candida abscess, were enrolled in our study but fungal infection did not recur in any of these six patients.

GVHD

Of the 35 patients who achieved donor engraftment and survived more than 21 days, 17 (48%) developed grade II–IV acute GVHD at a median of day 48 (range 21–98). GVHD was rated as grade II in nine, grade III in seven, and grade IV in one. None of the eight patients who received ATG developed grade II–IV acute GVHD. In contrast, 17 of the 27 patients who did not receive ATG developed grade II–IV acute GVHD. This difference was statistically significant ($P<0.01$). A total of 11 patients with acute GVHD responded to the administration of methylprednisolone. GVHD was refractory to corticosteroid in four patients, of whom one was successfully treated with additional mycofenolate mofetil. Other died of liver acute GVHD complicated with pneumonia and sepsis.

Of the 33 evaluable patients who achieved durable engraftment and survived more than 100 days, 27 developed chronic GVHD. Limited and extensive forms were found in five and 22 patients, respectively. Extensive forms were preceded by acute GVHD in 15 patients. Respiratory disorder-associated chronic GVHD was observed in four patients (two bronchiolitis obliterans, two bronchiolitis obliterans with organizing pneumonia). Two died of colon GVHD complicated systemic infection.

Disease response and relapse

All of the 15 patients with AML who were in remission at transplantation had sustained durable remission, although three patients have relapsed. While one patient with refractory MDS-AML developed engraftment failure because leukemic cells could not be eradicated by the preparative regimen, 14 of the 15 patients with measurable disease achieved remission after RIST, of whom four relapsed. Five MDS-RA patients achieved durable engraftment and remission, and one presented late graft failure.

We treated 14 patients with low-risk myeloid malignancy with one developing disease progression after transplant (Table 3). In contrast, 21 high-risk patients engrafted and six relapsed at a median of 282 days (78–728) after transplant. The difference was not significant ($P=0.11$). The outcomes of patients with high-risk disease are shown in Table 4. All of the five patients who received an ATG-containing preparative regimen relapsed at a median of 359 days (78–728). The incidences of acute (grade II–IV) and chronic GVHD were 0% (0/5) and 50% (2/4), respectively. On the other hand, one of 16 patients who did not receive an ATG-containing regimen relapsed on day 205 after transplant. The incidences of acute (grade II–IV) and chronic GVHD were 62% (10/16) and 94% (15/16), respectively. Median follow-up in the ATG and non-ATG groups was 838 days (347–1271) and 361 days (108–901 days).

Clinical courses after relapse of high-risk group are shown in Table 4. Patient 1 responded to DLI. Patient 2 had achieved CR following re-induction chemotherapy, but

Table 3 Clinical outcomes of patients in the low-risk group

Pt. no.	*Acute GVHD*	*Chronic GVHD*	*Use of ATG as conditioning or GVHD treatment*	*Relapse*	*Current status, follow-up*	*Cause of death*
3	0	Limited	Yes		Alive in CR, 34 mo+	
4	0	0	Yes		Alive in CR, 33 mo+	
6	0	NE	Yes		Dead in NE, 2 mo	Sepsis, multiple organ failure
11	II-S,G	Limited	No		Dead in CR, 16 mo	Chronic GVHD (bronchitis obliterans)
14	III-S,G	Extensive	No		Alive in CR, 24 mo+	
17	0	Extensive	No	445	Alive on disease, 15 mo+	
20	II-S	0	No		Alive in CR, 14 mo+	
24	I-S	Extensive	No		Alive in CR, 12 mo+	
25	II-S	Extensive	No		Alive in CR, 11 mo+	
28	II-S	Extensive	No		Alive in CR, 10 mo+	
30	II-G	Extensive	No		Dead in CR, 5 mo	Hemorrhage associated with colon GVHD
32	I	Extensive	No		Alive in CR, 8 mo+	
33	0	Extensive	No		Alive in CR, 6 mo+	
36	III-G	0	No		Alive in CR, 4 mo+	

GVHD = graft-versus-host disease; NE = not evaluable; CR = complete remission; S = skin; G, gastrointestinal; L = liver; mo = months.
mo$^+$ = months at follow-up of live patients.

Table 4 Clinical outcomes of patients in the high-risk group

Pt. no.	*Acute GVHD*	*Chronic GVHD*	*Use of ATG as conditioning or GVHD treatment*	*Use of corticosteroid (duration)*	*Relapse*	*Treatment after relapse*	*Current status, follow-up*	*Remission duration from the second RIST or chemotherapy*	*Cause of death*
1	0	Limited	Yes	Seven course of steroid-pulse therapy for neuropathy	728	Chemotherapy followed by DLI	Alive in CR, 43 mo+	14 mo	
2	0	0	Yes	None	359	Chemotherapy followed by second RIST from an alternative family donor	Dead in CR, 26 mo	1 mo	Multiple organ failure after second RIST
5	0	0	Yes	None	136	Chemotherapy followed by second RIST from the same donor	Dead in CR, 28 mo	14 mo	Chronic GVHD Interstitial pneumonitis or bronchitis obliterans with organizing pneumonia
7	I-S	Extensive	Yes	None	524	Chemotherapy followed by second RIST from the same donor	Dead in CR, 29 mo	8 mo	Relapse after second RIST
8	II-S	Extensive	No	1 mg/kg for GVHD treatment (26 mo)			Alive in CR, 30mo+		
9	II-S,G	Extensive	No	1 mg/kg for GVHD treatment (5 mo)			Dead in CR, 12 mo		Diffuse alveolar hemorrhage following Pseudomonas pneumonia
10	NE	No data	Yes	None	78	Chemotherapy alone	Dead on disease, 11 mo		Disease progression
12	I-S	Extensive	No	0.5 mg/kg for GVHD treatment (6 mo)			Alive in CR, 27 mo+		
13	II-S	Extensive	No	None			Alive in CR, 25 mo+		
15	IV-S,L,G	Limited	No	1 mg/kg and steroid pulse for GVHD treatment (2 mo)			Dead in CR, 3 mo		MRSA septicemia associated with severe liver GVHD
16	III-S,G	Extensive	No	1 mg/kg for GVHD treatment (20 mo)			Dead in CR, 22 mo		Secondary metastatic adenocarcinoma
18	I-S	Limited	No	None	205	Chemotherapy followed by second RIST from the same donor	Alive in CR, 17 mo+	4 mo	
19	0	Extensive	No	mPSL (details unkown)			Alive in CR, 15 mo+		
21	0	Extensive	No	0.5 mg/kg for GVHD treatment (16 days)			Alive in CR, 12 mo+		
22	II-S	Extensive	No	2 mg/kg for pulmonary insufficiency (1.5 mo)			Dead in CR, 5 mo		Recurrence of interstitial pneumonia
23	III-S,G	Extensive	No	0.5 mg/kg for GVHD prophylaxis (17 days)			Alive in CR, 12 mo+		
26	0	Extensive	No	0.5 mg/kg for GVHD treatment (20 days)			Alive in CR, 11 mo+		
27	NE*	NE*	No	None	0	Observation	Alive on disease, 6 mo+		
29	III-S,G	Extensive	No	1 mg/kg for GVHD treatment (4 mo)			Alive in CR, 10 mo+		
31	III-S	Extensive	No	1 mg/kg for GVHD treatment (1 mo)			Alive in CR, 8 mo+		
34	III-S,G	Extensive	No	1 mg/kg for GVHD treatment (1 mo)			Dead in CR, 4 mo		Colon GVHD, multiple organ failure
35	0	0	No	None			Alive in CR, 4 mo+		

GVHD = graft-versus-host disease; NE = not evaluable; CR = complete remission; S = skin; G = gastrointestinal; L = liver; mo = months; mo+ = months at follow-up of live patients.
*Engraftment failure because of persistent blastic cells.

relapsed again. Therefore, she received a second RIST from an HLA one-antigen mismatched alternative family donor, but died of multiple organ failure 29 days after the second transplant. Two patients (patients 5 and 7) had sustained CR following a second RIST using the same donor. However, patient 5 died from complication associated with chronic GVHD. Patient 7 relapsed 8 months after second RIST and died. Patient 10 received chemotherapy alone and died from progressive disease. Patient 18 did not respond to chemotherapy, but cleared blasts from bone marrow following a second RIST with the same donor, although she developed graft failure thereafter. She received a third transplantation with cord blood stem cells. Although one patient (patient 2) received ATG because of HLA disparity, ATG was not included in the preparative regimens in the second or third transplantation in the remaining four patients. In the low-risk group, patient 17 with MDS RA developed RAEB after remission. She is alive with receiving supportive chemotherapies.

