Immunohistochemistry for Survivin and Proliferating Cells

Tissue sections (4 μm thick) of bone marrow from the control, AML, ALL, and AMLL cases were cut on slides covered with adhesive. The sections were deparaffinized, and endogenous peroxidase was quenched with 1.5% hydrogen peroxide in methanol for 10 min. Antibodies were applied to identify survivin and to characterize proliferating cells. The primary antibodies included polyclonal rabbit antibody against human survivin (SURV 11-A, Alpha Diagnostic International, Inc., San Antonio, TX) and monoclonal antibody Ki-67 (DAKO). All sections were developed using biotin-conjugated secondary antibodies against rabbit IgG or mouse IgG followed by a sensitive peroxidase-conjugated streptavidin system (DAKO) with DAB as the chromogen. Negative control staining was performed using rabbit or mouse immunoglobulin of irrelevant specificity substituted for the primary antibody. The proportion of Ki-67-positive cells was determined in the same way as the proportion of TUNEL-positive cells.

Statistical Analysis

Statistically significant differences in the quantitative analysis were determined using the Mann–Whitney *U*-test for comparisons between the control, AML, ALL, and AMLL samples.

RESULTS

Clinicopathological Characteristics of Cases With Acute Mixed Lineage Leukemia

To determine the clinicopathological characteristics of cases with AMLL, the clinical data for cases including laboratory findings are summarized in Table I. As indicated by the flow-cytometric data, bone marrow blasts in these cases exhibited a high frequency of B-cell lineage antigen (CD19) and myeloid cell marker (CD13 and/or CD 33) expression. Thus, blasts of these cases were "biphenotypic." Chromosomal abnormalities were identified in 5 cases (cases 1, 3, 6, 7, and 8), and the Philadelphia chromosome was identified in two cases (cases 1 and 7). Although abnormalities involving chromosome 11q were identified in two cases (cases 1 and 8), the molecular rearrangement of the *mixed lineage leukemia* (*MLL*) gene located on chromosome 11q23 [32,33] was not observed at the chromosome level.

In spite of AML- and ALL-directed therapy (cytarabine, vincristine, etoposide, adriamycin, predonin, etc.), five patients failed to exhibit complete hematological remission, having blast persistence in bone marrow above 10%. Although complete remission could be induced by chemotherapy in four cases (cases 3, 6, 7, and 8), relapse with leukemic blast proliferation occurred within 6 months in two cases (cases 3 and 6, Table II). Overall, most cases exhibited a poor prognosis and the survival times after diagnosis were shorter than 14 months for 5 cases. However, one patient who received a bone marrow transplant (case 6) and the other patients who received chemotherapy (cases 7 and 8) lived.

Double Immunostaining for Myeloid and Lymphoid Cell Markers on AMLL Cells

To confirm the biphenotypic nature of blasts in the AMLL samples, double immunostaining for myeloid and lymphoid cell markers was performed. The majority of AMLL cells exhibited positive signals for B-cell markers such as CD20 or CD79a, while the myeloid cell marker (myeloperoxidase) was partially observed for many of the cases examined

TABLE II. Treatment and Outcome of Cases With Adult AMLL*

Case no.	First treatment	Response and status	Second treatment	Response status	Survival (months)
1	A-VVV	Failure	H-CPM/VP-16	Failure	3
2	H-CPM/VP-16, H-AraC+MIT	Failure	TBI + CPM	Failure	5
3	DCM, H-AraC+MIT	CR, relapse	A-VVV, H-AraC	Failure	6
4	L-AdVP, MVP	Failure	B-VVV, H-CPM/VP-16, H-AraC, L-AdVP	Failure	11
5	AdVP	Failure	A-VVV, VP-16, CAG	Failure	14
6	DC, A-VVV, H-CPM/VP-16	CR, relapse	H-AraC + MIT, BMT	CR and alive	>6
7	A-VVV	CR	H-AraC + MTX	CR and alive	>6
8	CAG	CR	DC	CR and alive	>9

*Abbreviations: A-VVV, AraC (cytarabine) + VCR (vincristine) + VLB (vinblastine) + VP-16 (etoposide); H-CPM, high-dose CPM (cyclophosphamide); H-AraC, high-dose AraC; MIT, mitoxantrone; TBI, total body irradiation; DCM, DNR (daunorubicin) + AraC + 6-MP (mercaptopurine); CR, complete remission; L-AdVP, L-Asp (L-asparaginase) + ADR (doxorubicin) + VCR + PDN (predonin) + CPM; MVP, MIT + VP-16 + PDN; B-VVV, BHAC (enocitabine) + VCR + VLB + VP-16; CAG, AraC + ACR (acurabicin) + G-CSF (lenograstim); DC, DNR + AraC; BMT, bone marrow transplantation; MTX, methotrexate.

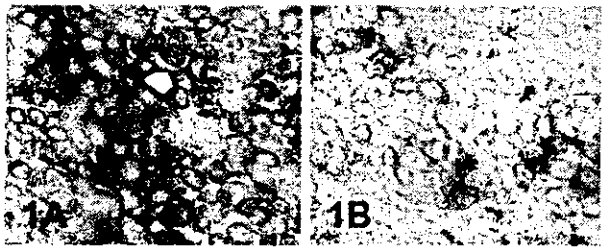

Fig. 1. Double immunostaining for a B-cell marker (CD79a) and myeloid cell marker (myeloperoxidase) in cases with AMLL (A, case 8; and B, case 6; original magnification 400×). Note that the majority of blasts stained positively for CD79a (brown) and a portion of them also stained positive for myeloperoxidase (blue) in both cases. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(Fig. 1A for case 8 and Fig. 1B for case 6). These findings were consistent with the flow-cytometric analytical data shown in Table I.

Apoptotic Frequency and Proliferation Activity of Acute Mixed Lineage Leukemia

To identify the apoptotic and proliferative cells present in the bone marrow samples, the TUNEL method and immunohistochemistry for Ki-67 were performed on paraffin-embedded sections. As expected from our previous studies [7,10], the frequency of apoptosis was significantly lower in AML (median, 0.769; range, 1.06–0.219) ($P < 0.001$) and ALL bone marrow cells (median, 0.543; range, 1.18–0.072) ($P < 0.01$) than control cells (median, 2.03; range, 2.81–0.848), and the proliferative cell ratio in AML/ALL bone marrow (median, 39.7; range, 47.8–32.4/median, 45.9; range, 71.9–34.2) was significantly higher than that in control cases (median, 19.2; range, 24.3–10.0) ($P < 0.0001$ and $P < 0.001$, respectively). As shown in Table III, AMLL cells exhibited a tendency similar to AML and ALL cells in that the apoptotic ratio (median, 0.176; range, 1.69–0.021) was significantly lower than the control ($P < 0.01$) and the proliferative cell ratio (median, 26.7; range, 49.1–18.3) was significantly higher ($P < 0.01$). However, AMLL cells exhibited a relatively lower apoptotic index and also significantly lower proliferative index compared with the AML ($P < 0.05$) or ALL samples ($P < 0.05$).

