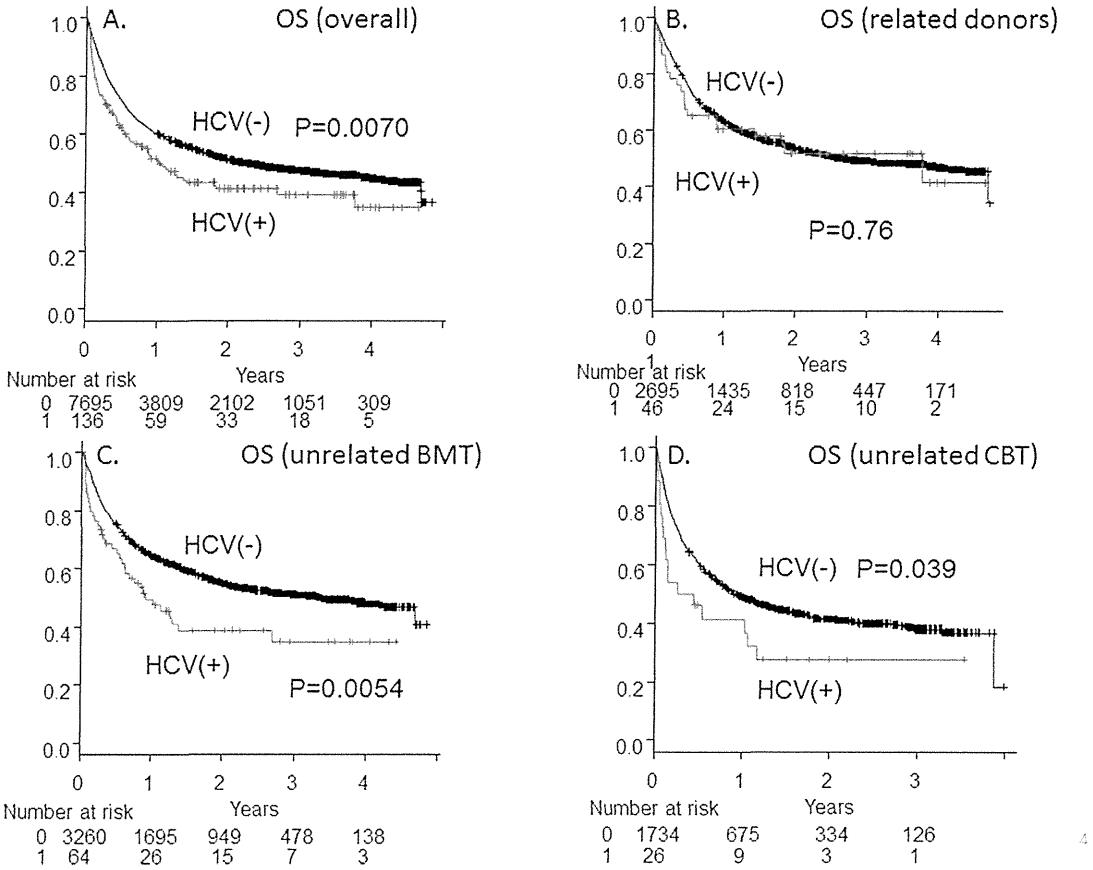

Figure 4. Comparison of the probabilities of overall survival between the HCV-positive and -negative groups: (A) overall patients, (B) in the subgroup of unrelated related donors, (C) in the subgroup of unrelated BMT, and (D) in the subgroup of unrelated CBT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of RIC, which is at least partially because the recent use of RIC and the development of supportive therapies might reduce the risk for SOS [24]. In addition, HCV is also known to alter intrahepatic cytokine profiles and deteriorate hepatic injuries [25,26]. Therefore, hepatic problems, including SOS and liver GVHD, might tend to become severe and fatal in recipients with HCV. Another possible explanation is that prolonged immunosuppression, particularly in unrelated BMT, may enhance the replication of HCV and liver dysfunction, which may result in fatal hepatitis or liver failure [23,27,28]. Otherwise, reduced and dysfunctional T cells after HCT might not be able to suppress HCV reactivation. A long-term observation reported that transplant recipients with HCV developed liver cirrhosis more rapidly than nontransplant patients with HCV [13]. The adverse impact of HCV on hepatic problems including cirrhosis and hepatocellular carcinoma might become further prominent after long-term follow-up even in our cohort.

To the best of our knowledge, this study is the first to show that pretransplant HCV infection was associated with a lower rate of platelet recovery. Thrombocytopenia is frequently observed in HCV-infected patients with chronic hepatitis [29]. The presence of hypersplenism and a low thrombopoietin level are thought to be responsible for the thrombocytopenia in HCV-infected patients [30]. In addition, HCV is known to infect CD34-positive hematopoietic progenitor cells and to suppress their maturation like megakaryocytes [31,32]. These facts might also contribute to the delay of platelet recovery following HCT in the HCV-positive group.

The current analysis further suggested that recipients with HCV would be susceptible to fatal bacterial infections, especially unrelated CBT recipients. An excess of bacterial infections in HCT recipients with HCV has been reported previously [13,14], and a similar susceptibility to bacterial infection has also been described in HCV-positive patients who receive solid organ transplantation or dialysis [33,34]. HCV-infected patients have been reported to have dysfunctional phagocytes, T cells, and B cells [35,36], as well as the impaired maturation of hematopoietic progenitor cells [31,32]. These findings suggest that the defense mechanisms against bacterial infections are impaired in recipients with HCV. The appropriate strategy for preventing infection in recipients with HCV should be explored further.