Disease-free and overall survival

As of April 20, 2003, 24 patients were alive for 4.4–43.5 months after transplantation, with a median follow-up of 12.4 months. A total of 12 patients died during the study period (patients 2, 5, 6, 7, 9, 10, 11, 15, 16, 22, 30, and 34). Four died of disease progression including multiple organ failure following the second RIST. Six patients died of complications including systemic infection, colon GVHD, and respiratory dysfunction associated with GVHD. One died of recurrence of interstitial pneumonia. The other died of secondary metastatic liver adenocarcinoma.

The Kaplan–Meier estimated 1-year OS in the low- and high-risk groups was 85% (95% CI 66–100%) and 80% (95% CI 62–98%), respectively (not significant; $P=0.98$) (Figure 1a). Estimated 1-year DFS was 85% (95% CI 66–100%) and 64% (95% CI 42–86%), respectively (Figure 1b). These differences were not significant ($P=0.59$).

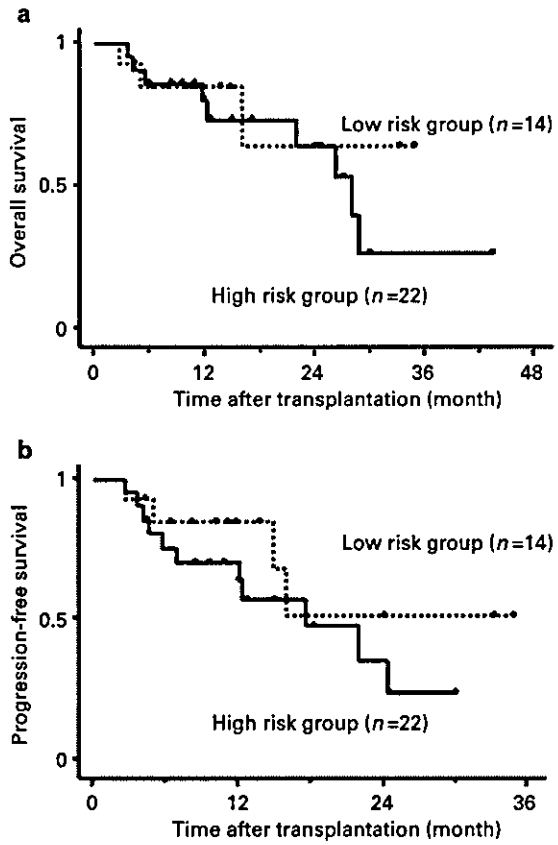

Figure 1 OS and DFS in the high- and low-risk groups. (**a**) Comparison of OS between low- ($n=14$) and high-risk patients ($n=22$). Estimated 1-year OS is 85% (95% CI 66–100%) and 80% (95% CI 62–98%) in the low- and high- risk groups, respectively ($P=0.98$). (**b**) Comparison of DFS between low- ($n=14$) and high-risk patients ($n=21$). Estimated 1-year DFS is 85% (95% CI 66–100%) and 64% (95% CI 42–86%) in the high- and low-risk groups, respectively ($P=0.59$).

Discussion

This is a small pilot study from which it is difficult to draw a definitive conclusion on the usefulness of RIST for myeloid malignancies. However, regarding the primary end point, we achieved complete chimerism without early transplant-related mortality in 29 of the 32 evaluated patients. Only one patient developed primary graft failure with persistent leukemic cells after the preparative regimen. Notably, serial chimerism analysis revealed that half the patients achieved complete chimerism without DLI by day 28 after transplantation and most achieved complete chimerism by day 90. Bornhauser *et al*[23] reported early induction of complete chimerism using a regimen consisting of fludarabine/busulfan, which is similar to ours. These findings were in contrast to those in previous studies with different combination regimens.[24]

When primary malignant diseases are refractory or beyond salvage therapy, it is widely believed that a nonmyeloablative preparative regimen is insufficient to establish stable engraftment. Giralt *et al* reported 35 patients with AML or MDS who were treated with purine analog, cytarabine, and idarubicin followed by allogeneic HSCT from an HLA-identical or one-antigen mismatched sibling donor. Two of the 23 evaluable patients failed to achieve initial engraftment, and two patients with >90% engraftment had late autologous reconstitution by 3 months without evidence of relapse. Four of the 35 patients were finally diagnosed with graft failure.[12] Childs *et al*[24] investigated the kinetics of donor chimerism after RIST using the fludarabine/cyclophosphamide regimen. Complete donor chimerism was achieved more slowly, and DLI was occasionally required in their study. Thus, our busulfan-containing reduced-intensity preparative regimen appears to be beneficial for achieving early and sustained engraftment.

In this study, the nonrelapse mortality rate within 100 days of transplantation was 2.7%, which was much lower than those reported in previous studies on RIST or conventional HSCT.[2,13] According to Bearman *et al*'s criteria,[21] only one patient developed grade III–IV regimen-related toxicities, which were difficult to distinguish

from the pre-transplant physical condition and a septicemia episode. Since the dose-limiting toxicity of busulfan in the setting of high-dose chemotherapy with HSCT is hepatic veno-occlusive disease,[25] hepatic dysfunction was frequently observed in this study. However, in this study, most of the hepatic damage was mild to moderate, and was rated as grade I ($n=11$) or grade II ($n=9$). None of the patients developed veno-occlusive disease of the liver during their clinical course. This study demonstrates that the combination of purine analog and 8 mg/kg busulfan is feasible for use in elderly patients or those with organ dysfunction.

Both fludarabine and cladribine have potent immunosuppressive and mild myeloablative effects.[26,27] The non-hematological toxicities of these agents are mild to moderate, while neurotoxicity sometimes develops at higher than the recommended dose.[28] However, Giralt *et al*[13] reported severe renal toxicity in patients receiving a cladribine-containing preparative regimen. In their study, fatal regimen-related toxicity, mostly renal toxicity, developed in four of eight patients, leading to the early closure of this treatment arm. They noted that the synergistic effects of melphalan and cladribine might cause toxic effects on the kidney. Based on their report,[13] we decreased the dose of 2-CdA to 0.11 mg/kg for 6 days, which was equal to the dose used in the single-agent treatment of lymphoid malignancies,[14] and approximately half of that used by Giralt *et al*.[13] In this study, seven of the eight patients who received a cladribine-based preparative regimen achieved sustained engraftment without developing grade III–IV regimen-related toxicities, although one patient with infectious complications developed late graft failure. Hence, this study suggests that a reduced dose of 2-CdA is sufficient for sustained engraftment, and may partly explain the lower toxicity than that reported by Giralt *et al*.[13]

The balance between GVHD and GVL is a significant issue in RIST. Since it has been hypothesized that the cytokine release caused by RRT enhances acute GVHD,[29] the use of a reduced-intensity preparative regimen may reduce the incidence of GVHD. However, we did not see any meaningful difference in the incidence of GVHD with a conventional transplant, within the context of RIST.[30–32] In this study, the incidences of grade II–IV acute and chronic GVHD were 43 and 82%, respectively. RIST is immunotherapy. It is difficult to separate GVL from GVHD. Martin *et al*[33] suggested the clinical grading of system of acute GVHD that represented a categorization of clinical management. Although some treatment protocols often specify 'grade II' GVHD as an indication for treatment, clinical management decisions actually entail evaluation of many risk factors in addition to overall severity. They suggested that treatment could be deferred even in patients with grade III GVHD, because GVHD was permissive and tolerable when risk factors were absent. Therefore, we often withheld the administration of steroids for GVHD if the patient was generally well. However, the GVHD-related mortality of 11% was lower than for CST, with the limited numbers of patients evaluated in this study. These findings suggest that GVHD is still a significant problem in RIST, and that RIST can exert a GVL effect as well as CST. Considering that augmentation of GVHD prophylaxis might hamper the GVL effect and that malignant cells cannot be eradicated by a reduced-intensity preparative regimen alone, we should be careful in the management of GVHD in RIST.

Until November 2000, 10 patients (five low risk, five high risk) had received ATG-containing regimens to harness stable engraftment.[14] Based on the report by Bornhauser *et al*[23] that ATG is not essential for engraftment in RIST, we excluded ATG from preparative regimens in December 2000. Since ATG has a long half-life in humans,[34] it can deplete both host-derived and donor-derived T lymphocytes, and inhibits not only graft rejection but also GVHD. As of December 2002, 26 patients (nine low risk, 17 high risk) had received a preparative regimen consisting of fludarabine and busulfan. Concerning the low-risk group, one of the 14 patients relapsed during the study periods with a median follow-up of 319 days (range 78–1010). Acute and chronic GVHD developed in six and eight patients, respectively. All five high-risk patients who had received ATG relapsed without developing grade II–IV acute GVHD. In contrast, nine and 15 of the 17 patients who had not been given ATG, with a median follow-up of 374 days (range 108–1271), developed grade II–IV acute and chronic GVHD, respectively. Only one patient relapsed.