Expression of IAP-Family Proteins Determined by Real-Time Quantitative PCR

To quantitate the mRNA expression levels of the IAP-family members in AMLL cells, real-time quantitative RT-PCR was performed using bone marrow samples from control, AML, ALL, and AMLL cases.

TABLE III. Apoptotic Frequency and Proliferation Activity of Bone Marrow Cells From Control and Acute Leukemia Cases*

	TUNEL$^+$ cell ratio (%)	Ki-67$^+$ cell ratio (%)
Cases	Median (max-min)	Median (max-min)
Control	2.03 (2.81–0.848)^{a,b,c}	19.2 (24.3–10.0)^{d,e,f}
AML	0.769 (1.06–0.219)^{a}	39.7 (47.8–32.4)^{d,g}
ALL	0.543 (1.18–0.072)^{b}	45.9 (71.9–34.2)^{e,h}
AMLL	0.176 (1.69–0.021)^{c}	26.7 (49.1–18.3)^{f,g,h}

*Values indicate the median, maximum, and minimum. Differences were significant between the TUNEL-positive cell ratio for control and AML ($^aP < 0.001$), control and ALL ($^bP < 0.01$), and control and AMLL ($^cP < 0.01$) as seen by the Mann–Whitney U-test. The Ki-67-positive cell ratio exhibited significant differences between control and AML ($^dP < 0.0001$), control and ALL ($^eP < 0.001$), control and AMLL ($^fP < 0.01$), AML and AMLL ($^gP < 0.05$), and ALL and AMLL ($^hP < 0.05$) as seen by the Mann–Whitney U-test.

As shown in Fig. 2, the expression of survivin ($P < 0.05$), cIAP1 ($P < 0.05$), NAIP ($P < 0.01$), and XIAP ($P < 0.01$) exhibited significant up-regulation in AMLL compared with the controls. The mRNA for survivin ($P < 0.05$) showed significantly higher levels of expression in AMLL than AML, while the expression levels of survivin ($P < 0.05$), NAIP ($P < 0.05$), and XIAP ($P < 0.05$) in AMLL were significantly higher than those in ALL.

In summary, survivin expression in AMLL was significantly higher than the expression in control, AML, and ALL. The expression level of cIAP1 in AMLL was significantly higher than that in control, but similar with the expression in AML and ALL. Regarding cIAP2, the AMLL cases exhibited stronger expression than the control, AML, and ALL samples although the differences were not significant. NAIP expression in AMLL was significantly higher than control and ALL. The expression level of XIAP in AMLL was significantly higher than control and ALL but similar with AML. No remarkable differences were found between IAP protein expression and patients' age, sex, phenotype, or genotype for AMLL, although further analysis would be necessary because the number of cases was rather small.

These results indicate that the overall expression of IAP-family proteins in AMLL subjects tended to be higher than that for the control, AML, or ALL samples. Specifically, survivin expression in AMLL was significantly higher than that for the control, AML, and ALL samples.

Immunohistochemical Detection of Survivin in the Bone Marrow of AMLL Subjects

To investigate the distribution of survivin, immunohistochemical staining was performed on bone

