absorption of the drug. Voriconazole and caspofungin have not yet been approved, and micafungin was approved only recently. Regarding *Aspergillus* infections, it is difficult to conclude that the CDC's recommendation for the prophylactic administration of fluconazole is useful. With the present availability of all of these alternative agents, a comparative study to identify a suitable procedure will be required.

While all of the patients received prophylactic fluconazole at least until engraftment, only 44% received the recommended dose of 400 mg/day. Moreover, only 20% of both the CST and RIST recipients received fluconazole beyond 75 days following transplantation, as recommended in a previous study.[6] It has been reported that *C. albicans* can be controlled at a lower dose of 200 mg/day.[35,36] Many physicians believe that 400 mg of fluconazole is not required for prophylactic use, and optimal duration of fluconazole prophylaxis remains to be established. Since fluconazole is expensive and costs about 100 000 yen ($850) when used at 400 mg/day to cover from the commencement of pre-transplant treatment and engraftment, validation of the adequate dose and the duration for prophylactic use is important.

With an increasing number of patients undergoing transplant, establishment of fungal management is important in RIST. The practice for the prevention and treatment of fungal infection varies among institutions. Mortality of invasive aspergillosis was 50% in this survey, which were far lower than reported previously.[1] The differences might be attributable to diagnostic approaches. In Japan, diagnostic measures using computed tomography and blood tests such as beta-D-glucan assay or an enzyme-linked immunosorbent test detecting galactomannan antigen are widely used.[37–39] These tests might have contributed to make an early diagnosis of aspergillosis, improving its prognosis. These situations are similar to antifungal prophylaxis. The guidelines for antifungal prophylaxis, which were prepared based on previous clinical studies, should be updated, since the circumstances surrounding transplantation have been changing. However, there are little data to make new recommendations for guidelines of antifungal prophylaxis, and more information is needed regarding fungal infections following RIST. Further investigation is needed to determine what measures are effective to accommodate the changes in transplant practices.

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Appendix

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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.

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

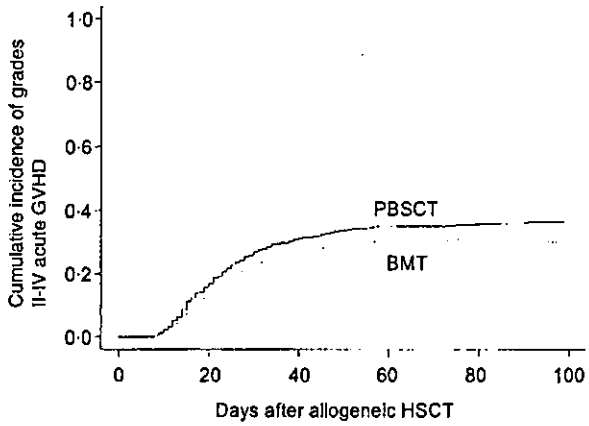

Fig 1. Cumulative incidences of grades II–IV acute graft *versus* host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model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

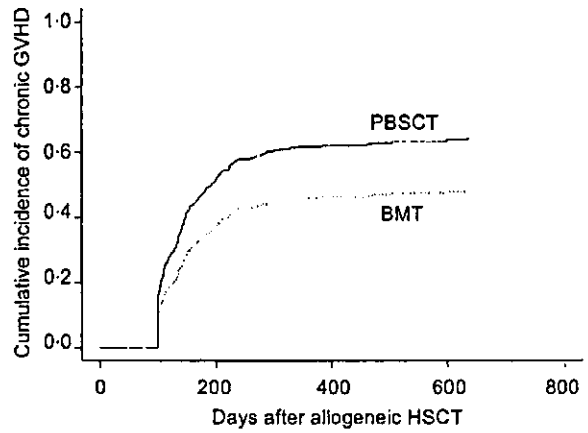

Fig 2. Cumulative incidences of any grade chronic graft *versus* host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and 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 × 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 (%).