The development of GVHD may not be associated with the control of low-risk myeloid malignancies. GVHD and infection, rather than relapse, are more important problems to be addressed in these patients. Indeed, our study showed that two in the low-risk group died of systemic complications associated with extensive GVHD. One developed lethal bronchiolitis obliterans as a manifestation of chronic GVHD. Adequate management for GVHD based on risk of primary disease may be required. Augmentation of GVHD prophylaxis such as with CAMPATH-1H,[35] ATG, mycofenolate mofetil,[36] tacrolimus,[37] and methotrexate[37] may be an option. We are now conducting a prospective randomized trial comparing cyclosporine alone with cyclosporine and short-term methotrexate as GVHD prophylaxis in RIST for low-risk hematological malignancies. Furthermore, it should be noted that none of the low-risk patients with a median age of 58 relapsed in this study. Since the genetic backgrounds of myeloid leukemia tended to be worse in elderly patients,[38] these findings support the feasibility of applying RIST in young patients with low-risk myeloid malignancies. It is possible that RIST will also be beneficial in young patients, since organ damage including infertility seems to be mild and less frequent in RIST than in CIST. A prospective randomized controlled trial comparing RIST with CST in young patients is warranted.

This study showed that some patients in the high-risk group can expect long-term survival following RIST, and that relapse-free survival is associated with the development of GVHD. However, it is difficult to differentiate a GVL effect from GVHD. The induction of GVHD through early tapering of cyclosporine may improve survival.[39,40] Severe, sometimes fatal, GVHD will be unavoidable in some patients. However, such a strategy will be justified considering the poor prognosis of elderly patients with chemorefractory myeloid malignancies.[41] In the future,

tumor vaccination against leukemia-specific antigen[42] or adoptive transfer of cytotoxic T cells against minor antigens[43] will be a choice for these patients. Some animal and human studies have shown a strong anti-leukemia effect without causing GVHD.[43]

In conclusion, although the number of patients is small and the follow-up is short, our results show that purine analog-based RIST can produce durable engraftment, lower TRM, and a high response rate in patients with advanced myeloid malignancies. These observations provide a rationale for continuing our clinical trials, which should be modified to focus more on minimizing toxicities, preserving a GVL effect, and establishing optimal strategies for GVHD prophylaxis according to the risk of the primary diseases.

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bjh research paper

Comparative analysis of clinical outcomes after allogeneic bone marrow transplantation *versus* peripheral blood stem cell transplantation from a related donor in Japanese patients

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Summary

A reduced incidence of graft *versus* host disease (GvHD) has been documented among Japanese allogeneic bone marrow transplantation (BMT) patients, as the Japanese are genetically more homogeneous than western populations. To clarify whether this ethnic difference affects the results of allogeneic peripheral blood stem cell transplantation (PBSCT), we conducted a nationwide survey to compare clinical outcomes of allogeneic PBSCT ($n = 214$) and BMT ($n = 295$) from a human leucocyte antigen-identical-related donor in Japanese patients. The cumulative incidence of grades II–IV acute GvHD was 37·4% for PBSCT and 32·0% for BMT. The cumulative incidence of extensive chronic GvHD at 1 year was significantly higher after PBSCT than BMT (42% vs. 27%; $P < 0·01$). The organ involvement patterns of GvHD were different between the two groups. By multivariate analyses, the incidence of chronic GvHD was significantly increased in PBSCT, whereas the stem cell source did not affect the incidence of acute GvHD, transplant-related mortality, relapse or survival. We concluded that Japanese PBSCT patients have an increased risk of chronic GvHD compared with BMT patients, but the incidence of acute GvHD was still lower than in western populations. Thus, the choice of haematopoietic stem cell source should be considered based on data for individual ethnic populations.

Keywords: Japanese, marrow transplantation, stem cell transplantation, graft *versus* host disease.

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During the past decade, peripheral blood stem cell transplantation (PBSCT) has been explored in the autologous as well as the allogeneic haematopoietic stem cell transplantation (HSCT) setting as an alternative to bone marrow transplantation (BMT). Although there were some inconsistencies in the early reports, it appears that haematological recovery is faster, but the incidence of acute graft *versus* host disease (GvHD) is similar, and chronic GvHD is more frequent in allogeneic PBSCT patients than in BMT patients (Schmitz *et al*, 1998, 2002; Blaise *et al*, 2000; Champlin *et al*, 2000; Heldal *et al*, 2000; Powles *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001; Couban *et al*, 2002; Ringden *et al*, 2002). Additionally, some investigators have reported improved survival after PBSCT compared with BMT (Powles *et al*, 2000; Bensinger *et al*, 2001; Couban *et al*, 2002).

Although a number of small prospective randomized-controlled trials (RCTs) have been published, cautious interpretation is required because the primary end points of these studies were safety (Schmitz *et al*, 1998), engraftment (Blaise *et al*, 2000; Heldal *et al*, 2000; Powles *et al*, 2000) and equivalency of acute GvHD (Bensinger *et al*, 2001). Because of the small sample size in these studies, the statistical power was not enough to detect differences in important, clinically relevant outcomes between PBSCT and BMT, such as chronic GvHD, relapse rate, transplant-related mortality (TRM) and survival. In an attempt to clarify this, several large RCTs and meta-analyses have recently been published (Cutler *et al*, 2001; Couban *et al*, 2002; Schmitz *et al*, 2002; Horan *et al*, 2003).

However, findings in western populations cannot be directly transferred to other ethnic populations, where the incidence of GvHD differs. Most previous studies that compared BMT and PBSCT were from western countries. While detailed information on the ethnics of the study population was not provided, most patients would have been Caucasian. In Japanese BMT patients, the incidence of acute GvHD is considered to be lower than in western countries because of the relative genetic homogeneity of the population (Morishima *et al*, 1989; Oh *et al*, 2002; Lin *et al*, 2003). Whether this ethnic difference also affects the results of PBSCT, as reflected in differences in the incidence of GvHD, relapse and survival, has not been established. Apart from the intense eradication of malignant cells by the conditioning regimen, the main therapeutic benefit of allogeneic HSCT relies on the induction of immune-mediated graft *versus* leukaemia (GVL) effect (Horowitz *et al*, 1990). This GVL effect may also have a different impact in different ethnic groups. Therefore, to survey outcomes after allogeneic HSCT in Japan, we conducted a retrospective, multi-centre study comparing allogeneic PBSCT with BMT from a human leucocyte antigen (HLA)-identical related donor in 509 patients with leukaemia or myelodysplastic syndrome (MDS). We also aimed to determine the impact of GvHD on relapse and survival after transplantation.

Patients and methods

Methods

Transplantation centres across Japan were contacted and asked to provide data on all consecutive allogeneic HSCT from a family donor using report forms with specific addenda. Recipients of T-cell-depleted blood stem cell transplants, those receiving reduced-intensity stem cell transplantation, and those who had received bone marrow together with PBSC were not reported. Between January 1999 and October 2001, a total of 629 adult patients with leukaemia or MDS received a myeloablative preparative regimen and allogeneic BMT or PBSCT from an HLA-identical-related donor (matched at HLA-A, -B, -DR by serological or molecular testing) in 82 participating centres (Appendix A). Patients who did not receive GvHD prophylaxis using ciclosporin A (CsA) and methotrexate (MTX) ($n = 41$), those who did not receive granulocyte colony-stimulating factor (G-CSF) postallograft ($n = 75$), those who had undergone autografting previously ($n = 3$) and those who had double cancer ($n = 3$) were excluded. Finally, a total of 509 patients were included in this analysis. The stem cell source was decided according to the protocol of each transplantation centre. The medical records were reviewed retrospectively for patients' demographic data, date of engraftment, onset of acute and chronic GvHD, grading and organ involvement from the date of transplantation to the date of death or last contact. Computerized error checks and physician review of submitted data were performed to ensure data quality.