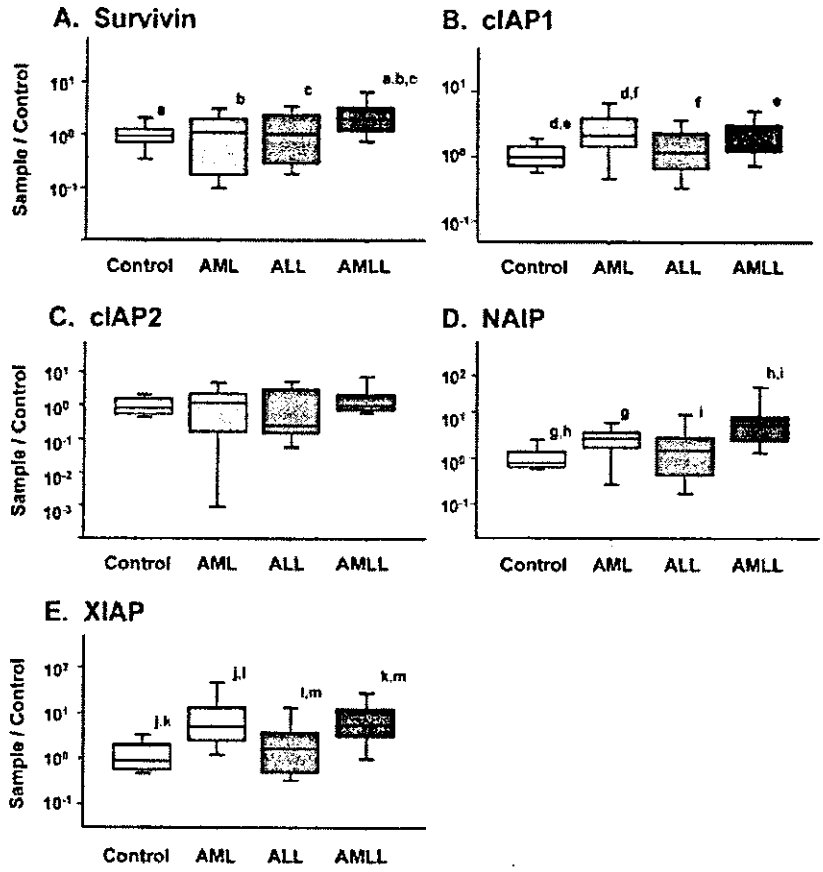

Fig. 2. Expression of IAP-family proteins in control bone marrow and acute leukemias determined by real-time quantitative RT-PCR. The relative intensity was calculated as (intensity of the reaction of IAP-family members [total Raji RNA, ng])/(intensity of the reaction of GAPDH [total Raji RNA, ng]). The intensities of the expressions from the AML, ALL, and AMLL samples are indicated as the ratios to the intensity of the control subjects. The box–bar graphs indicate the value of the control, AML, ALL, and AMLL cases: the bars indicate the 90th and 10th percentiles, and the box indicates the 75th to 25th percentiles. Differences were significant between samples as seen by the Mann–Whitney *U*-test as follows: (A) survivin—control and AMLL ($^{a}P < 0.01$), AML and AMLL ($^{b}P < 0.05$), and ALL and AMLL ($^{c}P < 0.05$). (B) cIAP1—control and AML ($^{d}P < 0.01$), control and AMLL ($^{e}P < 0.05$), and AML and ALL ($^{f}P < 0.05$). (C) cIAP2—differences were not significant. (D) NAIP—control and AML ($^{g}P < 0.05$), control and AMLL ($^{h}P < 0.01$), and ALL and AMLL ($^{i}P < 0.05$). (E) XIAP—control and AML ($^{j}P < 0.01$), control and AMLL ($^{k}P < 0.01$), AML and ALL ($^{l}P < 0.05$), and ALL and AMLL ($^{m}P < 0.05$).

marrow samples from AMLL subjects. As we previously showed [13], survivin was detected in only a few scattered myeloid cells in the control bone marrow samples and subcellular localization was mainly cytoplasmic but partly nuclear. The staining pattern and intensity in the control bone marrow was constant between different samples. All of the AMLL samples showed positive staining for survivin, although the staining intensity and frequency varied for each case. At the cellular level, survivin signals in AMLL cells were predominantly localized in the nucleus and also weakly in the cytoplasm (Fig. 3A). However, one case exhibited prominent cytoplasmic staining with mildly positive staining in the nucleus (Fig. 3B). The tissue sections that reacted with preimmune rabbit antibody of nonrelevant specificity showed no significant staining for any of the samples (not shown).

DISCUSSION

AMLL blasts are expected to possess more immature or intermediate characters of AML and ALL blasts because they express both myeloid and lymphoid phenotypes. Regarding the expression of survivin in myeloid neoplasms, previous studies have revealed the significant expression of survivin in AML [34,35]. Adida et al. [35] reported that survivin expression frequently occurs in AML, detecting it in 60% of a series of 125 patients analyzed, and survivin expression was found to be an unfavorable prognostic factor. In contrast, in lymphoid neoplasms, several

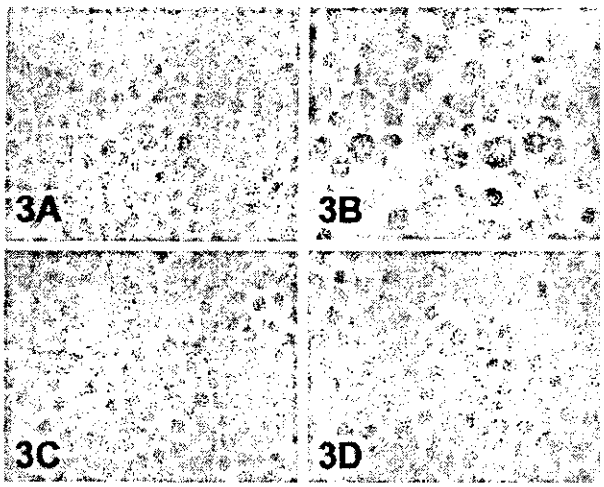

Fig. 3. Immunostaining for survivin in the bone marrow of AMLL (A, case 5; and B, case 8) in comparison with AML (C) and ALL (D) (original magnification 400×). Development was performed using the peroxidase–DAB system (brown) with hematoxylin counterstaining. Note the positive signals in the nucleus as well as the cytoplasm of AMLL cells (A) in contrast to the cytoplasmic staining (B). AML (C) and ALL (D) cases exhibited nuclear and partial cytoplasmic staining. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

studies investigated the dynamics of survivin expression in association with cell proliferation. The in vitro data on mononuclear cells from peripheral blood or bone marrow indicated that B-CLL cells expressed survivin in concert with CD40 and that survivin was the only IAP whose expression was induced by the CD40 ligand (CD40L) [36]. CD40 belongs to the TNF receptor superfamily [37], and its stimulation rescues B-CLL cells from apoptosis and induces proliferation [38]. We recently found that ALL as well as CLL cells exhibited significant expression of survivin and cIAP2 [13]. Thus, both in myeloid and lymphoid neoplasms, IAPs are expressed and seemed to influence the prognosis of patients. Therefore, we can imagine that IAPs would have functions also in AMLL blasts; however, little is known about the potential roles of survivin and other IAPs in the pathogenesis of AMLL.

A major problem with leukemia treatment is drug resistance to chemotherapeutic agents, which may already be present upon diagnosis or after chemotherapy for minimal residual blasts. Resistance originates from genetic or epigenetic mutations during growth of the leukemic clone. Anti-apoptosis mechanisms, alterations of tumor suppressor genes, altered immunogenicity, and drug-resistance mechanisms act in combination [39]. AMLL exhibits strong resistance against chemotherapy, resulting in poor patient prognosis [40,41]. In the present study, expression levels of IAPs in AMLL blasts were higher than those in control samples. Furthermore, several IAPs, such as survivin, NAIP, and XIAP, exhibited stronger expression in AMLL compared with conventional acute leukemias. Thus, the IAP expression level is one criterion that can be used to explain the strong drug resistance in this category of leukemia. The IAP might function probably via the inhibition of caspase-dependent apoptotic signaling. Although we have yet to clarify the caspase-independent pathway of apoptosis in AMLL, the findings of the present study suggest that the regulation of IAPs may become a possible target of AMLL therapy in the future.

In addition to its anti-apoptotic function, survivin also helps regulate cell-cycle progression during mitosis [20]. The highly proliferative activity of AMLL bone marrow cells as well as AML/ALL cells might be associated with survivin expression. As for the expression of IAPs in AML/ALL, the present study found strong expression in some cases and control levels in others, suggesting that AML/ALL cases are heterogeneous in terms of IAP expression.

The human *MLL* gene is involved in about 50 different chromosomal translocations associated with the acute leukemia phenotype [42]. Although chromosomal rearrangement involving chromosome 11q23 was not identified, the cases in the present study were not examined for the presence of *MLL* gene rearrangement by PCR analysis at the DNA level. Further studies are necessary to clarify the interaction of the *MLL* gene and IAP-family genes in association with apoptotic signaling in AMLL blasts.