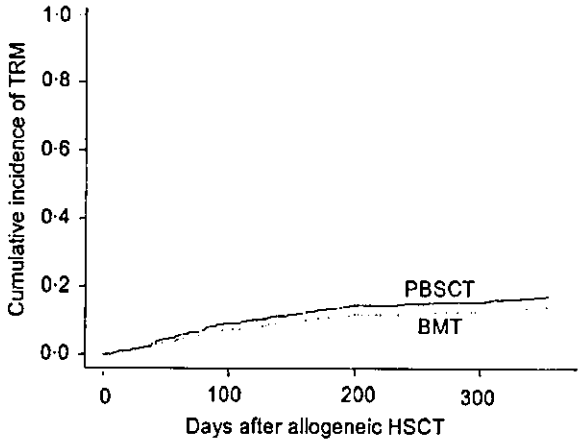

Fig 3. Cumulative incidences of treatment-related mortality after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of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

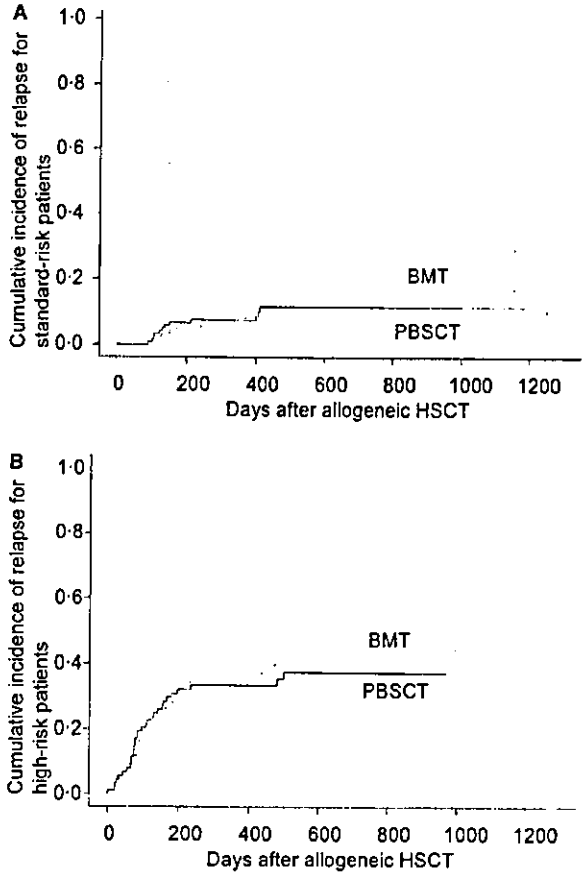

Fig 4. Cumulative incidences of relapse after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions (A: standard-risk group; B: high-risk group) were predicted from the proportional subdistribution hazards model and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

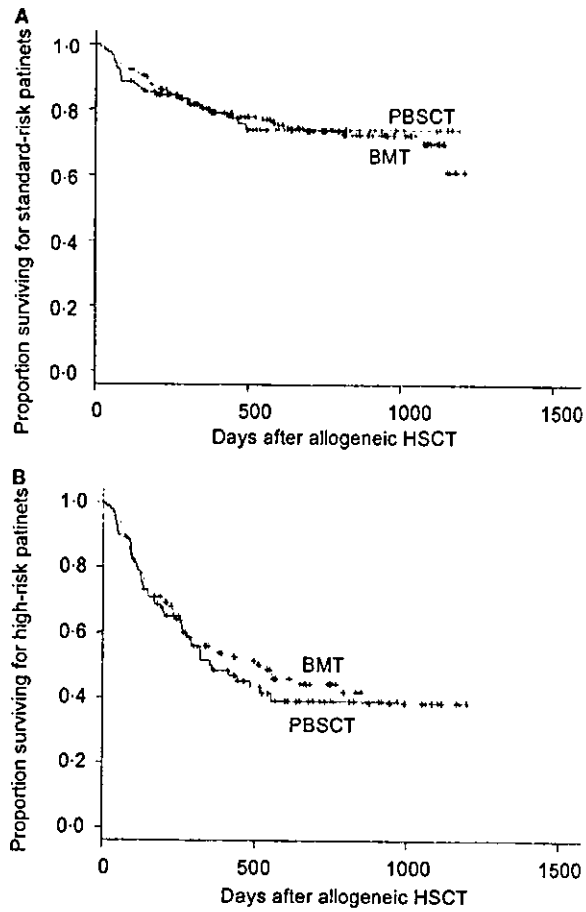

Fig 5. Probabilities of overall survival after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Probabilities were derived from Kaplan–Meier estimates [A: overall survival (OS) for standard risk group; B: OS for high-risk group].