This analysis had several limitations as a result of its retrospective nature, and all information was based on the reports by attending physicians, not on a central review. The HCV-positive group might include more patients with a worse disease status, such as those who had to receive HCT despite the presence of HCV infection and liver dysfunction, although there were no differences in the patient backgrounds including disease risk, performance status, and EBMT score before HCT. In addition, the registry database had no information on baseline liver functions, viral loads of HCV, pathological grades, and the presence of cirrhosis at clinical events, which are supposed to be important for risk stratification of HCV-positive patients. Therefore, the HCV-positive group might include more recipients with highly damaged liver functions such as cirrhosis. However, when we analyzed the association

between HCV seropositivity and the presence of "hepatic-moderate/severe" in HCT-comorbidity index [37], on which we have started to gather information since 2008, we did not find any significant associations (data not shown), although HCV-seropositive patients in this cohort might be equal to patients with possible chronic HCV-hepatitis. On the other hand, strength of this study is that it involved the largest number of recipients with HCV of all studies to date and is the first to reveal the impact of HCV seropositivity on the clinical outcome of HCT in subgroups stratified according to the donor source. These findings in addition to liver functional tests could help a further risk stratification and management of HCV-positive recipients.

To date, the strategy against HCV infection, such as peginterferon and ribavirin therapy, has shown a sustained viral remission rate of close to 50% [38,39]. Regarding HCT recipients, treatment for HCV infection after HCT has been reported to be efficient in the specific population [40]. Recent progress in novel direct-acting antiviral agents might also be beneficial among HCV-positive recipients [41]. These anti-HCV therapies would play a critical role for the control and prevention of possible HCV-induced complications after HCT, as well as risk stratification by liver functional tests.

In summary, HCV seropositivity had an adverse impact on the clinical outcome following HCT, especially in the setting of unrelated HCT and in older patients. Careful evaluation before embarking on HCT and intensive assessment against complications are warranted in HCV-infected recipients. We may need to pay more attention to hematopoietic recovery and bacterial infections as well as hepatic problems in recipients with HCV. Based on these findings, a further prospective observation is warranted to overcome the adverse impact of HCV.

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Author Contributions

H.N. designed the study, analyzed data, and wrote the manuscript. S.K. and K.Y. gave advice regarding the methods, analyzed data, and wrote the manuscript. S.T., M.M., K.I., T.K., T.E., and K.M. collected data. H.S., Y.M., and T.N. collected data and were responsible for the management of data from JSHCT, JMDP, and JCBBN, respectively. R.S. analyzed and managed the unified registry database and wrote the manuscript. T.F. analyzed data, wrote the manuscript, and was responsible for the study and the Complication-WG of the JSHCT.

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TABLE III. Causes of death

	Overall			Related donors			Unrelated BMT			Unrelated CBT		
	HCV (136)	HCV-negative (7,695)	*P*	HCV (46)	HCV-negative (2,695)	*P*	HCV (64)	HCV-negative (3,260)	*P*	HCV (26)	HCV-negative (1,734)	*P*
Total death incidence	57% (77)	46% (3,571)	0.019	48% (22)	45% (1,222)	0.77	58% (37)	42% (1,383)	0.015	69% (18)	55% (962)	0.17
Fatal hepatic problems	8.1% (11)	2.2% (173)	0.00034	2.2% (1)	1.9% (52)	0.6	14% (9)	2.6% (85)	<0.0001	3.8% (1)	2.1% (36)	0.43
SOS	2.2% (3)	1.1% (82)	–	0.0% (0)	0.9% (24)	–	3.1% (2)	1.1% (37)	–	3.8% (1)	1.2% (21)	–
Liver aGVHD	0.7% (1)	0.1% (7)	–	0.0% (0)	0.1% (2)	–	1.6% (1)	0.2% (5)	–	0% (0)	0% (0)	–
Liver cGVHD	0% (0)	0.0% (2)	–	0.0% (0)	0.1% (2)	–	0% (0)	0% (0)	–	0% (0)	0% (0)	–
Hepatic failure due to uncertain cause	4.4% (6)	0.9% (71)	–	2.2% (1)	0.8% (22)	–	7.8% (5)	1.1% (36)	–	0% (0)	0.7% (13)	–
MOF with hepatic failure	0.7% (1)	0.1% (11)	–	0.0% (0)	0.1% (2)	–	1.6% (1)	0.2% (7)	–	0% (0)	0.1% (2)	–
Fatal bacterial infection	10% (14)	4.4% (336)	0.0048	6.5% (3)	3.2% (87)	0.19	7.8% (5)	3.9% (128)	0.11	23.1% (6)	7.0% (121)	0.0087
Death due to graft Failure	5.1% (7)	2.4% (187)	0.084	2.2% (1)	1.8% (48)	0.57	3.1% (2)	2.1% (68)	0.39	15% (4)	4.1% (71)	0.022

"–" indicates that statistical assessment was not performed in a subcategory of fatal hepatic problems.
BMT, bone marrow transplantation; CBT, cord blood transplantation; SOS, sinusoidal obstruction syndrome; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; MOF, multiple organ failure.