In conclusion, we showed that strong expression of IAPs, especially survivin and NAIP, occurs in AMLL. Further studies are warranted to clarify the regulatory mechanisms of IAP expression in AMLL in association with drug resistance in this leukemia.

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Regimen-related toxicity following reduced-intensity stem-cell transplantation (RIST): comparison between Seattle criteria and National Cancer Center Common Toxicity Criteria (NCI-CTC) version 2.0

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Summary:

Acute regimen-related toxicity (RRT) is minimal in reduced-intensity stem-cell transplantation (RIST). However, the Seattle RRT grading (Bearman *et al*), developed in the context of conventional-intensity transplantation, is frequently applied to RIST. We compared the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 with the Seattle criteria after RIST in 86 patients. RRT within 30 days of transplant graded by both sets of criteria were significantly associated with the outcome confirming the predictive value of both the systems. A total of 15 patients died of disease progression, and 12 of transplant-related mortality: RRT ($n=2$), graft-versus-host disease (GVHD) ($n=7$), infection ($n=1$), and others ($n=2$). GVHD-related deaths primarily resulted from infections after steroid treatment ($n=6$) and bronchiolitis obliterans ($n=1$). This study shows that NCI-CTC is appropriate in toxicity evaluation of RIST, and that its application to RIST enables a toxicity comparison between RIST and other types of cancer treatments. Since GVHD is a significant problem in RIST, modifications are required to evaluate immunological complications following RIST.

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Reduced-intensity stem-cell transplantation (RIST) is associated with lower acute regimen-related toxicity (RRT).[1–6] The recognition and grading of toxicity caused by RIST is important in practice and in designing clinical trials.

After conventional-intensity stem-cell transplantation (CIST), patients can die of disease progression or complications of therapy. RRT is toxicity that is directly attributable to the conditioning regimen, but usually excludes graft-versus-host disease (GVHD), infection, and hemorrhage. It is often difficult to separate RRT from other toxicities. The Seattle group proposed a toxicity grading system specifically for allogeneic HSCT based upon a retrospective review of 195 patients who underwent CIST.[7] RRT in RIST is minimal and a significant proportion of morbidity and mortality is secondary to GVHD.[8] However, the Seattle criteria have been used to evaluate RIST.

The National Cancer Institute Common Toxicity Criteria version 2.0 (NCI-CTC ver. 2.0) has been widely used for development and evaluation of chemotherapeutic agents. If NCI-CTC ver. 2.0 can be applied to RIST, toxicity comparison between RIST and various other cancer treatments would be possible. We studied 86 patients who underwent RIST to see if NCI-CTC ver. 2.0 could be used to predict transplant-related mortality (TRM) and overall survival (OS) after RIST.

Patients and methods

Patients

The medical records of all of the patients ($n=86$) who underwent RIST at the National Cancer Center Hospital between January 1999 and April 2002 were reviewed. 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.

The median age was 51 years (range, 4–67). The underlying disease was AML ($n=26$), lymphoma ($n=21$), MDS ($n=11$), CML ($n=5$), ALL ($n=2$), other hematologic diseases ($n=3$), and solid tumors ($n=18$). The hematological malignancies were refractory to chemotherapy in 33 cases, and were in remission or sensitive to

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treatment in the remaining 35 cases. All of the patients with solid tumors were refractory to conventional treatments.

Preparative regimens

The preparative regimens comprised busulfan 4 mg/kg daily for 2 days with fludarabine 25 mg/kg daily for 6 days ($n = 64$)[9] or cladribine 0.11 mg/kg daily for 6 days ($n = 22$).[5] Rabbit antithymocyte globulin (ATG, Thymoglobulin, IMTIX-SANGSTAT, Lyons, France) 2.5 mg/kg for 2 or 4 days and TBI (4 Gy) were added to the preparative regimen in 49 and three patients, respectively.

Stem-cell source

A total of 64 patients had an HLA-identical related donor and 17 had a one-locus mismatched related donor.[10] Peripheral blood was used for these 81 patients. Five patients received bone marrow from a matched unrelated donor (MUD).

Prophylaxis and treatment of GVHD

Patients who were transplanted from an HLA-identical related donor received cyclosporin alone (3 mg/kg). Those who were transplanted from an HLA-mismatched related donor or MUD received cyclosporin and short-course methotrexate.

The diagnosis of GVHD was made on clinical grounds in conjunction with biopsy of the skin and digestive tract. Acute and chronic GVHD were graded according to the consensus criteria.[11,12] Grade II–IV acute GVHD was treated with 2 mg/kg/day of methylprednisolone in addition to cyclosporin.

Management of infections

All of the patients stayed in reverse isolation in a laminar airflow-equipped room, and received prophylaxis with trimethoprim/sulfamethoxazole or pentamidine inhaler, ciprofloxacin, and fluconazole against *Pneumocystis carinii*, bacterial, and fungal infection, respectively. Herpes virus prophylaxis with acyclovir was also given as previously described.[13] CMV pp65 antigenemia was routinely monitored once a week. When antigenemia was detected, preemptive therapy with ganciclovir was initiated as previously reported.[14]

Toxicity grading

The Seattle criteria assess post transplant RRT in eight organs: the heart, bladder, kidneys, lungs, liver, mucosa, central nervous system (CNS), and gut. As the criteria exclusively assess RRT, they exclude adverse events attributable to GVHD and infection. Similarly, renal failure is excluded when it coincides with the administration of known nephrotoxic agents. RRT was graded with the Seattle criteria on the day of initiation of conditioning regimens and days 0, 7, 14, and 28 (and on day 100 for lungs) post transplant (Table 1).

NCI-CTC ver. 2.0 assesses more than 260 adverse events in 24 organ systems. To make a comparison, 16 items in NCI-CTC ver. 2.0 equivalent to those in the Seattle criteria were regraded. These included arrhythmia, cardiovascular dysfunction, hematuria, renal dysfunction/creatinine levels, lung toxicity, serum levels of bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), weight gain/ascites, neurological dysfunction, mucositis, and diarrhea (Table 2). All of the observed adverse events were evaluated daily with NCI-CTC ver. 2.0 from the day of initiation of conditioning regimens until day 30 post transplant (and on day 100 for lungs).

Two or more independent physicians graded RRT based on the medical records. If there was discordance between their diagnoses, another physician (MK) made a final diagnosis.