(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

above, we needed an RCT in Japan. We have therefore launched a prospective, open-label RCT comparing allogeneic BMT *versus* PBSCT for adult patients with leukaemia. The primary end point of this trial is leukaemia-free survival based on time-to-event analysis. We plan the sample size per one arm to be 160, in order to detect the difference of 1·6 to 1·7 in HR for leukaemia-free survival. If this study can be completed, the impact of stem cell source on survival will be defined more accurately than the previous studies.

In summary, we observed faster engraftment and increased incidence of chronic GvHD in PBSCT compared with BMT for Japanese patients. The incidence of GvHD was lower than the western populations, but there were no differences in relapse, TRM, PFS and OS between PBSCT and BMT. These results suggest that the choice of haematopoietic stem cell source should be considered based on the data for individual ethnic populations. More detailed analysis and future trials may reveal the differential applicability of stem cells from these different sources in each disease category and hence enable us to choose appropriately between BMT and PBSCT based on reliable evidence.

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Appendix A

This study was conducted at the following institutions under the auspices of the following investigators in Japan: M. Sakai (Tokyo Metropolitan Hospital, Tokyo), T. Hamaki (National Cancer Centre, Tokyo), T. Karasuno (Osaka Medical Centre for Cancer and Cardiovascular diseases, Osaka), M. Kasai (Japanese Red Cross Nagoya first Hospital, Aichi), K. Kishi (Tokai University School of Medicine, Kanagawa), S. Okamoto (Keio University School of Medicine, Tokyo), N. Maseki (Saitama Cancer Centre Hospital, Saitama), S. Morishima (Meitetsu Hospital, Aichi), S. Yamasaki (Municipal Kitakyushu Medical Centre, Fukuoka), M. Kasai (Sapporo Hokuyu Hospital, Hokkaido), T. Kamimura (Harasanshin Hospital, Fukuoka), K. Shinagawa (Okayama University Medical School, Okayama), T. Yamane (Osaka City University, Osaka), S. Miyawaki (Saiseikai Maebashi Hospital, Gunma), Y. Miyazaki (Kansai Medical University, Osaka), T. Yamashita (National Medical Defence College, Saitama), N. Uike (National Kyushu Cancer Centre, Fukuoka), A. Maruta (Kanagawa Cancer Centre, Kanagawa), M. Misawa (Hyogo College of Medicine, Hyogo), K. Mitani (Dokkyo University School of Medicine, Tochigi), K. Kamezaki (Kyushu University Graduate School of Medical Sciences, Fukuoka), M. Masuda (Ryukyu University, Okinawa), J. Ishikawa (Osaka University, Osaka), A. Wake (Kokura Memorial Hospital, Fukuoka), A. Kohno (JA Aichi Showa Hospital, Aichi), M. Hara (Ehime Prefectural Central Hospital, Ehime), M. Kuroiwa (Hamanomachi Hospital, Fukuoka), E. Kusumi (Toranomon Hospital, Tokyo), K. Nishiwaki (Jikei University School of Medicine, Tokyo), M. Imamura (Hokkaido University Graduate School of Medicine, Hokkaido), Y. Takemoto (Jiaikai Imamura Hospital, Kagoshima), K. Fujimaki (Yokohama City University School of Medicine, Kanagawa), T. Tamaki (Rinku General Medical Centre, Osaka), Y. Takamatsu (Fukuoka University School of Medicine, Fukuoka), T. Murayama (Hyogo Medical Centre for Adults, Hyogo), M. Hirokawa (Akita University School of Medicine, Akita), T. Kobayashi (Tsuchiura Kyodo General Hospital, Ibaraki), K. Ozawa (Jichi Medical School, Tochigi), T. Ashida (Kinki University School of Medicine, Osaka), S. Imamura (Fukui Medical University, Fukui), Y. Kimura (Tokyo Medical