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Comparison of Intravenous with Oral Busulfan in Allogeneic Hematopoietic Stem Cell Transplantation with Myeloablative Conditioning Regimens for Pediatric Acute Leukemia

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Recent reports revealed that intravenous (iv) busulfan (BU) may not only reduce early nonrelapse mortality (NRM) but also improve overall survival (OS) probability in adults. Therefore, we retrospectively compared outcomes for 460 children with acute leukemia who underwent hematopoietic stem cell transplantation with either iv-BU (n = 198) or oral busulfan (oral-BU) (n = 262) myeloablative conditioning. OS at 3 years was 53.4% ± 3.7% with iv-BU and 55.1% ± 3.1% with oral-BU; the difference was not statistically significant ($P = .77$). OS at 3 years in 241 acute lymphoblastic leukemia and 219 acute myeloid leukemia patients was 56.4% ± 5.5% with iv-BU and 54.6% ± 4.1 with oral-BU ($P = .51$) and 51.0% ± 5.0% with iv-BU and 55.8% ± 4.8% with oral-BU ($P = .83$), respectively. Cumulative incidence of relapse at 3 years with iv-BU was similar to that with oral-BU (39.0% ± 3.6% and 36.4% ± 3.1%, respectively; $P = .67$). Cumulative incidence of NRM at 3 years was 16.6% ± 2.7% with iv-BU and 18.3% ± 2.5% with oral-BU ($P = .51$). Furthermore, multivariate analysis showed no significant survival advantage with iv-BU. In conclusion, iv-BU failed to show a significant survival advantage in children with acute leukemia.

INTRODUCTION

Busulfan (BU) is an alkylating agent that plays an important role in myeloablative preconditioning regimens in hematopoietic stem cell transplantation (HSCT) for patients with malignant diseases [1]. Although it has a potent antileukemic effect and excellent central nervous system penetration, wide inter- and intrapatient variability in absorption and metabolism has been observed with oral BU (oral-BU) [2]. However, its therapeutic potential has been compromised with unpredictable adverse events because overdosing leads to severe toxicity, and underdosing can potentially cause a relapse [3,4] or graft failure [5].

In children, BU is an important substitute for total body irradiation (TBI) [6], which is often associated with a higher incidence of late complications [7]; however, the range of heterogeneity in bioavailability with oral-BU is problematic. Blood concentrations and clearance may vary up to 6-fold among children receiving oral-BU [8,9], and age-dependent BU metabolism results in further complications. Therefore, therapeutic drug monitoring (TDM) of oral-BU has been considered an essential practice in children undergoing stem cell transplantation [10,11].

Intravenous BU (iv-BU) has recently replaced oral-BU because iv-BU avoids oral-BU's variable bioavailability. Furthermore, iv-BU showed less hepatic toxicity by avoiding the hepatic first-pass effect of oral-BU [12]. Previous reports revealed that the use of iv-BU reduced early

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Table 1
Patients' Characteristics

Characteristics	Total, n	iv-BU, n (%)	Oral-BU, n (%)	*P* Value
All patients	460	198	262	
Age at HSCT				.23
<1 yr	83	34	49	
1 to 10 yrs	278	128	150	
>10 yrs	99	36	63	
Gender				.19
Male	237	95	142	
Female	223	103	120	
Year of HSCT				<.001
2000 to 2003	159	4	155	
2004 to 2007	155	52	103	
2008 to 2010	146	142	4	
Underlying disease				.01
ALL, total	241	90	151	
B lineage	198	76	122	
T lineage	22	9	13	
Not determined	21	5	16	
AML, total	219	108	111	
M0	7	4	3	
M1	31	11	20	
M2	37	18	19	
M3	3	2	1	
M4	31	12	19	
M5	46	24	22	
M6	6	3	3	
M7	48	28	20	
Not determined	7	5	2	
Disease status				.29
CR1	205	85	120	
CR2	74	28	46	
>CR2 and non-CR	179	85	94	
Unknown	2	0	2	
Prior HSCT				
No	343	147	196	
Yes	117	51	66	
Donor type				<.001
Related donor	189	71	118	
BM	131	54	77	
PB	58	17	41	
Unrelated donor BM	67	43	24	
Cord blood	199	83	116	
Unknown	5	1	4	
Usage in preconditioning regimens				
CY	219	87	132	.19
VP16	150	59	91	.27
L-PAM	226	101	125	.51

ALL indicates acute myeloid leukemia; AML, acute myeloid leukemia; iv-BU, intravenous busulfan; oral-BU, oral busulfan; CR, complete remission; HSCT, hematopoietic stem cell transplantation; BM, bone marrow; PB, peripheral blood; CY, cyclophosphamide; VP16, etoposide; L-PAM, melphalan.

complications, including hepatic sinusoidal obstruction syndrome (SOS) [13,14], and decreased early nonrelapse mortality (NRM) [13-15]. Some reports demonstrated that iv-BU may provide better overall survival (OS) in adults with malignant diseases [14-17].

Although several reports have been published on children with iv-BU [18-20], the number of patients included was small, and the reports mainly focused on acute toxicity or early clinical outcome because of a short follow-up period. Therefore, the role of iv-BU in HSCT for children with acute leukemia is yet to be determined.

In this study, to compare the clinical outcome of HSCT with iv-BU and oral-BU, we performed a retrospective analysis in 460 children who underwent myeloablative conditioning regimens including BU after allogeneic transplantation for acute leukemia.