Statistical analysis

The probability of OS was determined with the Kaplan–Meier method as of June 31, 2002. The median follow-up period after transplantation was 252 days (range, 82–1046 days). Surviving patients were censored on the last day of follow-up.

A univariate analysis using the χ^2 test and Mann–Whitney test were performed to identify the risk factors for RRT. A multivariable Cox proportional-hazards analysis was conducted to determine whether the development of RRT was independent of other clinical variables in predicting overall mortality. In RIST for solid tumors and malignant lymphoma, some lesions persist following preparative regimens. Sizes of these lesions frequently show a transient increase until the development of alloimmune responses. When patients die without disease progression, it is difficult to determine whether these deaths are attributable to disease progression or TRM. We therefore used overall mortality instead of nonrelapse deaths.

Clinical variables examined in a univariate analysis were entered in a backward, stepwise Cox proportional-hazards model to identify predictors of mortality. Variables with a *P*-value of less than 0.50 were entered into the model, and those with a *P*-value of less than 0.10 were retained. The *P*-values less than 0.05 were considered to be significant.

Results

Toxicity grading

Grade 3–4 toxicity by the Seattle criteria was observed in the lung (5%), CNS (2%), kidney (1%), and heart (1%) (Table 3). The maximal toxicity of grades 0, 1, 2, 3, and 4 was noted in eight (9%), 38 (44%), 35 (41%), four (5%), and one patient (1%), respectively.

Grade 3–4 toxicity by NCI-CTC ver. 2.0 was observed in all of the organs (Table 4): liver (31%), lung (21%), stomatitis (13%), gastrointestinal tract (9%), heart (6%), CNS (6%), kidney (2%), and bladder (1%). The maximal toxicity of grades 0, 1, 2, 3, and 4 was observed in two

Table 1 Regimen-related toxicity according to the Seattle criteria

Toxicity	*Grade 1*	*Grade 2*	*Grade 3*
Heart	Mild electrocardiogram abnormality, not requiring medical intervention; or noted heart enlargement on CXR with no clinical symptoms	Moderate electrocardiogram abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment; or congestive heart failure responsive to digitalis or diuretics	Severe electrocardiogram abnormalities with no or only partial response to medical intervention; or heart failure with no or only minor response to medical intervention: or decrease in voltage by more than 50%
Bladder	Macroscopic hematuria after 2 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection; or hematuria after 2 days with subjective symptoms of cystitis not caused by infection	Hemorrhagic cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedures
Kidney	Increase in creatinine up to twice the baseline value	Increase in creatinine above twice baseline but not requiring dialysis	Requirement of dialysis
Lung	Dyspnea without CXR changes not caused by infection or congestive heart failure; or CXR showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	CXR with extensive localized infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF; or decrease of PO_2 (>10% from baseline) but not requiring mechanical ventilation or >50% O_2 on mask and not caused by infection	Interstitial changes requiring mechanical ventilatory support or >50% oxygen on mask and not caused by infection or CHF
Liver	Mild hepatic dysfunction with 2.0 mg/dl <bilirubin <6.0 mg/dl or weight gain >2.5% and <5% from baseline, of noncardiac origin; or serum AST increase more than two-fold but less than five-fold from lowest preconditioning	Moderate hepatic dysfunction with bilirubin >6 mg/dl <20 mg/dl; or serum AST increase >five-fold from preconditioning; or clinical ascites or image-documented ascites >100 ml; or weight gain >5% from baseline of noncardiac origin	Severe hepatic dysfunction with bilirubin >20 mg/dl; or hepatic encephalopathy; or ascites compromising respiratory function
CNS	Somnolence but the patient is easily arousable and oriented after arousal	Somnolence with confusion after arousal; or other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding, or CNS infection	Seizures or coma not explained (documented) by other medication, CNS infection, or bleeding
Stomatitis	Pain and/or ulceration not requiring a continuous i.v. narcotic drug	Pain and/or ulceration requiring a continuous i.v. narcotic drug (morphine drip)	Severe ulceration and/or mucositis requiring preventive intubation; or resulting in documented aspiration pneumonia with or without intubation
GI toxicity	Watery stools >500 ml but <2000 ml every day not related to infection	Watery stools >2000 ml every day not related to infection; or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or subileus not related to infection	Ileus requiring nasogastric suction and/or surgery and not related to infection; or hemorrhagic enterocolitis affecting cardiovascular status and requiring transfusion

Grade IV regimen-related toxicity is defined as fatal toxicity.
CXR = chest X ray; i.v. = intravenous; CNS = central nervous system; GI = gastrointestinal; CHF = congestive heart failure.

Table 2 Regimen-related toxicity according to NCI-CTC version 2.0

Toxicity		*Grade 1*	*Grade 2*	*Grade 3*	*Grade 4*
Heart	Arrhythmia	Asymptomatic, not requiring treatment	Symptomatic, but not requiring treatment	Symptomatic and requiring treatment	Life-threatening (eg, arrhythmia associated with CHF, hypotension, syncope, shock)
	Ischemia/infarction	Nonspecific T-wave flattening or changes	Asymptomatic, ST- and T-wave changes suggesting ischemia	Angina without evidence of infarction	Acute myocardial infarction
	Left ventricular function	Asymptomatic decline of resting ejection fraction of > 10% but < 20% of baseline value; shortening fraction > 24% but < 30%	Asymptomatic but resting ejection fraction below LLN for laboratory or decline of resting ejection fraction > 20% of baseline value; < 24% shortening fraction	CHF responsive to treatment	Severe or refractory CHF or requiring intubation
Bladder	Hematuria	Microscopic only	Intermittent gross bleeding, no clots	Persistent gross bleeding or clots; may require catheterization or instrumentation, or transfusion	Open surgery or necrosis or deep bladder ulceration
Kidney	Renal dysfunction	Not defined	Not defined	Requiring dialysis, but reversible	Requiring dialysis and irreversible
	Creatinine levels	> ULN–1.5 × ULN	> 1.5–3.0 × ULN	> 3.0–6.0 × ULN	> 6.0 × ULN
Lung	Hypoxia	Not defined	Decreased O_2 saturation with exercise	Decreased O_2 saturation at rest, requiring supplemental oxygen	Decreased O_2 saturation, requiring pressure support (CPAP) or assisted ventilation
	Pneumonitis/pulmonary infiltrates	Radiographic changes but asymptomatic or symptoms not requiring steroids	Radiographic changes and requiring steroids or diuretics	Radiographic changes and requiring oxygen	Radiographic changes and requiring assisted ventilation
Liver	Bilirubin	> ULN–1.5 × ULN	> 1.5–3.0 × ULN	> 3.0–10.0 × ULN	> 10.0 × ULN
	Aspartate aminotransferase (AST)	> ULN–2.5 × ULN	> 2.5–5.0 × ULN	> 5.0–20.0 × ULN	> 20.0 × ULN
	Alanine aminotransferase (ALT)	> ULN–2.5 × ULN	> 2.5–5.0 × ULN	> 5.0–20.0 × ULN	> 20.0 × ULN
	Alkaline phosphatase (ALP)	> ULN–2.5 × ULN	> 2.5–5.0 × ULN	> 5.0–20.0 × ULN	> 20.0 × ULN
	Weight gain/asites	2–5%	5–10%	⩾ 10% or as ascites	⩾ 10% or fluid retention resulting in pulmonary failure
CNS	Depressed level of consciousness	Somnolence or sedation not interfering with function	Somnolence or sedation interfering with function, but not interfering with activities of daily living	Obtundation or stupor; difficult; interfering with activities of daily living	Coma
Stomatitis	Stomatitis/pharyngitis	Painless ulcers, erythema, or mild soreness in the absence of lesions	Painful erythema, edema or ulcers but can swallow	Painful erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support	Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia
GI toxicity	Diarrhea	Increase of < 4 stools/day over pretreatment	Increase of 4–6 stools/day, or nocturnal stools	Increase of ⩾ 7 stools/day or incontinence; or need for parenteral support for dehydration	Physiologic consequences requiring intensive care; or hemodynamic collapse