University, Tokyo), K. Hodohara (Shiga Medical University, Shiga), H. Ago (Shimane Prefectural Central Hospital, Shimane), C. Shimazaki (Kyoto Prefectural University of Medicine, Kyoto), H. Teshima (Osaka City General Hospital, Osaka), A. Kubota (National Kyushu Medical Centre, Fukuoka), J. Tsukada (University of Occupational and Enviromental Health, School of Medicine, Fukuoka), C. Hashimoto (Yokohama City University Medical Centre), A. Yokota (Chiba Municipal Hospital, Chiba), H. Tsurumi (Gifu University, Gifu), M. Yamaguchi (Ishikawa Prefectural Central Hospital, Ishikawa), T. Endo (Hokkaido University Graduate School of Medical Sciences, Hokkaido), T. Chujo (Kanazawa University Graduate School of Medical Sciences, Ishikawa), M. Masuda (Tokyo Women's Medical College, Tokyo), S. Murakami (Social Insurance Kyoto Hospital, Kyoto), N. Emi (Nagoya University School of Medicine, Aichi), T. Fujisaki (Matsuyama Red Cross Hospital, Ehime), E. Matsuishi (Saga Prefectural Hospital Koseikan, Saga), F. Sano (St Mariannna University School of Medicine, Yokohama City Seibu Hospital, Kanagawa), Y. Torimoto (Asahikawa Medical College, Hokkaido), K. Yakushiji (Kurume University School of Medicine, Fukuoka), N. Uoshima (Matsushita Memorial Hospital, Osaka), H. Takamatsu (Kurobe City Hospital, Toyama), Y. Kobayashi (Kyoto Prefectural University of Medicine, Kyoto), K. Sunami (National Okayama Medical Centre, Okayama), K. Naito (Hamamatsu University School of Medicine, Shizuoka), H. Taguchi (Kochi Medical School, Kochi), S. Tsuchiya (Institute of Development, Aging and Cancer, Tohoku University, Miyagi), Y. Itoh (National Beppu Hospital, Oita), S. Doi (Kyoto Katsura Hospital, Kyoto), H. Kobayashi (Kyoto Prefectural Hospital, Kyoto), K. Tanimoto (Shin-koga Hospital, Fukuoka), K. Hayashi (Hoshigaoka Koseinenkin Hospital, Osaka), K. Kawachi (Takamatsu Red Cross Hospital, Kagawa), A. Urabe (NTT Kanto Medical Centre, Tokyo), R. Okamoto (Tokyo Metropolitan Komagome Hospital, Tokyo), T. Nishiura (National Kure Medical Centre, Hiroshima), H. Kimura (Kita-Fukushima Medical Centre,

Fukushima), T. Matsunaga (Sapporo Medical University School of Medicine, Hokkaido), N. Masauzi (Hakodate Municipal Hospital, Hokkaido), and T. Ishida (Sapporo Medical School, Hokkaido).

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Case Report

EBV-Positive Burkitt Lymphoma as a Late-Onset Posttransplantion Lymphoproliferative Disorder after Allogeneic Stem Cell Transplantation

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Abstract

Posttransplantation lymphoproliferative disorder (PTLD) is one of the well-recognized complications after allogeneic stem cell transplantation (SCT). It generally occurs early after SCT, and only a few reports of late-onset cases are available. We report a 58-year-old male patient who developed lymphoma 4 years after allogeneic SCT for chronic myeloid leukemia. The presence of *c-myc* translocation and Epstein-Barr virus–encoded RNA in the lymphoma cells, without rearrangement of the 3'-bcr region, confirmed the histopathologic diagnosis of Burkitt lymphoma. DNA chimerism analysis revealed that the lymphoma cells were of donor origin. The patient achieved complete response with intensive chemotherapy. To our knowledge, this is the first report of Burkitt lymphoma as a PTLD occurring after allogeneic SCT.
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Key words: Burkitt lymphoma; Posttransplantation lymphoproliferative disorder; PTLD; Allogeneic stem cell transplantation; Epstein-Barr virus