End point definitions

End points were assessed on the date of last patient contact and were analysed as of 31 May 2002. The study focused on haematopoietic recovery, acute and chronic GvHD, target organs of GvHD, TRM, progression-free survival (PFS) and overall survival (OS) after PBSCT compared with BMT. The day of neutrophil engraftment was defined as the first of three consecutive days on which the patient's absolute neutrophil count was above $0{\cdot}5 \times 10^9$/l. 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 without platelet transfusion. Engraftment failure was diagnosed as when engraftment was not achieved at any time after transplantation. The diagnosis of GvHD was based on clinical evidence with histological confirmation whenever possible. Acute GvHD within the first 100 d after transplantation was graded according to standard criteria by attending physicians of each hospital (Przepiorka *et al*, 1995). Patients who survived at least 100 d without relapse or disease progression, with sustained donor engraftment, were evaluated for chronic GvHD. Chronic GvHD was graded as limited (localized skin or single organ involvement) or clinically extensive (Shulman *et al*, 1980).

Patients without GvHD were censored at the time of relapse, disease progression, death or last follow-up. GvHD after donor leucocyte infusion was not included in this analysis.

Standard risk diseases were defined as acute myeloid leukaemia (AML) or acute lymphoblastic leukaemia (ALL) in first remission; chronic myeloid leukaemia (CML) in chronic phase; and refractory anaemia without excess of blasts (Bensinger *et al*, 2001). All other stages of these diseases and all other types of leukaemia were considered as high risk. The Eastern Cooperative Oncology Group (ECOG) scale was used to evaluate performance status (PS) at the time of transplantation. PFS was measured as the time from the day of transplantation until disease relapse or progression, death from any cause or second transplantation for graft failure or rejection. Both relapse and progression were defined as disease progression with TRM being censored. TRM included all causes of death other than disease progression or relapse occurring at any time after transplantation. Reported causes of death were reviewed and categorized. Patients who died as a result of relapse or disease progression after transplantation were considered to have died of their original disease. Similarly, patients who died of active GvHD were considered to have died of this complication even if other complications (e.g. infection) were recorded as the proximate cause. All deaths were considered for estimating the OS.

Statistical analysis

The primary end point of the comparison was the cumulative incidence of acute and chronic GvHD. The secondary end points included the incidence of relapse, TRM, PFS and OS. The following patient or transplant characteristics were analysed for their prognostic value on each of the outcomes: patient and donor age (less than or more than 40 years), sex, sex matching, ECOG PS, disease risk, cytomegalovirus serology, stem cell source, conditioning regimen and doses of MTX. To compare the two groups of patients receiving PBSC or BM, we used the chi-square test for categorical variables and the non-parametric Mann–Whitney *U*-test for ordered categorical and continuous variables. The unadjusted probabilities of PFS and OS were estimated from the time of transplantation using the Kaplan–Meier product limit method, according to the risk group, and 95% confidence intervals (CIs) were calculated using the Greenwood formula (Kaplan & Meier, 1958). To compare these two outcomes between the graft types, the log-rank test was used. In calculating the time-to-event for analysis of neutrophil/platelet engraftment, acute/chronic GvHD, TRM or relapse where competing risks alter the assessment of frequency, cumulative incidences were estimated (Gooley *et al*, 1999).

Association of graft type and each of the outcomes were mainly evaluated with multivariate Cox proportional hazards models (Cox, 1972). The occurrence of acute and/or chronic GvHD was included as a time-dependent covariate. The proportional hazards assumption of the Cox model was assessed mainly by a graphical approach. To confirm the results concerning the effects of graft type obtained from Cox analyses, we also presented results that adjusted the baseline confounding by the inverse probability-of-treatment weighted (IPTW) method (Robins *et al*, 2000). This method is less restrictive than the Cox model because we did not need to correctly specify any assumption between time to each event and baseline factors. We modelled the probability that a patient received PBSCT using the logistic regression with all the baseline factors described above as explanatory variables. From this logistic regression model, estimates of the patient specific weight, i.e. the inverse of the conditional probability of receiving his/her own graft type, were obtained. The subject-specific weight was used to estimate the effect of graft type. This weight is the probability that a subject would have his/her own observed transplantation. For IPTW estimates, the conservative robust variance estimates were used to construct confidence intervals (Lin & Wei, 1989). For end points other than relapse, cumulative incidence functions were predicted from the proportional (subdistribution) hazards model (Fine & Gray, 1999) and adjusted for effects of significant covariates in the multivariate Cox models explained above. The weights were the sample population value for each prognostic factor. SAS version 8.2 (SAS Institute Inc., Cary, NC, USA) and S Plus 2000 (Mathsoft, Seattle, WA, USA) were used for all statistical analyses.

Results

Patient and transplantation characteristics

Patient and transplantation characteristics are summarized in Table I; 295 patients received BMT and 214 received PBSCT. Regarding the diagnosis of their disease, 188 (36·9%) had AML, 144 (28·3%) had CML, 108 (21·2%) had ALL, 50 (9·8%) had MDS, and 19 (3·7%) had other types of leukaemia. The standard risk disease cohort consisted of 307 patients (60·3%), and the remaining 202 (39·7%) were of high-risk disease status. Conditioning before transplantation was a total body irradiation (TBI)-based regimen (74·9% in BMT, 64·5% in PBSCT), most often TBI plus cyclophosphamide, or a chemotherapy-based regimen (25·1% in BMT, 35·5% in PBSCT), most often busulphan plus cyclophosphamide. The median dose of nucleated cells given in the BMT group was $3{\cdot}0 \times 10^8$/kg recipient body weight (range $0{\cdot}3$–$18{\cdot}4 \times 10^8$/kg). The median number of $CD34^+$ cells infused was $5{\cdot}0 \times 10^6$/kg recipient body weight ($1{\cdot}0$–$19{\cdot}7 \times 10^6$/kg) in the PBSCT group. Prophylaxis for GvHD mainly consisted of a combination of CsA and three doses of short-term MTX (90·2% in BMT, 87·4% in PBSCT). The remaining patients received the four doses (day +1, +3, +6, +11) of MTX (6·8% in BMT, 8·9% in PBSCT) or less than two doses (3·1% in BMT, 3·7% in PBSCT). There were significant differences in the following variables: both patients and donors were older, and chemotherapy-based conditioning regimen was more frequent

Table I. Patient, donor and graft characteristics.

	BM		PBSC		
	n	%	*n*	%	*P*-value
No. of patients	295		214		
Median patient age, years (range)	38 (16–58)		41 (15–67)		0·028
Patient sex (male/female)	179/116		113/101		0·076
Female donor	137		114		0·137
Female to male	78		58		0·886
Median donor age, years (range)	37 (12–80)		41 (11–71)		0·045
ECOG PS					0·060
0–1	287	97·3	201	93·9	
2–4	8	2·7	13	6·1	
Risk group					0·352
Standard risk	183	62·0	124	57·9	
High risk	112	38·0	90	42·1	
Diagnosis					
Standard risk					0·485
AML	49	26·8	36	29·0	
CML	74	40·4	47	37·9	
ALL	42	23·0	34	27·4	
MDS	18	9·8	7	5·6	
High-risk					0·920
AML	57	50·9	46	51·1	
CML	14	12·5	9	10·0	
ALL	16	14·3	16	17·8	
MDS	15	13·4	10	11·1	
Others	10	8·9	9	10·0	
Conditioning regimen					0·011
TBI-based	221	74·9	138	64·5	
Chemotherapy-based	74	25·1	76	35·5	
Schedule of MTX					0·528
Abbreviated (one or two doses)	9	3·1	8	3·7	
Three doses	266	90·2	187	87·4	
Four doses	20	6·8	19	8·9	
Patient and donor CMV seronegative	23	7·8	6	2·8	**0·014**

BM, bone marrow; PBSC, peripheral blood stem cell; ECOG PS, Eastern Cooperative Oncology Group performance status; HLA, human leucocyte antigen; AML, acute myeloid leukaemia; ALL, acute lymphoid leukaemia; CML, chronic myeloid leukaemia; TBI, total body irradiation; MDS, myelodysplastic syndrome; GvHD, graft *versus* host disease; MTX, methotrexate; CMV, cytomegalovirus.

Standard risk disease included AML or ALL in first remission, CML in chronic phase and refractory anaemia. High-risk diseases included all other disease and stages.

in the PBSCT group. However, the two groups did not differ significantly for other patient, disease and transplant-related characteristics. Median follow-up period for the surviving patients at the time of analysis was 15 months in the PBSCT group (3–40 months) and 23 months in the BMT group (1–40 months).