ULN = upper limit of normal values; LLN = lower limit of normal value; WNL = within normal limits; CHF = congestive heart failure; GI = gastrointestinal.

Table 3 Toxicity grading using the Seattle criteria in 86 patients undergoing RIST

Grade	*0*	*1*	*2*	*3*	*4*
Gut	69	17	0	0	0
Stomatitis	47	29	10	0	0
Central nervous system	78	4	2	2	0
Liver	23	31	31	1	0
Lung	75	4	3	3	1
Kidney	51	31	3	1	0
Bladder	84	2	0	0	0
Heart	70	8	7	1	0
Maximal grades	8	38	35	4	1

Table 4 Toxicity grading using NCI-CTC ver. 2.0 in 86 patients undergoing RIST

Grades	*0*	*1*	*2*	*3*	*4*
Gut	50	18	10	7	1
Stomatitis	35	8	32	1	0
Central nervous system	74	5	2	2	3
Liver	5	27	27	22	5
Lung	56	12	0	17	1
Kidney	47	26	11	2	0
Bladder	61	23	1	1	0
Heart	72	6	3	3	2
Maximal grades	2	16	25	35	8

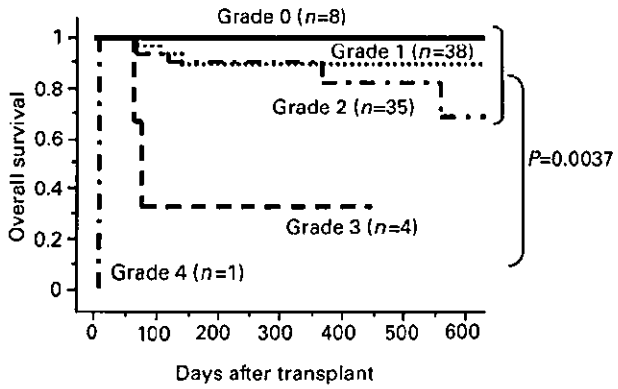

Figure 1 Overall survival evaluated by the Seattle criteria. Five patients had grade 3–4 toxicity in at least one organ, of whom three died (60%). The estimated 1-year OS was 25.0% (95% confidence interval (CI) 0.0–61.7%). In contrast, of the 81 patients with grade 0–2 toxicity in all the organ systems, eight died (9.9%). The estimated 1-year OS was 74.2% (95% CI, 64.2–84.2%). The one-year OS was significantly lower in the patients with grade 3–4 toxicity ($P = 0.0037$).

(2%), 16 (19%), 25 (29%), 35 (41%), and eight patients (9%), respectively.

The lung toxicity on day 100 using either the Seattle criteria or NCI-CTC ver. 2.0 was not maximal in any of the 86 patients.

There were 10 cases of the 'up-staging' of the toxicity from the Seattle criteria to NCI-CTC version 2.0: liver ($n = 6$), and kidney ($n = 4$).

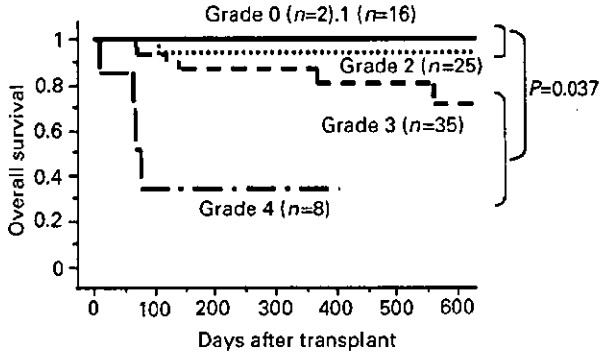

Figure 2 Overall survival evaluated by NCI-CTC version 2.0. In total, 43 patients had grade 3–4 toxicity in at least one organ, of whom 10 patients (23.2%) died of TRM. The estimated 1-year OS was 64.7% (95% CI, 50.2–79.2%). In contrast, of the remaining 43 patients with grade 2 toxicity in all the organ systems, one died of GVHD, resulting in 78.5% (95% CI, 64.8–92.2%) of estimated 1-year OS and 8.8% of TRM. The 1-year OS was significantly lower in the patients with grade 3–4 toxicity ($P = 0.037$).

Table 5 Variables influencing the grades of regimen-related toxicity according to NCI-CTC ver. 2.0

Variables	*Grade 0–2 (n = 43)*	*Grade 3–4 (n = 43)*	*P-value*
Age			
Median (range)	53 (4–65)	50 (19–67)	0.459
Sex			
Male/female	26/17	31/12	0.362
Risk of primary diseases			
High/low	13/30	20/23	0.183
Preparative regimens			
Fludarabine-based/cladribine-based	34/9	30/13	0.459
ATG-containing yes/no	22/21	27/16	0.384
TBI-containing yes/no	1/42	2/41	0.999
GVHD prophylaxis			
Cyclosporine alone/cyclosporine and methotrexate	38/5	32/11	0.165
Donors			
Related/unrelated	41/2	40/3	0.999
Matched/mismatched	37/6	32/11	0.278

ATG = anti-thymocyte globulin, TBI = total body irradiation, GVHD = graft-versus-host disease.
Any variables were significant on multivariate analysis.