1. Introduction

Posttransplantation lymphoproliferative disorder (PTLD) is one of the well-recognized complications occurring after allogeneic stem cell transplantation (SCT) [1-5]. PTLD typically develops within 1 year after allogeneic SCT as an early-onset disorder and is frequently fatal [1,2]. Although Burkitt lymphoma has been reported as a rare subtype of PTLD in solid organ transplant recipients [3], there have been no reports of Burkitt lymphoma as a PTLD after allogeneic SCT. We report here a case of Epstein-Barr virus (EBV)-positive Burkitt lymphoma that developed after allogeneic SCT.

2. Case Report

A 54-year-old man visited the National Cancer Center Hospital because of leukocytosis, and his illness was diagnosed as chronic myeloid leukemia (CML) in the chronic phase. Allogeneic SCT from his HLA-matched brother was performed, because cytogenetic response was not obtained by administration of interferon (IFN)-α. When he received IFN-α for the treatment of CML, imatinib mesylate was not available. The preparative regimen consisted of 16 mg/kg busulfan and 120 mg/kg cyclophosphamide. Neither T-cell depletion nor the administration of antithymocyte globulin was performed. Cyclosporine-A (CsA) and short-term methotrexate were given for the prophylaxis of graft-versus-host disease (GVHD).

The peripheral blood leukocyte count reached more than 1.0×10^9/L on day +14 post-SCT. Acute grade II GVHD symptoms consisting of skin eruption and mild hyperbilirubinemia were controlled by 2 mg/kg methylprednisolone. Cytogenetic complete response (CR) was achieved on day +190. Oral administration of CsA was continued for mild extensive chronic GVHD, and it was discontinued on day +511. No recurrence of chronic GVHD was recognized throughout the patient's clinical course. On day +637, daily oral administration of 10 mg of pravastatin was started for the treatment of hyperlipidemia.

On day +1392, the patient developed lymphadenopathy in his neck and supraclavicular region, and tonsillar swelling was also noted. A biopsy specimen from his supraclavicular

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lymph node showed diffuse and dense infiltration of medium- to-large-sized, atypical lymphoid cells with hyperchromatic nuclei, and a starry sky pattern was recognized (Figures 1A and 1B). Mitotic figures were numerous. Immunohistochemical staining showed that the tumor cells were positive for CD10 and CD20 and negative for CD3, CD68, bcl-2, myeloperoxidase, latent membrane protein-1 (LMP-1), and EBV nuclear antigen-2 (EBNA-2), whereas EBV-encoded RNA-1 (EBER-1) was detected in tumor cells by in situ hybridization (Figure 1C). More than 99% of the tumor cells were Ki-67 positive.

Southern blot analysis revealed no rearrangement of the *3'-bcr* gene, a finding indicating that it was highly unlikely that the patient had lymphoid crisis of CML (data not shown). Fusion of the *c-myc* and Ig heavy chain (IgH) genes was shown by fluorescence in situ hybridization analysis (Figure 2), and the bone marrow was not infiltrated with lymphoma cells. Therefore, this case was diagnosed as EBV-positive Burkitt lymphoma, a subtype of late-onset PTLD, according to the World Health Organization (WHO) classification [2]. The clinical stage was II according to both the Ann Arbor and Murphy staging systems. DNA chimerism analysis revealed that the lymphoma cells were of donor origin (data not shown).

The patient achieved CR under intensive chemotherapy administered according to the National Cancer Institute protocol for Burkitt lymphoma, CODOX-M/IVAC [6]. At the time of this report, CR had been maintained without therapy for 17 months.