METHODS

This study was approved by the institutional ethics committee of Saitama Children's Medical Center. A total 460 patients were analyzed based on the data reported in the Japan Society for Hematopoietic Cell Transplantation registry [21] (Table 1). The patients were selected based on the following criteria: (1) patients diagnosed with either acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML); (2) age 15 years or younger when receiving HSCT; (3) BU-based myeloablative (more than 8 mg/kg) preconditioning regimens; and (4) HSCT performed between 2000 and 2010.

The OS probability was calculated using Kaplan-Meier estimates. Cumulative incidence curves were used in competing risks settings to calculate the probability of engraftment, graft-versus-host disease, and NRM. Univariate analyses of OS were performed using the log-rank test, and Gray's test was used for group comparisons of cumulative incidences. Multivariate analysis was performed using the Cox proportional hazard regression model, and the variables considered were patient's age, underlying diseases (ALL or AML), disease status (low risk: first complete remission [CR] or second CR, or high risk: nonremission or later than second CR), prior HSCT, donor type, and form of BU. All statistical analyses were performed using R software 2.13.0 (The R Foundation for Statistical Computing, Vienna, Austria). A 2-sided *P* value of less than .05 was considered to be statistically significant.

RESULTS

The patients' characteristics are listed in Table 1. The median age at HSCT was 4 years (range, 0 to 15 years). Of the 460 HSCT patients, 198 used iv-BU and 262 used oral-BU. The iv-BU replaced oral-BU in most of the cases after the iv-BU approval in Japan in 2006 (Table 1). The median follow-up period was 1828 days (range, 85 to 4619 days) after HSCT in all the surviving patients and 1185 days (range, 100 to 3759 days) after HSCT in the iv-BU patients.

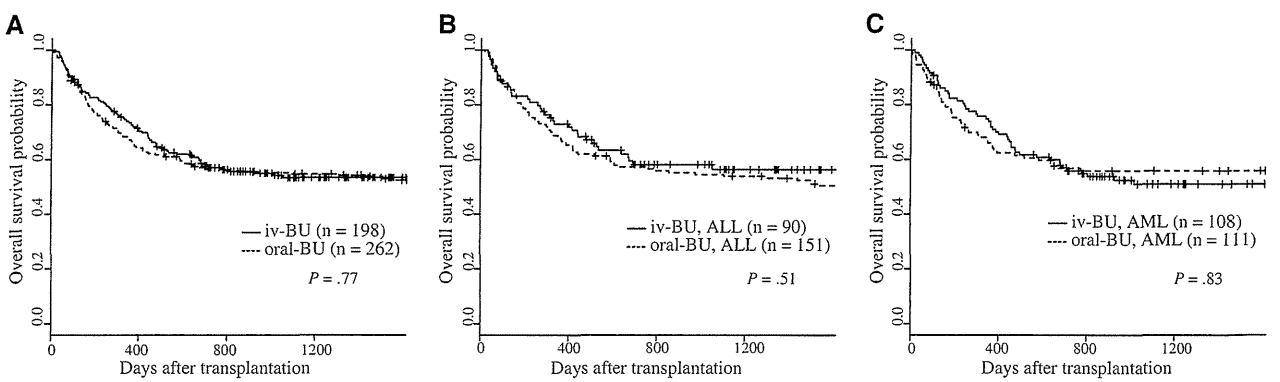

Figure 1. Overall survival probability of transplantation. Overall survival probability are shown according to busulfan form, (A) in all patients, (B) in patients with ALL, and (C) in patients with AML.

Table 2
Multivariate Analysis of the Risk Factors for Overall Mortality

Characteristics	No. of Patients	Overall Mortality	
		Hazard Ratio (95% CI)	P Value
Patient age at HSCT			
<1 yr	83	1	
1 to 9 yrs	278	1.18 (.76 to 1.86)	.45
>10 yrs	99	1.39 (.83 to 2.33)	.21
Underlying disease			
ALL	241	1	
AML	219	1.00 (.75 to 1.34)	.98
Disease status			
Low risk (CR1 and CR2)	279	1	
High risk (>CR2 and nonCR)	179	3.92 (2.86 to 5.39)	<.0001
Prior HSCT			
No	343	1	
Yes	117	1.47 (1.06 to 2.03)	.02
Donor type			
Related donor	189	1	
Unrelated donor (bone marrow)	67	1.09 (.72 to 1.64)	.68
Cord blood	199	1.22 (.90 to 1.66)	.19
Form of busulfan			
Oral	262	1	
Intravenous	198	.80 (.60 to 1.06)	.12

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; CI, confidence interval; CR, complete remission; HSCT, hematopoietic stem cell transplantation.

The estimated OS probability and standard error at 3 years after HSCT was 54.6% ± 2.4%, whereas the cumulative incidence of relapse and NRM were 37.5% ± 2.3% and 17.6% ± 1.8%, respectively.

Although OS with iv-BU and oral-BU at day 100 after HSCT was 72.5% ± 3.2% and 66.9% ± 2.9%, respectively, OS at 3 years after HSCT was similar (iv-BU, 53.4% ± 3.7%; oral-BU, 55.1% ± 3.1%), and the log-rank test for OS did not show a statistically significant difference ($P = .77$) (Figure 1A). The result was concordant even when the analysis was limited to patients with ALL or AML. OS at 3 years for patients with ALL using iv-BU (n = 90) and oral-BU (n = 151) was 56.4% ± 5.5% and 54.6% ± 4.1%, respectively ($P = .51$) (Figure 1B). OS at 3 years for patients with AML using iv-BU (n = 108) and oral-BU (n = 111) was 51.0% ± 5.0% and 55.8% ± 4.8%, respectively ($P = .83$) (Figure 1C).