Haematopoietic recovery

Among the patients surviving more than 28 d (BMT, $n = 287$; PBSCT, $n = 208$), engraftment occurred in 286 (99·7%) of the BMT patients and in 206 (99·0%) of the PBSCT patients. Patients who received PBSCT had significantly faster neutrophil and platelet recovery. The median time to a neutrophil count of at least $0{\cdot}5 \times 10^9$/l was 16 d (interquartile range 14–19 d) for the BMT group and 14 d (interquartile range 12–16 d) for the PBSCT group. The median time to a platelet count of at least 20×10^9/l was 22 d (interquartile range 18–28 d) for the BMT group and 18 d (interquartile range 13–25 d) for the PBSCT group. In multivariate Cox analyses, PBSCT was significantly associated with faster neutrophil recovery to at least $0{\cdot}5 \times 10^9$/l compared with BMT [hazard ratio (HR) = 1·84, 95% CI 1·53–2·22, $P < 0{\cdot}001$; Table II]. On the contrary, the high-risk disease (HR = 0·73, 95% CI 0·61–0·89, $P = 0{\cdot}001$) was associated with slower neutrophol recovery. Likewise, the significant factor associated

Outcomes	Analysis	Variables	HR (95% CI)	*P*-value
Neutrophils >0·5 × 10^9/l	Cox	Stem cell source: PBSCT	1·84 (1·53–2·22)	<0·001
		Disease risk: high	0·73 (0·61–0·89)	0·001
	IPTW	Stem cell source: PBSCT	1·77 (1·57–2·00)	<0·001
Platelets >20 × 10^9/l	Cox	Stem cell source: PBSCT	1·52 (1·25–1·84)	<0·001
		Donor age: ≥40 years	0·75 (0·57–0·98)	0·033
	IPTW	Stem cell source: PBSCT	1·46 (1·29–1·66)	<0·001
Grades II–IV acute GvHD	Cox	Stem cell source: PBSCT	1·13 (0·83–1·53)	0·454
	IPTW	Stem cell source: PBSCT	1·14 (0·93–1·41)	0·217
Any grade chronic GvHD	Cox	Stem cell source: PBSCT	1·41 (1·06–1·87)	0·017
		Donor age: ≥40 years	1·56 (1·06–2·29)	0·026
		Disease risk: high	1·40 (1·06–1·87)	0·020
		Prior acute GvHD: grades II–IV	1·66 (1·26–2·20)	<0·001
	IPTW	Stem cell source: PBSCT	1·56 (1·30–1·88)	<0·001
Extensive chronic GvHD	Cox	Stem cell source: PBSCT	1·65 (1·15–2·36)	0·007
		Donor age: ≥40 years	1·65 (1·01–2·70)	0·046
		Disease risk: high	1·45 (1·01–2·07)	0·043
		Prior acute GvHD: grades II–IV	2·36 (1·68–3·33)	<0·001
	IPTW	Stem cell source: PBSCT	1·88 (1·49–2·39)	<0·001

Table II. Multivariate Cox regression analysis and inverse probability-of-treatment weighted (IPTW) method analysis comparing haematopoietic reconstitution and graft *versus* host disease (GvHD) after bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT).

The following covariates were included in the Cox models as explanatory variables; patient and donor age (less than or more than 40 years), sex, sex matching, ECOG PS, disease risk, cytomegalovirus (CMV) serology, stem cell source, conditioning regimen, and doses of MTX. The values of stem cell source and significant covariates are shown.

with faster recovery to a platelet count of at least 20 × 10^9/l was PBSCT (HR = 1·52, 95% CI 1·25–1·84, $P < 0·001$; Table II). Significant factors for slower platelet recovery were donor age less than 40 years (HR = 0·75, 95% CI 0·57–0·98, $P = 0·033$) and high-risk disease (HR = 0·77, 95% CI 0·64–0·94, $P = 0·008$). Using the IPTW method, we confirmed that PBSCT was significantly associated with faster neutophil and platelet recovery (Table II).

Acute GvHD

Table III summarizes clinical characteristics of patients with acute GvHD and the adjusted cumulative incidence of grades II–IV acute GvHD in the two treatment groups is shown in Fig 1. The cumulative incidence of grades II–IV acute GvHD was 37·4% (95% CI 30·9–43·9) in the PBSCT group and 32·0% (95% CI 26·8–37·2) in the BMT group. By multivariate Cox analysis, haematopoietic stem cell source was not a significant factor for the incidence of grades II–IV acute GvHD (BMT vs. PBSCT: HR = 1·13, 95% CI 0·83–1·53, $P = 0·454$; Table II). We found no significant factor for the incidence of grades II–IV acute GvHD in our model. This result was the same when we used the IPTW method (Table II). The prevalence of organ involvement was different depending on the stem cell source (Table III). Liver and gastrointestinal involvement was more frequent in PBSCT patients than BMT (liver: 14·1% vs. 7·6%, $P < 0·019$; gut: 27·3% vs. 19·0%, $P < 0·014$; Table III), whereas skin involvement was similar between the two groups (46·8% vs. 52·6%, $P = 0·207$).

Table III. Clinical characteristics of patients with acute GvHD.

	BMT (n = 289)	PBSCT (n = 205)	*P*-value
Acute GvHD			0·213
Grade 0	125 (43·3)	88 (42·9)	
Grade I	70 (24·2)	37 (18·0)	
Grade II	69 (23·9)	44 (21·5)	
Grade III	22 (7·6)	24 (11·7)	
Grade IV	3 (1·0)	12 (5·9)	
Onset after transplantation among patients with grades II–IV acute GvHD			
Median	21	22	
Interquartile range	13·5–28·5	13–31	
Organ involvement			
Skin	152 (52·6)	96 (46·8)	0·207
Liver	22 (7·6)	29 (14·1)	0·019
Gut	52 (17·9)	56 (27·3)	0·014

GvHD, graft *versus* host disease; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation.
Values are given as n (%).

Chronic GvHD

The adjusted cumulative incidence of any grade chronic GvHD is shown in Fig 2 and the data on the incidence, severity and organ involvement of chronic GvHD are summarized in Table IV. The risk of any grade chronic GvHD in the first year after transplantation was higher in PBSCT than BMT

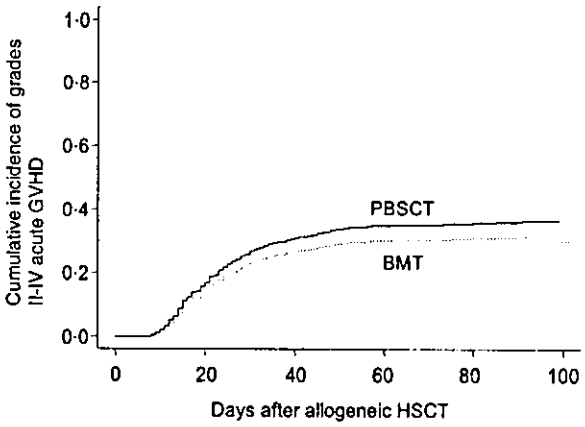

Fig 1. Cumulative incidences of grades II–IV acute graft *versus* host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates.

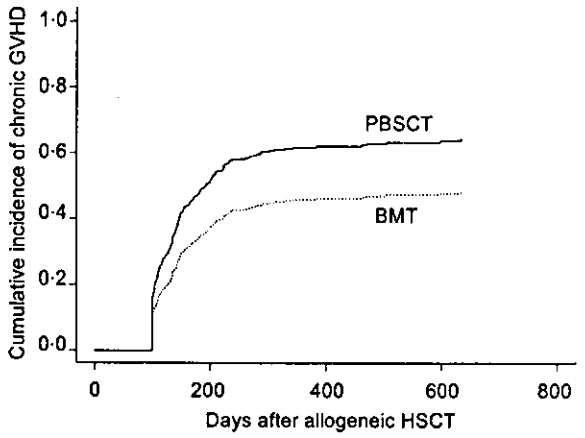

Fig 2. Cumulative incidences of any grade chronic graft *versus* host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates, except occurrence of prior grades II–IV acute GvHD.