3. Discussion

PTLD is one of the well-recognized complications after allogeneic SCT; however, its cumulative incidence is reported to be relatively low: 1.0% ± 0.3% at 10 years [1]. Curtis et al reported that the incidence of PTLD varied markedly with time after SCT, with particularly high rates occurring in the first 5 months, followed by a steep decline in incidence between 6 and 12 months post-SCT [1]. In the Curtis et al report, 14 patients with late-onset PTLD occurring among 18,014 patients who underwent allogeneic SCT were documented, and the only risk factor identified for late-onset PTLD was extensive chronic GVHD. In contrast, in PTLD after solid organ transplantation, late-onset cases are not rare. The risk of PTLD development in solid organ transplant recipients varies depending on the type of allograft and immunosuppressive therapy. In solid organ transplant recipients treated with azathioprine, the mean interval between transplantation and development of PTLD is 48 months, whereas in those treated with CsA it is 15 months [2]. The reasons for the infrequency of late-onset PTLD after allogeneic SCT might include the following: almost all SCTs are performed for malignant neoplasms; some patients undergoing SCT die of disease progression or SCT-related complications; most long-term survivors after SCT are free from immunosuppressive therapy.

In the present case, the patient had been given pravastatin, an inhibitor of hydroxy-methyl-glutaryl coenzyme A reductase (or statin), which is a popular agent for the treatment of hyperlipidemia. A recent in vitro study by Kwak et al showed that statins act as direct inhibitors of the induction