The similarity of OS was reproduced even with the limited cohort of 247 patients who received HSCT after the first CR or second CR without prior HSCT. OS at 3 years was 78.3% ± 4.2% for iv-BU patients (n = 98) and 78.7% ± 3.4% for oral-BU patients (n = 149) and the difference was not statistically significant ($P = .66$).

In addition, the hazard ratio of overall mortality between iv-BU and oral-BU was not statistically significant based on the multivariate analysis (Table 2).

The cumulative incidence curve of relapse after 3 years of iv-BU (39.0% ± 3.6%) was superimposed on the oral-BU curve (36.4% ± 3.1%) (Figure 2A). The difference did not show statistical significance after limiting the analyses to each disease. Relapse incidence at 3 years was 41.7% ± 5.5% with iv-BU and 39.1% ± 4.0% with oral-BU in the ALL cohort ($P = .56$) (Figure 2B), and 37.0% ± 4.8% with iv-BU and 33.6% ± 4.7% with oral-BU in the AML cohort ($P = .70$) (Figure 2C).

The cumulative incidence of NRM at 100 days after HSCT was 6.8% ± 1.8% with iv-BU and 8.3% ± 1.7% with oral-BU, and NRM at 3 years after HSCT was 16.6% ± 2.7% with iv-BU and 18.3% ± 2.5% with oral-BU ($P = .51$) (Figure 3A). The SOS occurrence was evaluable in 173 patients. Twenty-seven (30.3%) of 89 patients using iv-BU and 23 (27.4%) of 84 patients using oral-BU had SOS ($P = .74$). One patient using iv-BU and 4 patients using oral-BU succumbed to SOS. No significant difference in the causes of death was noted between the iv-BU and oral-BU groups.

The iv-BU group showed a tendency toward higher engraftment probability at day 60 (96.0% ± 1.5%) compared with the oral-BU group (89.3% ± 2.0%), but the difference was not statistically significant ($P = .22$) (Figure 3B). The cumulative incidence of acute graft-versus-host disease (grade II to IV) at day 100 was similar (iv-BU, 37.7% ± 3.5%; oral-BU, 35.4% ± 3.0%; $P = .98$).

DISCUSSION

Busulfan is widely used as an alternative myeloablative agent to TBI. Although previous randomized studies and a meta-analysis comparing BU with TBI revealed that TBI-based regimens were at least as good for survival and disease-free survival [22], it should be noted that majority of BU in these studies was oral-BU, and recent reports showed that targeted BU with TDM or iv-BU could improve HSCT outcome [10,11,14-17,23]. However, this study included the largest number of children to date and demonstrated that the advantage of iv-BU on survival probability was not significant. The result was reproduced in subgroups, such as patients with ALL, patients with AML, and patients who received iv-BU at first or second CR without prior HSCT.

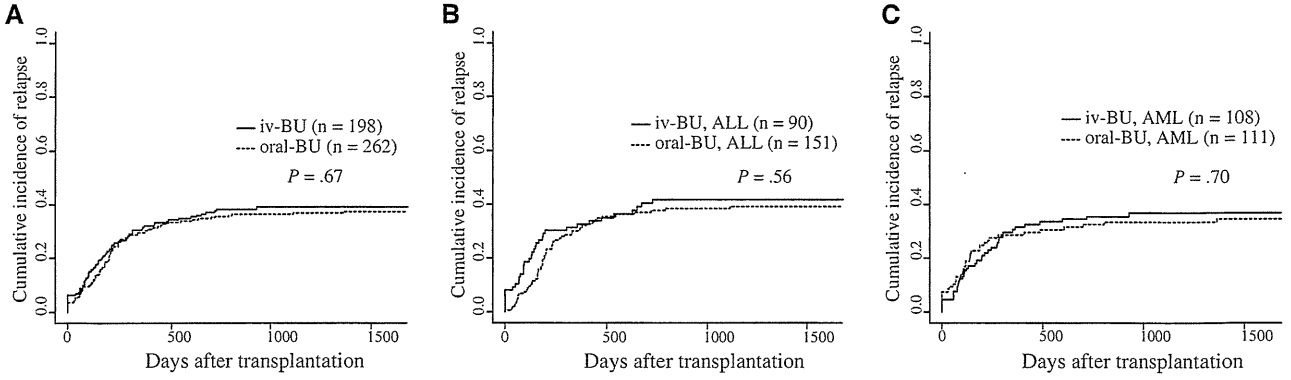

Figure 2. Cumulative incidences of relapse. Cumulative incidences of relapse are shown according to busulfan form, (A) in all patients, (B) in patients with ALL, and (C) in patients with AML.