Association between toxicity grading and survival following RIST

A total of 27 patients died: 16 of disease progression (19%) and 11 of TRM (13%). The 11 patients who died of TRM had the maximal toxicity of grade 2 ($n = 1$), grade 3 ($n = 6$), and grade 4 ($n = 4$) by NCI-CTC ver. 2.0, which was also graded with the Seattle criteria to be grade 1 ($n = 3$), grade 2 ($n = 5$), grade 3 ($n = 2$), and grade 4 ($n = 1$). The causes of TRM were GVHD/steroid-related infection ($n = 6$), GVHD (bronchiolitis obliterans) ($n = 1$), infection ($n = 2$), and

Table 6 Regimen-related toxicities (RRT) in studies reported previously

Authors/Reference	*n*	*Preparative regimens*	*Age years*	*Primary diseases*	*GVHD prophylaxis*	*RRT*				*Transplant-related mortality (TRM)*
						Criteria	*III–IV*	*III*	*IV*	
Carella *et al*[15]	15	Flu/CY[a]	34 (19–60)	HD, NHL	CSP/MTX	Details not described				Not described
Childs *et al*[3]	19	Flu/CY[b]	48 (37–65)	RCC	CSP	Details not described				2/19 (11%)
Giralt *et al*[16]	86	Flu/Mel[c] ($n=78$)	52 (22–70)	AML/MDS, CML, ALL/ lymphoma	FK506/MTX	Bearman's criteria	19	13	6	38.0%
		Clad/Mel[d] ($=8$)					12	5	7	88.0%
Khouri *et al*[4]	6	Flu/CY[a]	62 (51–71)	CLL	FK506/MTX	Details not described	0%	0%	0%	
	4	PFA[e]	55 (47–61)	Intermediate-grade lymphoma or in Richter's transformation						
	5	Flu/CY[f]	50 (47–57)	Low-grade lymphoma						
Khouri *et al*[17]	20	Flu/CY[a] or Flu/CY[f]	51 (31–68)	Follicular lymphoma	FK506/MTX	Bearman's criteria	0%[g]	0%	0%	3/20 (15%)
Slavin *et al*[2]	26	Flu/BU/ATG	33.5 (1–61)	Acute leukemia, chronic leukemia, NHL, MDS, MM, and genetic diseases	CSP	WHO criteria				4/26 (15%)
Nagler *et al*[18]	23	Flu/BU/ATG	41 (13–63)	HD, NHL	CSP	Bearman's criteria	17%	13%	4%	7/23 (30%)
McSweeney *et al*[6]	45	TBI 200 cGy	56 (31–71)	ALL, AML, CLL, CML, HD, MM, NHL, WM, CLL, MDS	CSP/MMF	Details not described[i]	0%	0%	0%	3/56 (5%)[h]
This study	86	Clad or Flu/ BU ± ATG[j]	51 (4–67)	AML, MDS, CML, ALL, lymphoma, TLBL, ATL, solid tumor	CSP or CSP/ MTX	Bearman's criteria	6%	5%	1%	11/86 (13%)
						NCI-CTC ver. 2.0	50%	41%	9%	

HD = Hodgkin's lymphoma; NHL = non-Hodgkin's lymphoma; RCC = renal cell carcinoma; AML = acute myeloid leukemia; MDS = myelodysplastic syndrome; CML = chronic myelogenous leukemia; ALL = acute lymphocytic leukemia; CLL = chronic lymphocytic leukemia, MM = multiple myeloma; WM = Waldenstrome's macroglobulinemia.

[a]Flu/CY: fludarabine 30 mg/m^2 with cyclophosphamide 300 mg/m^2 daily for 3 days.

[b]Flu/CY: fludarabine 25 mg/m^2 given daily for 5 days and cyclophosphamide 60 mg/kg for 2 days.

[c]Flu/Mel: fludarabine 25 mg/m^2 given daily for 5 days and melphalan 90 mg/m^2 or 70 mg/m^2 for 2 days.

[d]Clad/Mel: cladribine 12 mg/m^2 given daily for 5 days and melphalan 90 mg/m^2 or 70 mg/m^2 for 2 days.

[e]PFA: cisplatin 25 mg/m^2 daily for 4 days; fludarabine 30 mg/m^2; and cytarabine 500 mg/m^2 daily for 2 days.

[f]Flu/CY: fludarabine 30 mg/m^2 given daily for 5 days and cyclophosphamide 1000 mg/m^2 for 2 days.

[g]Moderate VOD was observed in two and was severe in two subjects.

[h]Four with disease progression died from DLI-induced GVHD and infections and three died of transplantation complications without disease progression.

[i]No patient experienced regimen-related painful mucositis, severe nausea and vomiting, pulmonary toxicity, cardiac toxicity, hemorrhagic cystitis, or new-onset alopecia.

[j]Flu/BU/ATG: fludarabine 30 mg/m^2 for 6 consecutive days, oral busulfan 4 mg/kg/day for 2 days, and anti-T-lymphocyte globulin 10 mg/kg/day for 4 days.

others ($n=2$). Maximal grades of GVHD in fatal cases were IV ($n=1$), III ($n=1$), II ($n=3$), and I ($n=2$).

Figure 1 demonstrates the association between the maximal toxicity of the Seattle criteria and OS. Five patients had grade 3–4 toxicity in at least one organ, of whom three died (60%). Estimated 1-year OS was 25.0% (95% confidence interval (CI) 0.0–61.7%). In contrast, of the 81 patients with grade 0–2 toxicity in all the organ systems, eight died (9.9%). The estimated 1-year OS was 74.2% (95% CI, 64.2–84.2%). The 1-year OS was significantly lower in the patients with grade 3–4 toxicity ($P=0.0037$).