(cumulative incidence at 1 year: 46·2%, 95% CI 40·4–52·4 with BMT vs. 62·1%, 95% CI 54·8–69·4 with PBSCT). The cumulative incidence of limited chronic GvHD was similar in the two groups (19·2%, 95% CI 14·4–24·0 with BMT and 20·2%, 95% CI 14·3–26·0 with PBSCT). However, the extensive form of chronic GvHD was more prevalent in PBSCT than BMT (27·1%, 95% CI 21·5–32·6 with BMT and 41·9%, 95% CI 34·6–49·3 with PBSCT). Progressive and *de novo* forms of chronic GvHD were more frequent in PBSCT. In the multivariate Cox analysis, PBSCT, donor age 40 years or older, high-risk disease and prior grades II–IV acute GvHD were significantly associated with increased risk for any grade chronic GvHD (BMT vs. PBSCT: HR = 1·41, 95% CI 1·06–1·87, $P = 0·017$; donor age <40 years vs. ≥40 years: HR = 1·56, 95% CI 1·06–2·29, $P = 0·026$; standard-risk vs. high-risk disease, HR = 1·40, 95% CI 1·06–1·87, $P = 0·02$; prior grades 0–I acute GvHD vs. grades II–IV acute GvHD: HR = 1·66, 95% CI 1·26–2·19, $P < 0·001$; Table II). The extensive form of chronic GvHD was associated with the same risk factors (BMT vs. PBSCT: HR = 1·65, 95% CI 1·15–2·36, $P = 0·007$; donor age <40 years vs. ≥40 years: HR = 1·65, 95% CI 1·01–2·70, $P = 0·046$; standard-risk vs. high-risk disease: HR = 1·45, 95% CI 1·01–2·07, $P = 0·043$; prior grades 0–I acute GvHD vs. grades II–IV acute GvHD: HR = 2·36, 95% CI 1·68–3·33, $P < 0·001$; Table II). Using the IPTW method, we confirmed a significantly increased incidence of any grade and extensive chronic GvHD in PBSCT group. There were differences in the distribution of organ involvement in chronic GvHD during the course of the disease. Rash/scleroderma (38·9% vs. 25·2%, $P = 0·006$), oral mucositis (45·0% vs. 22·3%, $P < 0·001$), ocular sicca (28·9% vs. 15·0%, $P = 0·002$), and liver abnormality (47·0% vs. 30·6%, $P = 0·002$) were more frequent in PBSCT patients than in BMT patients. The prevalence of organ involvement was otherwise similar in the two groups (Table IV).

Transplantation-related mortality

The cumulative incidence of TRM at 100 d was 9·7% (95% CI 7·0–12·5) with BMT and 15·0% (95% CI 11·6–18·4) with PBSCT, and at 1 year 16·2% (95% CI 12·3–20·1) with BMT and 19·3% (95% CI 14·1–24·4) respectively (Fig 3; Table V). The stem cell source did not affect TRM in the multivariate Cox, or the IPTW method, analysis. The significant adverse risk factor was grades II–IV acute GvHD (HR = 4·92, 95% CI 2·57–9·42, $P < 0·001$) at 100 d. At 1 year, donor age 40 years or older (HR = 1·98, 95% CI 1·03–3·80, $P = 0·040$) and grades II–IV acute GvHD (HR = 2·58, 95% CI 1·65–4·05, $P < 0·001$) increased the risk of TRM. There were 104 deaths in the BMT group and 75 deaths in the PBSCT group (Table VI). The number of TRM was 51 following BMT (49·0%) and 44 following PBSCT (58·7%), and there was a higher incidence of GvHD-related death in the PBSCT group than in the BMT group (17·3% vs. 3·8%). On the contrary, the number of deaths from relapse was lower in PBSCT ($n = 31$, 41·3%) than in BMT ($n = 53$, 51·0%). Time to non-relapse death was similar in the two groups.

Relapse

For the standard-risk group, the cumulative incidence of relapse at 1 year was similar (8·1%, 95% CI 4·2–12·0 with BMT vs. 7·5%, 95% CI 3·1–11·9 with PBSCT; Fig 4A). For the high-risk group, this was 37·1% (95% CI 28·0–46·4) with BMT and 33·3% (95% CI 23·3–43·4) with PBSCT respectively (Fig 4B). In multivariate Cox analysis, there was no statistical difference in the risk of relapse after PBSCT and BMT (HR = 0·95, 95%

Table IV. Clinical characteristics of patients with chronic GvHD.

	BMT (n = 206)	PBSCT (n = 149)	P-value
The incidence of chronic GvHD			0·001
All grade	113 (54·9)	107 (71·8)	
Limited	47 (22·8)	33 (22·1)	
Extensive	66 (32·0)	74 (49·7)	
Onset after transplantation among patients with chronic GvHD (days)			
Median	131	127	
Range	100–634	100–598	
Type			0·003
Progressive	12 (5·8)	15 (10·1)	
Quiescent	59 (28·6)	43 (28·9)	
De novo	42 (20·4)	49 (32·9)	
Organ involvement			
Rash/scleroderma	52 (25·2)	58 (38·9)	0·006
Oral mucositis	46 (22·3)	67 (45·0)	<0·001
Ocular sicca	31 (15·0)	43 (28·9)	0·002
Pulmonary disease	14 (6·8)	19 (12·8)	0·057
Liver abnormalities	63 (30·6)	70 (47·0)	0·002
Nausea/vomiting	6 (2·9)	10 (6·7)	0·089
Diarrhoea	7 (3·4)	7 (4·7)	0·534
Esophagitis	2 (1·0)	3 (2·0)	0·411
Arthralgias/arthritis	5 (2·4)	6 (4·0)	0·112
Effusions	1 (0·5)	1 (0·7)	0·818
Auto-antibody	2 (1·0)	2 (1·3)	0·744
Thrombocytopenia ($<100 \times 10^9$/l)	38 (19·3)	38 (26·6)	0·112

GvHD, graft *versus* host disease; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation.
Values are given as n (%).

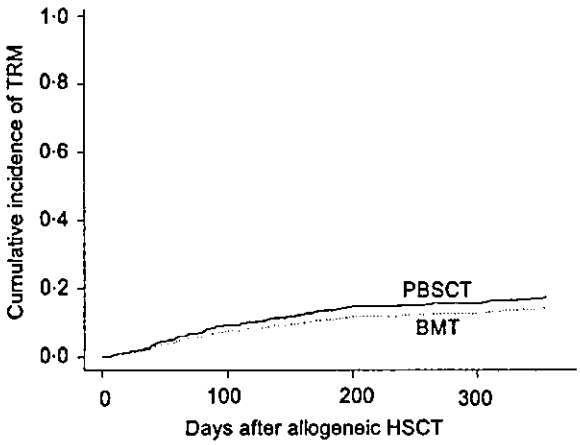

Fig 3. Cumulative incidences of treatment-related mortality after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates.

CI 0·64–1·41, P = 0·806; Table V). We found that the high-risk disease (HR = 3·97, 95% CI 2·66–5·94, P < 0·001) and ECOG PS 2–4 (HR = 3·42, 95% CI 1·73–6·77, P < 0·001) had a significantly increased risk of relapse. We did not observe any difference of relapse between the PBSCT and BMT groups using the IPTW method.

Progression-free and overall survival

In standard risk patients, the 2-year PFS and OS in PBSCT and BMT were, respectively, 68·2% (95% CI 58·8–77·5) and 64·7% (95% CI 57·0–72·5) (P = 0·993), and 74·1% (95% CI 65·2–83·1) and 73·8% (95% CI 66·9–80·6) (P = 0·991). In high-risk patients, PFS and OS in PBSCT and BMT were, respectively, 34·9% (95% CI 23·7–46·0) and 37·7% (95% CI 27·7–47·7) (P = 0·539), and 39·1% (95% CI 27·5–50·8) and 44·5% (95% CI 34·3–54·6) (P = 0·555; Fig 5A,B). In the multivariate Cox analysis, the use of PBSCT was not a significant factor for both PFS and OS (Table V). We obtained the same result using the IPTW method. The following variables were significant adverse risk factors for both PFS and OS, respectively: high-risk disease (HR = 2·41, 95% CI 1·82–3·21, P < 0·001; HR = 2·45, 95% CI 1·79–3·34, P < 0·001), ECOG PS 2–4 (HR = 2·83, 95% CI 1·63–4·92, P < 0·001; HR = 3·31, 95% CI 1·88–5·84, P < 0·001), and grades II–IV acute GvHD (HR = 1·33, 95% CI 1·00–1·78, P = 0·05; HR = 1·57, 95% CI 1·15–2·13, P = 0·004).

Discussion

This is the first large comparative study from an Asian area on the outcome of allogeneic HSCT using different sources of stem cells (BMT or PBSCT). We analysed the outcome of allogeneic HSCT from related donors in 509 Japanese patients with leukaemia and MDS. All of the patients in our cohort were given G-CSF postgrafting and we confirmed the more rapid haematological recovery after PBSCT than in BMT, which is in line with many previous studies (Schmitz *et al*, 1998, 2002; Champlin *et al*, 2000; Heldal *et al*, 2000; Powles *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001; Couban *et al*, 2002; Ringden *et al*, 2002).