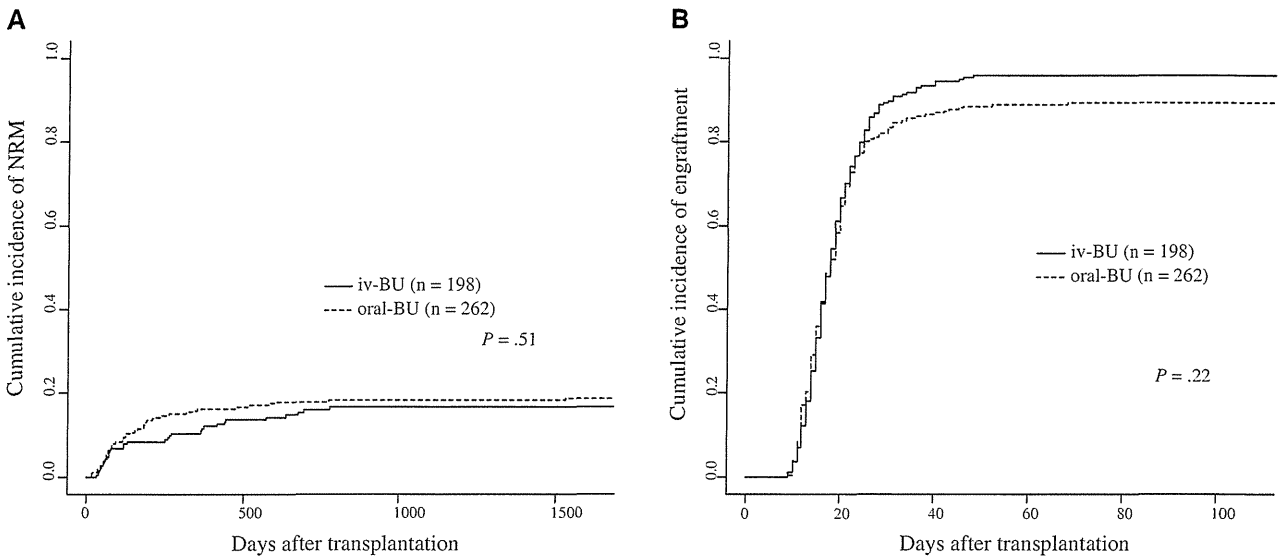

Figure 3. Cumulative incidences of nonrelapse mortality and engraftment. Cumulative incidences of (A) nonrelapse mortality and (B) engraftment are shown.

Moreover, multivariate analysis failed to show any survival benefit with iv-BU.

Our results regarding the iv-BU usage were discordant with the previous studies in adults. Although the reason is unclear, a possible explanation could be that the optimal dosing was already achieved in most patients, even those on oral-BU. In children, TDM of oral-BU has been considered an essential practice [10,11]. Therefore, the administration dose in oral-BU patients was usually determined based on the results of the test dose administration result and it was also adjusted according to TDM result.

Dosing schedule based on body weight using iv-BU provided adequate therapeutic targeting in children [24]. In our study, iv-BU failed to show superior outcomes compared with oral-BU, but it could provide a comparable survival outcome with a reduced requirement for TDM.

Concordant with previous studies, short-term NRM and OS in our study seemed to be superior in the iv-BU group compared with the oral-BU group, although it was caused by improvement of support therapy. Therefore, iv-BU may be advantageous for patients with high risk for treatment-related mortality, such as poor performance status, uncontrolled infectious diseases, or organ dysfunction.

This retrospective study, using the registry data, has some limitations. For example, the selection of iv-BU or oral-BU was strongly associated with the transplantation period, which may have introduced bias. Further prospective studies are required to establish an optimal allogeneic HSCT treatment strategy for children with acute leukemia.

In conclusion, our study provides valuable information on the role of iv-BU in myeloablative HSCT for pediatric acute leukemia. In children, iv-BU failed to improve the survival outcome of acute leukemia.

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Authorship statement: M.K, Y.T, K.Kudo and K.Kato designed the research. J.I, K.Koh, A.O, K.O, H.S, H.Y, K.Kawa, R.S and K.Kato collected the data. M.K analyzed the data, and M.K, Y.T, D.T, Y.O, K.Kudo and K.Kato wrote the manuscript. All authors discussed the results and commented on the manuscript.

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Pediatr Blood Cancer 2013;60:1513–1519

PBSCT Is Associated With Poorer Survival and Increased Chronic GvHD Than BMT in Japanese Paediatric Patients With Acute Leukaemia and an HLA-Matched Sibling Donor

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Background. Peripheral blood stem cells (PBSC) may be used as an alternative to bone marrow (BM) for allogeneic transplantation. Since peripheral blood stem cell bank from unrelated volunteer donor has been started in Japan, use of PBSC allografts may be increased. Therefore we surveyed the outcomes of Japanese leukemia children after PBSC and BM transplantation. ***Procedure.*** This retrospective study compared the outcomes of 661 children (0–18 years) with acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) who received their first allogeneic peripheral blood stem cell transplantation (PBSCT; n = 90) or bone marrow transplantation (BMT; n = 571) from HLA-matched siblings between January 1996 and December 2007. ***Result.*** Neutrophil recovery was faster after PBSCT than after BMT (ALL: $P < 0.0001$; AML: $P = 0.0002$), as was platelet recovery (ALL: $P = 0.0008$; AML: $P = 0.0848$). However, the cumulative incidence of chronic graft-versus-host disease (GvHD) was higher after PBSCT than after BMT (ALL: 26.0% vs. 9.9%, $P = 0.0066$; AML: 41.6% vs. 11.1%, $P < 0.0001$). The 5-year disease-free survival (DFS) was lower after PBSCT than after BMT for ALL (40.6% vs. 57.1%, $P = 0.0257$). The 5-year overall survival (OS) was lower after PBSCT than after BMT for ALL (42.4% vs. 63.7%, $P = 0.0032$) and AML (49.8% vs. 71.8%, $P = 0.0163$). Multivariate analysis revealed the use of PBSC was a significant risk factor for DFS and OS. PBSCT and BMT did not differ in relapse rate, acute GvHD for ALL and AML, or in DFS for AML. ***Conclusion.*** PBSC allografts in Japanese children engraft faster but are associated with poorer survival and increased chronic GvHD. Pediatr Blood Cancer 2013; 60:1513–1519.