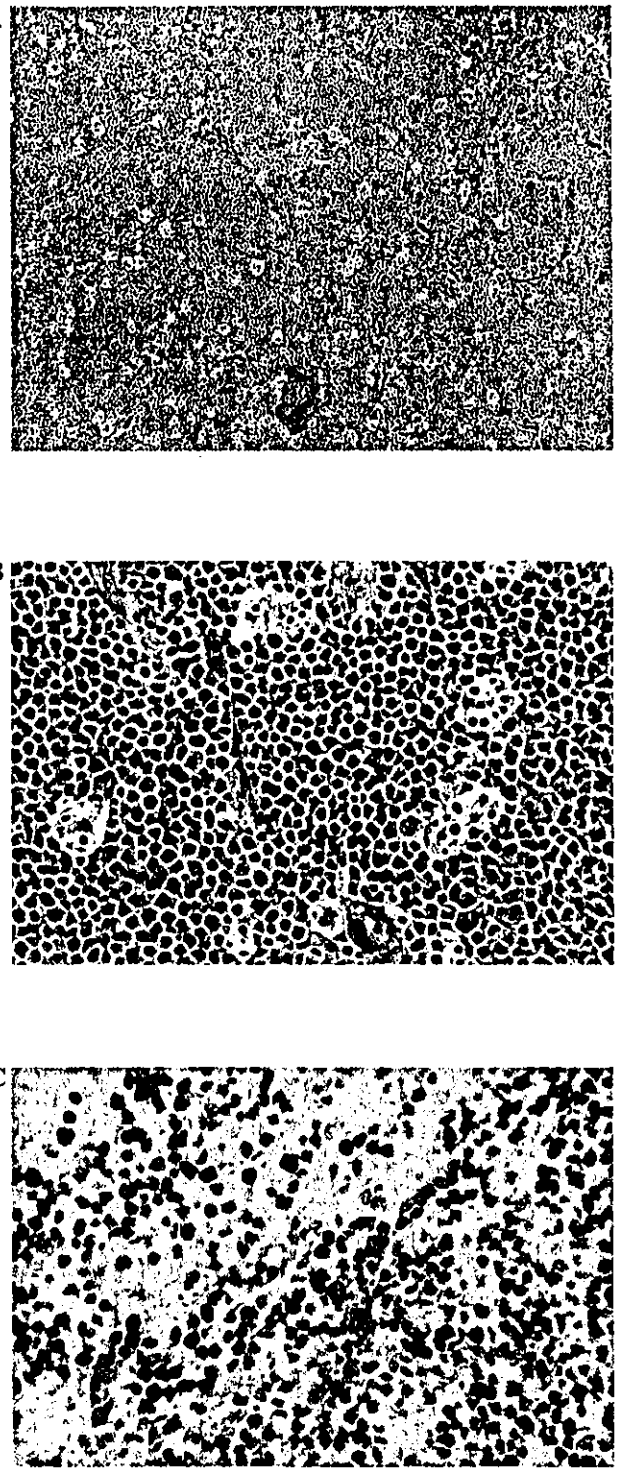

Figure 1. Histopathologic findings of the biopsied supraclavicular lymph node. A, A prominent starry sky pattern was recognized (hematoxylin and eosin, original magnification ×100); B, diffuse and dense infiltration of medium- to-large-sized atypical lymphoid cells with hyperchromatic nuclei was recognized (hematoxylin and eosin, original magnification ×400); C, Epstein-Barr virus–encoded RNA 1 was detected in neoplastic cells (in situ hybridization, original magnification ×400).

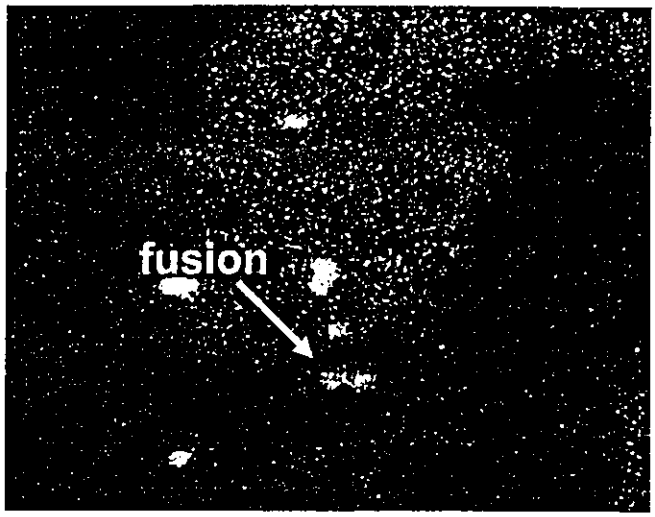

Figure 2. Fluorescence in situ hybridization analysis of the biopsied supraclavicular lymph node. The immunoglobulin (Ig)H/c-myc fusion signal was recognized. The signals represented IgH/c-myc rearrangement (yellow), c-myc (orange), IgH (green) and CEP8 (aqua). To detect IgH/c-myc rearrangements, a dual fusion translocation probe (VYSIS) was used.

of MHC class II–mediated T-cell activation [7]. They proposed recognition of statins as a new type of immunomodulator. A review by Newman et al suggested the potential carcinogenicity of long-term administration of statins in humans based on rodent carcinogenicity studies [8]; however, a recent metaanalysis of 5 large, randomized, placebo-controlled clinical trials showed no association between the use of statins and the risk of fatal or nonfatal cancers including malignant lymphoma [9].

The lymphoma cells in this case were positive for EBER-1. The majority of PTLD cases are associated with EBV infection, although approximately 20% of PTLD cases are not [2,5]. Three clinical variants of Burkitt lymphoma have been recognized: endemic, sporadic, and immunodeficiency-associated variants (including human immunodeficiency virus–associated [10] and PTLD after solid organ transplantation). Interestingly, the frequency of EBV association is quite different among the variants of Burkitt lymphoma [3,10]. Moreover, all of the reported EBV-positive Burkitt lymphoma cases, including immunodeficiency-associated cases, showed a latency I form of EBV infection [3,10], which is lacking EBNA-2 and LMP-1, although most PTLD cases show a latency III pattern. Burkitt lymphoma after solid organ transplantation and EBV-negative PTLD tend to occur later than EBV-positive cases [3,5]. Considering these findings, it is likely that the essential factor in Burkitt lymphoma development as a PTLD is *c-myc* dysregulation caused by genetic instability after prolonged mild immunosuppression, independent of EBV stimulation.

Acknowledgments

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