It has been suggested that the increased incidence of acute GvHD in PBSCT patients is a consequence of PBSC grafts containing 1 log more T cells compared with bone marrow grafts, although this may be counterbalanced by the decreased potential of type 1 cytokine secretion from donor T cells in PBSC grafts (Mielcarek *et al*, 1997). In clinical studies, a statistically significant increase in acute GvHD after PBSCT has been reported in an RCT (Schmitz *et al*, 2002) and a meta-analysis (Cutler *et al*, 2001). On the contrary, there was no difference in other RCTs (Heldal *et al*, 2000; Powles *et al*, 2000; Bensinger *et al*, 2001; Couban *et al*, 2002). We also found no increased incidence of grades II–IV acute GvHD after PBSCT in the current study. Another important point to be discussed is the dose of MTX that was used as prophylaxis for GvHD. The most common regimen for MTX in Japanese institutions in HLA-identical-related donor transplantation is

Table V. Multivariate Cox regression analysis and inverse probability of treatment weighted (IPTW) method analysis comparing transplant-related mortality (TRM), progression-free survival (PFS) and overall survival (OS) after peripheral blood stem cell transplantation (PBSCT) and bone marrow transplantation (BMT).

Outcomes	Analysis	Variables	HR (95% CI)	*P*-value
TRM at 100 d	Cox	Stem cell source: PBSCT	1·18 (0·66–2·12)	0·584
		Acute GvHD: grades II–IV	4·92 (2·57–9·42)	<0·001
	IPTW	Stem cell source: PBSCT	1·33 (0·84–2·10)	0·230
TRM at 1 year	Cox	Stem cell source: PBSCT	1·07 (0·69–1·66)	0·773
		Donor age: 40 years or older	1·98 (1·03–3·80)	0·040
		Acute GvHD: grades II–IV	2·58 (1·65–4·05)	<0·001
	IPTW	Stem cell source: PBSCT	1·17 (0·82–1·66)	0·381
Relapse	Cox	Stem cell source: PBSCT	0·95 (0·64–1·41)	0·806
		Disease risk: high	3·97 (2·66–5·94)	<0·001
		ECOG PS: 2–4	3·42 (1·73–6·77)	0·004
	IPTW	Stem cell source: PBSCT	0·95 (0·73–1·23)	0·676
PFS	Cox	Stem cell source: PBSCT	1·03 (0·77–1·37)	0·868
		Disease risk: high	2·41 (1·82–3·21)	<0·001
		ECOG PS: 2–4	2·83 (1·63–4·92)	<0·001
		Acute GvHD: grades II–IV	1·33 (1·00–1·78)	0·05
	IPTW	Stem cell source: PBSCT	1·05 (0·87–1·27)	0·589
OS	Cox	Stem cell source: PBSCT	0·99 (0·73–1·36)	0·972
		Disease risk: high	2·45 (1·79–3·34)	<0·001
		ECOG PS: 2–4	3·31 (1·88–5·84)	<0·001
		Acute GvHD: grades II–IV	1·57 (1·15–2·13)	0·004
	IPTW	Stem cell source: PBSCT	1·05 (0·85–1·29)	0·659

The following covariates were included in the Cox models as explanatory variables; patient and donor age (less than or more than 40 years), sex, sex matching, Eastern Cooperative Oncology Group performance status (ECOG PS), disease risk, cytomegalovirus (CMV) serology, stem cell source, conditioning regimen, doses of methotrexate (MTX), grades II–IV acute graft *versus* host disease (GvHD) and chronic GvHD. The values of stem cell source and significant covariates are shown in this table. Grades II–IV GvHD and chronic GvHD were included as time-dependent covariate (HR, hazard ratio).

Table VI. Causes of mortality and time of death.

	BMT (n = 104)	PBSCT (n = 75)
Number of TRM	51 (49·0)	44 (58·7)
Causes of TRM		
GvHD	4 (3·8)	13 (17·3)
Non-infectious pneumonia	6 (5·8)	6 (8·0)
Veno-occlusive disease of the liver	5 (4·8)	1 (1·3)
Infection	25 (24·0)	14 (18·7)
Haemorrhage	1 (1·0)	3 (4·0)
Others	10 (9·6)	7 (9·3)
Time of TRM		
Days 0–30	7 (6·7)	4 (5·3)
Days 31–100	14 (13·5)	20 (26·7)
After day 100	30 (28·8)	20 (26·7)
Number of deaths in relapse	53 (51·0)	31 (41·3)

TRM, transplant-related mortality; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; GvHD, graft *versus* host disease.
Values are given as n (%).

three doses of MTX (day +1: 10 mg/m^2; day +3 and day +6: 7 mg/m^2) rather than four doses of MTX routinely used in other countries, because of the lower frequency of GvHD in Japan (Morishima *et al*, 1989). An RCT from the European Group for Blood and Marrow Transplantation (EBMT) study, in which increased incidence of acute and chronic GvHD was shown, also gave three doses of MTX (Schmitz *et al*, 2002). Omission of day +11, MTX may influence the incidence of acute and chronic GvHD (Nash *et al*, 1992; Cutler *et al*, 2001; Mehta & Singhal, 2002), although we did not find any difference among the different MTX dose groups. A recent report from the EBMT suggested that post-transplant G-CSF might increase the incidence of acute and chronic GvHD and TRM, resulting in lower leukaemia-free and OS rates after BMT (Ringden *et al*, 2004). Although the use of G-CSF postallografting is usually accepted as a standard care in Japan, we need to reconsider this indication, especially after BMT.

Notably, the observed cumulative incidence of grades II–IV acute GvHD in patients receiving HLA-identical transplants seemed lower in both groups (BMT 32·0%, PBSCT 37·4%) compared with rates reported from western countries (Powles *et al*, 2000; Bensinger *et al*, 2001; Couban *et al*, 2002; Schmitz *et al*, 2002). These data are consistent with previous reports on Japanese BMT patients (Morishima *et al*, 1989; Oh *et al*, 2002). Oh *et al* (2002) reported a multivariate analysis for adult allogeneic BMT patients showing that a Japanese cohort had a significantly lower risk of acute GvHD than white American, black American and Irish cohorts [relative risk

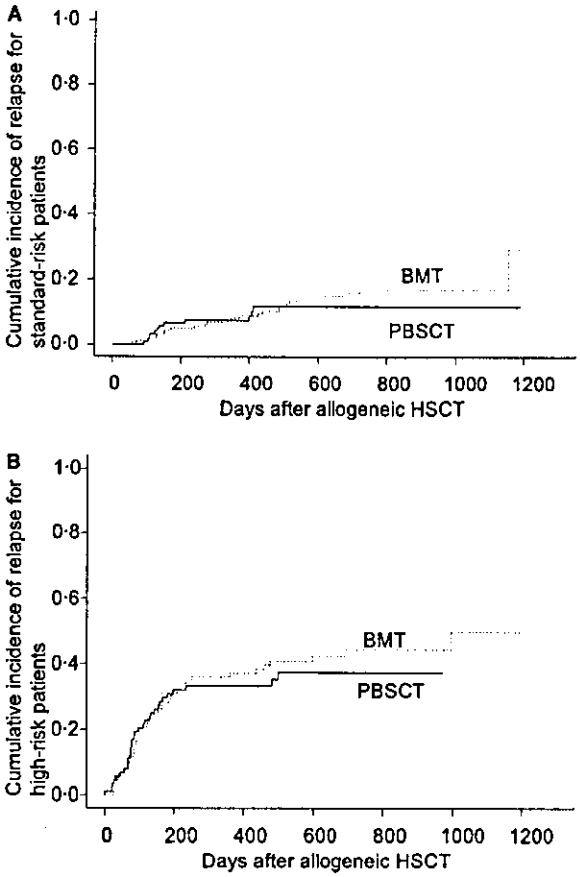

Fig 4. Cumulative incidences of relapse after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions (A: standard-risk group; B: high-risk group) were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates.

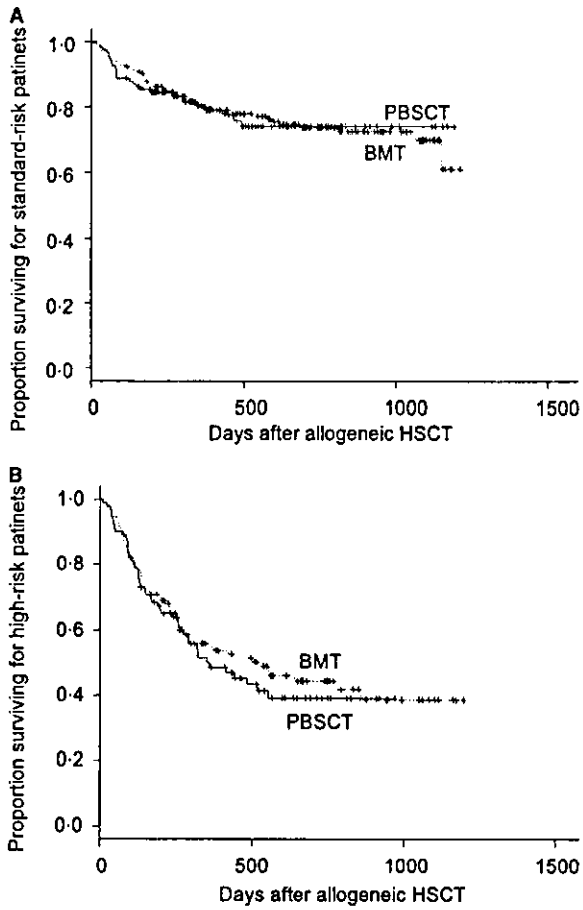

Fig 5. Probabilities of overall survival after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Probabilities were derived from Kaplan–Meier estimates [A: overall survival (OS) for standard risk group; B: OS for high-risk group].