Key words: acute leukaemia; bone marrow transplantation; children; chronic graft-versus-host disease; peripheral blood stem cell transplantation

INTRODUCTION

Allogeneic peripheral blood stem cell (PBSC) transplantation (PBSCT) was established along with allogeneic bone marrow (BM) transplantation (BMT) in the last decade [1–7]. In October 2010, a bank that stores PBSC from unrelated volunteer donors was established in Japan. Other progress in this area in Japan relates to the registration of haematopoietic stem cell transplantation (HSCT), which, until five years ago, involved four separate registry organisations. However, in 2006, the registers of these organisations were computerised and unified under the Transplantation Registry Unified Management Program (TRUMP) [8]. In 2008, the HSCT data of paediatric patients that had been stored on paper in the four registries were entered into TRUMP electronically. TRUMP has thus made it possible to analyse the paediatric HSCT data with greater accuracy.

While several prospective and retrospective randomised controlled trials (RCTs) and meta-analyses that compared BMT and PBSCT have been published [3,4,6,9–15], most have focused on adult patients only. To survey the outcomes of Japanese children after allogeneic HSCT from related donors, TRUMP data were used to conduct a retrospective, multi-centre study that compared the outcomes of 661 paediatric patients with leukaemia after their transplantation with allogeneic PBSC or BM from HLA-matched siblings. The impact of chronic graft-versus-host disease (GvHD) after transplantation was also examined.

PATIENTS AND METHODS

Study Population

The Japan Society for Haematopoietic Cell Transplantation uses a standardised reporting form to collect the data of individual transplant

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Conflict of interest: Nothing to declare.

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patients from each transplant centre. Follow-up reports are also submitted annually after transplantation. Between January 1996 and December 2007, the data of 1,048 paediatric patients with acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) who underwent a myeloablative preparative regimen and allogeneic BMT or PBSCT from a family donor were reported to the Japan Society for Haematopoietic Cell Transplantation. Patients were excluded from the study if their data were incomplete (n = 20), if they had received BM together with PBSC (n = 15), if they received grafts from father or mother (n = 322), or if they received grafts from anyone other than an HLA-identical sibling or a sibling whose HLA matched in all but one antigens (n = 30). As a result, 661 patients were included in this study. This study was approved by the Data Management Committee for the Nationwide Survey of the Japan Society for Haematopoietic Cell Transplantation.

Methods of HLA Typing

The method of HLA typing varied from time to time among participated institutes. In general, serological or low-resolution HLA typing was performed by lymphocyte cytotoxicity test until 2003 and reversed SSO after 2003. High-resolution HLA typing was done for class II alleles by PCR-RFLP from 1992 to 2000, and done by SBT for class I and II after 2000.

Engraftment Evaluation

Neutrophil engraftment was defined as an absolute neutrophil count greater than 0.5×10^9/L occurring on the first of three consecutive days. Platelet engraftment was defined as the first day with a platelet count greater than 50×10^9/L without further need for platelet transfusions.

End Points and Definition

End points were assessed on the date of last patient contact and were analysed on the 31 October 2009. The study end points were relapse rate (RR), disease-free survival (DFS), overall survival (OS), acute and chronic GvHD and transplantation-related mortality (TRM). Acute GvHD was graded according to standard criteria by the attending physicians of each hospital [16]. Chronic GvHD was graded as limited (localised skin or single organ involvement) or clinically extensive [17]. The reported causes of death were reviewed and categorised and TRM was defined as death with no evidence of disease. DFS was defined as survival without evidence of relapse, the event under study being death or relapse. Patients who died as a result of relapse or disease progression after transplantation were considered to have died of their original disease.

Risk status at transplantation was categorised as either standard or high. Standard-risk (SR) disease included ALL in first or second complete remission (CR1, CR2) except when it was Philadelphia chromosome-positive [Ph (+)] or exhibited mixed-lineage leukaemia gene (MLL) rearrangement. SR disease also included AML in CR1 or CR2. All other diseases status were categorised as high-risk (HR) disease.

Statistical Analysis

The cumulative incidences of neutrophil and platelet recovery, acute and chronic GvHD, relapse, survival and TRM of the PBSCT and BMT groups were compared by using the log-rank test to compare Kaplan and Meier curves. The 95% confidence intervals (CIs) were calculated by using the Greenwood formula [18]. The significance of all other differences between both groups was estimated by using the Chi-square test for categorical variables and the Kruskal–Wallis test for continuous variables. Multivariate Cox analysis [19] was used to study the potential effect of the following factors on OS and DFS: age, diagnosis, disease risk, stem cell source and donor-recipient HLA compatibility.