Figure 2 demonstrates the association between the maximal toxicity of NCI-CTC ver. 2.0 and OS. In all, 43 patients had grade 3–4 toxicity in at least one organ, of whom 10 patients (23.2%) died of TRM. The estimated 1-year OS was 64.7% (95% CI, 50.2–79.2%). In contrast, of the remaining 43 patients with grade 2 toxicity in all the organ systems, one died of GVHD, resulting in 78.5% (95% CI, 64.8–92.2%) of estimated 1-year OS and 8.8% of TRM. The 1-year OS was significantly lower in the patients with grade 3–4 toxicity ($P=0.037$).

Variables influencing RRT

No variables were found to be associated with RRT of NCI-CTC ver. 2.0 by univariate (Table 5) or multivariate analysis.

Variables influencing overall survival

Patients who survived longer than 30 days were included in this analysis. Multivariate analysis showed that survival was significantly different between unrelated *vs* related donors (hazard ratio 7.5, 95% CI 1.7–32.8, $P=0.0074$), HLA-mismatched *vs* matched (hazard ratio 3.8, 95% CI 1.1–12.9, $P=0.0295$), and the maximal toxicity grade 3–4 *vs* grade 2–3 of NCI-CTC ver. 2.0 within day 30 post transplant (hazard ratio 3.0, 95% CI 1.2–7.3, $P=0.0177$).

Discussion

Evaluation of RRT after RIST is not uniform. As a result, toxicity grades vary among studies (Table 6) (2–4, 6, 15–18). Our study shows that both the Seattle criteria and NCI-CTC ver. 2.0 are significantly associated with outcome, and have predictive value.

The prognosis of grade 3 by the Seattle criteria is comparable to that of grade 4 by NCI-CTC ver. 2.0, and the prognosis of grade 2 by the Seattle criteria is comparable to that of grade 3 by NCI-CTC ver. 2.0 (Figures 1 and 2). However, neither criteria can offer a cutoff to predict death since the sensitivity and specificity are insufficient; the threshold of $\leqslant$grade 2 by Seattle criteria and $\leqslant$grade 3 by NCI-CTC ver. 2.0 would be sensitive but not specific to predict TRM, whereas the threshold of $\geqslant$grade 3 by the Seattle criteria and $\geqslant$grade 4 by NCI-CTC ver. 2.0 would be specific but not sensitive. These findings suggest that these criteria need to be modified for use in RIST.

There are two types of complications associated with allogeneic HSCT. One is the organ toxicity directly caused by preparative regimens. The other is immunological complications, represented by GVHD. When anti-T-cell antibodies are included in conditioning regimens, the frequency of GVHD is decreased[19,20] showing that GVHD is influenced by the types of preparative regimens. Given the fact that GVHD is the most common cause of nonrelapse death after RIST,[8] GVHD should be considered in the safety evaluation of conditioning regimens.

Another common complication after RIST is early progression of the underlying malignancy. This phenomenon could potentially be considered a consequence (and therefore toxicity) of the reduced intensity of the conditioning regimen.

Another consideration is the follow-up duration in evaluating immunological complications following RIST. The duration of observation after chemotherapy is usually 30 days. In contrast, the onset of GVHD can be delayed. The period of 30 days of observation is not long enough to evaluate the safety of RIST. Although the day 100 TRM has been used in RIST, it is not sufficient in evaluation of the immunological complications. We propose that TRM until day 200 should be used in the evaluation criteria for RIST-related toxicity.

Our study shows that both the Seattle criteria and NCI-CTC ver. 2.0 are useful in evaluating toxicity of RIST. Prospective studies are required to establish a proper toxicity grading system for RIST.

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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

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).

Outcomes	Analysis	Variables	HR (95% CI)	*P*-value
Neutrophils $>0.5 \times 10^9$/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 $>20 \times 10^9$/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

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

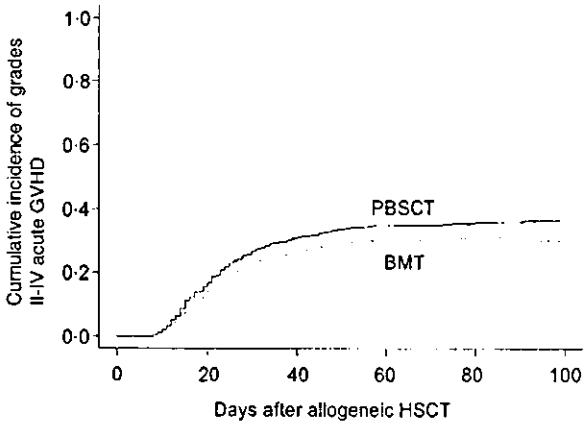

Fig 1. Cumulative incidences of grades II–IV acute graft *versus* host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model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

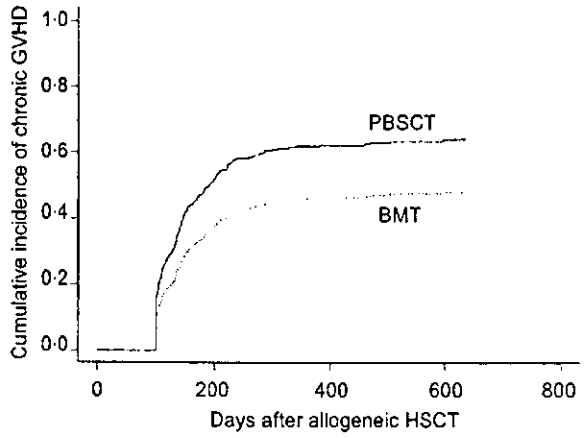

Fig 2. Cumulative incidences of any grade chronic graft *versus* host disease (GvHD) after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and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

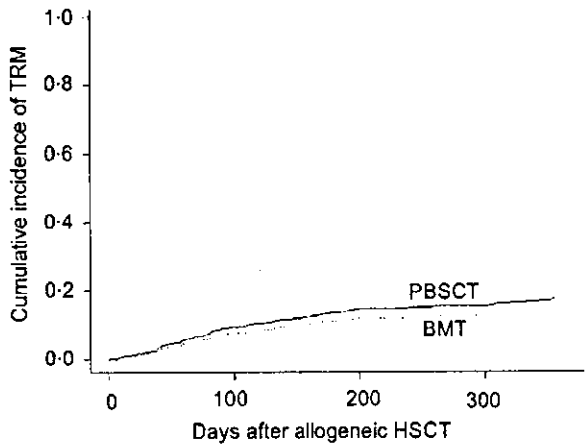

Fig 3. Cumulative incidences of treatment-related mortality after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions were predicted from the proportional subdistribution hazards model and adjusted for effects of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