(RR) = 1·77, $P < 0·01$; RR = 1·84, $P < 0·01$; RR = 2·22, $P < 0·01$ respectively]. Our data suggest that this trend might also apply to PBSCT. This difference has been speculated to reflect a lower degree of diversity for HLA and minor histocompatibility antigens among Japanese. However, a recent report revealed the influence of an interleukin-10 promotor polymorphism after allogeneic HSCT (Lin *et al*, 2003). The interleukin-10-592A/A genotype was associated with a decreased risk of grade III or IV acute GvHD. The frequency of this genotype is 67% in the Japanese population (Tegoshi *et al*, 2002), which is much higher than the frequency of 23% and 24% in two white populations (Lin *et al*, 2003). This finding may account for the decreased incidence and severity of acute GvHD in Japanese population than in white populations.

We found a significantly increased cumulative incidence of chronic GvHD among PBSCT patients in accord with several previous studies (Champlin *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001; Schmitz *et al*, 2002; Heldal *et al*, 2003). In particular, the extensive form of chronic GvHD was increased in the PBSCT cohort, whereas the incidence of the limited form was similar in the two cohorts. There is now considerable evidence that the preferential expansion of T-helper 2 (Th2) cells after allogeneic HSCT is associated with the development of chronic GvHD in both murine models and human beings (Doutrelepont *et al*, 1991; Umland *et al*, 1992; Allen *et al*, 1993; De Wit *et al*, 1993; Garlisi *et al*, 1993; Tanaka *et al*, 1997). A G-CSF-induced Th2 cytokine profile of donor T cells may be associated with increased incidence and severity of chronic GvHD (Pan *et al*, 1995). G-CSF also mobilized type 2 dendritic cells, which promote Th2 responses (Arpinati *et al*, 2000). Thus, G-CSF may have an important role in the development of chronic GHVD among PBSCT patients.

Another interesting point is the different distribution of organs affected by acute and chronic GvHD in BMT and PBSCT. Although previous reports demonstrated that skin and vaginal involvement (Bensinger *et al*, 2001; Flowers *et al*,

2002) or ocular involvement (Mohty *et al*, 2002) of chronic GvHD was more prevalent after PBSCT, the current study showed an increased incidence of skin, ocular sicca and oral mucositis, similar to Sjoegren syndrome. It is not well understood how selected organs become the targets of activated T cells. Inflammatory chemokines expressed in inflamed tissues upon stimulation by proinflammatory cytokines are specialized for the recruitment of effector cells (Moser & Loetscher, 2001). In mouse models, a comparative study of gene expression profiles of livers after experimental allogeneic and syngeneic BMT using oligonucleotide microarrays identified genes related to leucocyte trafficking that were upregulated at day 7 after allogeneic BMT when neither hepatic injury nor donor T-cell migration into the liver was evident (Ichiba *et al*, 2003). This study suggests that the interferon-γ produced by donor T cells in secondary lymphoid organs transactivates genes in target organs, stimulating the recruitment of effector cells to target organs and eventually rendering them vulnerable to effector cell attack. Thus, quantifiable and qualitative differences in immunological cells in PBSC grafts compared with bone marrow grafts may affect the chemokine environment, leading to the different distribution of affected organs. Alternately, increased numbers of affected organs in PBSC patients may simply reflect the increased severity of chronic GvHD.

Recent reports suggest that chronic GvHD with risk factors may negatively affect patients' survival (Akpek *et al*, 2001, 2003; Przepiorka *et al*, 2001). Long-term follow-up of an RCT showed that, although the cumulative incidence of chronic GvHD at 3 years was similar in BMT and PBSCT patients, chronic GvHD after PBSCT was more protracted and less responsive to treatment than after BMT (Bensinger *et al*, 2001; Flowers *et al*, 2002). With increasing numbers of long-term survivors, we need more information concerning the clinical characteristics of chronic GvHD after PBSCT (Przepiorka *et al*, 2001).

It has been postulated that a GVL effect may be observed, and the results of allogeneic HSCT may be improved in the presence of GvHD (Sullivan *et al*, 1989; Horowitz *et al*, 1990). However, the potential advantage of the GVL effect of allogeneic HSCT is often reduced by the GvHD-related morbidity and mortality (Weiden *et al*, 1981; Sullivan *et al*, 1989; Horowitz *et al*, 1990; Przepiorka *et al*, 2001; Lee *et al*, 2002). In most of the previous RCTs comparing BMT and PBSCT, the sample sizes were too small to detect meaningful survival increases (Schmitz *et al*, 1998; Blaise *et al*, 2000; Heldal *et al*, 2000; Powles *et al*, 2000). Even in the larger RCTs, survival was evaluated as a secondary end point (Bensinger *et al*, 2001; Couban *et al*, 2002; Schmitz *et al*, 2002). Bensinger *et al* (2001) and Couban *et al* (2002) have reported an OS benefit of PBSCT in patients with advanced disease. The former study included miscellaneous diseases and the observed advantage was derived from subgroup analysis, in which we were unable to draw reliable conclusions. The latter study, which involved 228 patients, included only myeloid malignancy but the improved survival was due to lower TRM with similar relapse rates, suggesting that faster haematological recovery accounts for this benefit. A meta-analysis reported by Cutler *et al* (2001), which involved 16 studies, and a large RCT from the EBMT (Schmitz *et al*, 2002) included 350 patients, and showed an increased incidence of acute and chronic GvHD, with no significant difference in relapse (Cutler *et al*, 2001; Schmitz *et al*, 2002) and survival rate (Schmitz *et al*, 2002). A recent meta-analysis suggested that any survival advantage of PBSCT is limited to patients with advanced disease (Horan *et al*, 2003). Thus, allogeneic PBSCT offered the prospect of a better outcome, but evidence for a survival benefit has been inconclusive. We must explicitly state that caution is highly advisable when interpreting *post hoc* subgroup analyses. These cannot be used for recommendations on treatment selection for individual patients, although they can be used in the development of new, empirically based research hypotheses. In addition, there might be a different impact on patient outcome after allogeneic HSCT according to stem cell source in this particular ethnic group, if the incidence of acute GvHD is lower than western countries. In the present study, multivariate analyses revealed that differences in stem cell source was not a significant factor for acute GvHD, relapse, TRM, PFS and OS despite the increased incidence of chronic GvHD after PBSCT. Early mortality within day 100 of PBSCT could be reduced because of faster engraftment (Champlin *et al*, 2000; Couban *et al*, 2002) but we did not observe this advantage. Our data showed that grades II–IV acute GvHD were significant adverse prognostic factors for TRM. The advantages of PBSCT may thus be counterbalanced by the increased incidence of GvHD. Treatment of acute and chronic GvHD was performed at the physician's discretion and immunosuppressive treatment may hamper the GVL effect in some cases. This may indicate the difficulty of separating GVL effects from GvHD clinically. We analysed the data according to each disease category and risk status, although there were no apparent differences between the two groups (data not shown). Therefore, in contrast to general belief, whether the GVL effect will improve survival after PBSCT remains unknown. Assessment of the overall benefits of PBSCT compared with BMT will require long-term follow-up of the morbidity of patients associated with chronic GvHD.

The retrospective nature, the heterogeneity of the diagnoses and the relatively short follow-up limit the power of this analysis. We cannot exclude the possibility that there are unmeasured confounders that could cause a bias between two groups. Analysis of the $CD34^+$ and $CD3^+$ cell dose was not performed because these are generally dependent on the source of stem cells, and in addition, we could not obtain enough data, especially in the BMT group. In multicentre studies, there is likely to be a variation among centres in both baseline risks and treatment effects that cannot be explained by the known prognostic factors (Frassoni *et al*, 2000; Matsuo *et al*, 2000; Loberiza *et al*, 2003). To resolve the limitations described