RESULTS

Patient Characteristics

The data of 661 paediatric patients with acute leukaemia who underwent their first stem cell transplantation were analysed. The patients were divided according to whether they had ALL (n = 411) or AML (n = 250). The characteristics of these two groups are shown in Table I. Of the ALL group, 60 (15%) received PBSCT and 351 (85%) received BMT. Of the AML group, 30 (12%) received PBSCT and 220 (88%) received BMT. The ages of both the ALL and AML groups ranged from 0 to 18 years and the median ages were both 9.0 years. For the ALL group, 247 (60%) and 164 (40%) had SR and HR disease respectively, while 202 (81%) and 48 (19%) of the AML group had SR and HR disease, respectively. The PBSC and BM recipients in the ALL and AML groups did not differ in terms of gender (ALL, $P = 0.28$; AML, $P = 0.23$) or conditioning regimen (ALL, $P = 0.37$; AML, $P = 0.42$). But for the ALL group, the PBSC recipients were significantly older than the BM recipients (ALL, $P < 0.01$; AML, $P = 0.20$).

These ALL and AML groups were also divided further according to the disease risk into SR and HR subgroups. For all four subgroups, the gender ratios of the PBSC and BM recipients did not differ significantly (ALL SR, $P = 0.29$; ALL HR, $P = 0.93$; AML SR, $P = 0.64$; AML HR, $P = 0.23$). The ages of the PBSC and BM recipients did not differ significantly either except for in the ALL HR subgroup, where PBSCT was associated with a significantly higher median age than BMT (ALL SR, $P = 0.13$; ALL HR, $P < 0.01$; AML SR, $P = 0.12$; AML HR, $P = 0.89$).

Engraftment Associated With PBSCT and BMT in ALL and AML

In the ALL group, 59/60 and 350/351 patients after PBSCT and BMT survived more than 28 days, while in the AML group, 30/30 and 220/220 patients after PBSCT and BMT survived more than 28 days. Of these surviving patients in ALL, engraftment occurred in 58 (98.3%) patients after PBSCT and 346 (98.9%) patients after BMT. Of the surviving patients in AML, 29 (96.7%) patients after PBSCT and 219 (99.5%) patients after BMT exhibited engraftment.

The median times to recovering a neutrophil count of $>0.5 \times 10^9$/L for the ALL and AML groups and their SR and HR subgroups are shown in Figure 1. For the ALL group, PBSCT and BMT were associated with median times of 13 and 16 days ($P < 0.0001$). Similarly, the median times after PBSCT and BMT in the ALL SR subgroup were 13 and 16 days ($P < 0.0001$), respectively. For the ALL HR subgroup, these times were 13 and 16 days ($P = 0.0003$), respectively. The median times after PBSCT and BMT in the AML group were 12.5 and 17 days, respectively ($P = 0.0002$), while for the AML SR subgroup, they were 12 and 17 days, respectively ($P < 0.0001$). However, in the AML HR subgroup, PBSCT (14.5 days), did not differ significantly from BMT (18 days) in terms of neutrophil recovery ($P = 0.1795$). Thus, PBSCT was associated with

TABLE I. Patient Characteristics

	ALL (n=411)				AML (n=250)			
	PBSC	BM	*P*-value	Total	PBSC	BM	*P*-value	Total
Number of patients	60 (15)	351 (85)		411	30 (12)	220 (88)		250
Median age, years [range]	11.0 [4–18]	8.0 [0–18]	<0.01	9.0 [0–18]	11.5 [0–17]	9.0 [0–18]	0.20	9.0 [0–18]
Sex (male/female)	33/27	222/129	0.28	255/156	14/16	132/88	0.23	146/104
Risk group			0.03				<0.01	
Standard risk	28 (47)	219 (62)		247 (60)	18 (60)	184 (84)		202 (81)
High risk	32 (53)	132 (38)		164 (40)	12 (40)	36 (16)		48 (19)
Conditioning regimen			0.37				0.42	
TBI-based	44 (73)	279 (79)		323 (79)	11 (37)	102 (46)		113 (45)
Chemotherapy-based	16 (27)	72 (21)		88 (21)	19 (63)	118 (54)		137 (55)
GVHD prophylaxis								
CSA+short MTX	32 (53)	167 (48)		199 (48)	18 (60)	108 (49)		126 (50)
CSA alone	9 (15)	49 (14)		58 (14)	5 (17)	23 (10)		28 (11)
Short MTX alone	7 (12)	94 (27)		101 (25)	1 (3)	65 (30)		66 (26)
FK506+short MTX	5 (8)	15 (4)		20 (5)	0 (0)	8 (4)		8 (3)
Standard risk								
Median age, years [range]	10.5 [4–17]	8.0 [1–18]	0.13	8.0 [1–18]	12.0 [0–17]	9.0 [0–18]	0.12	9.0 [0–18]
Sex (male/female)	15/13	144/75	0.29	159/88	9/9	108/76	0.64	117/85
High risk								
Median age, years [range]	11.0 [5–18]	8.0 [0–18]	<0.01	9.0 [0–18]	9.0 [1–16]	10.0 [0–17]	0.89	10.0 [0–17]
Sex (male/female)	18/14	78/54	0.93	96/68	5/7	24/12	0.23	29/19

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BM, bone marrow; PBSC, peripheral blood stem cell; TBI, total body irradiation; CSA, cyclosporine; MTX, methotrexate. Standard-risk disease includes ALL in first or second complete remission (unless Philadelphia chromosome positivity or mixed-lineage leukemia gene rearrangement was present) and AML in first or second complete remission. High-risk disease includes all other diseases statuses. Values are given as n (%).

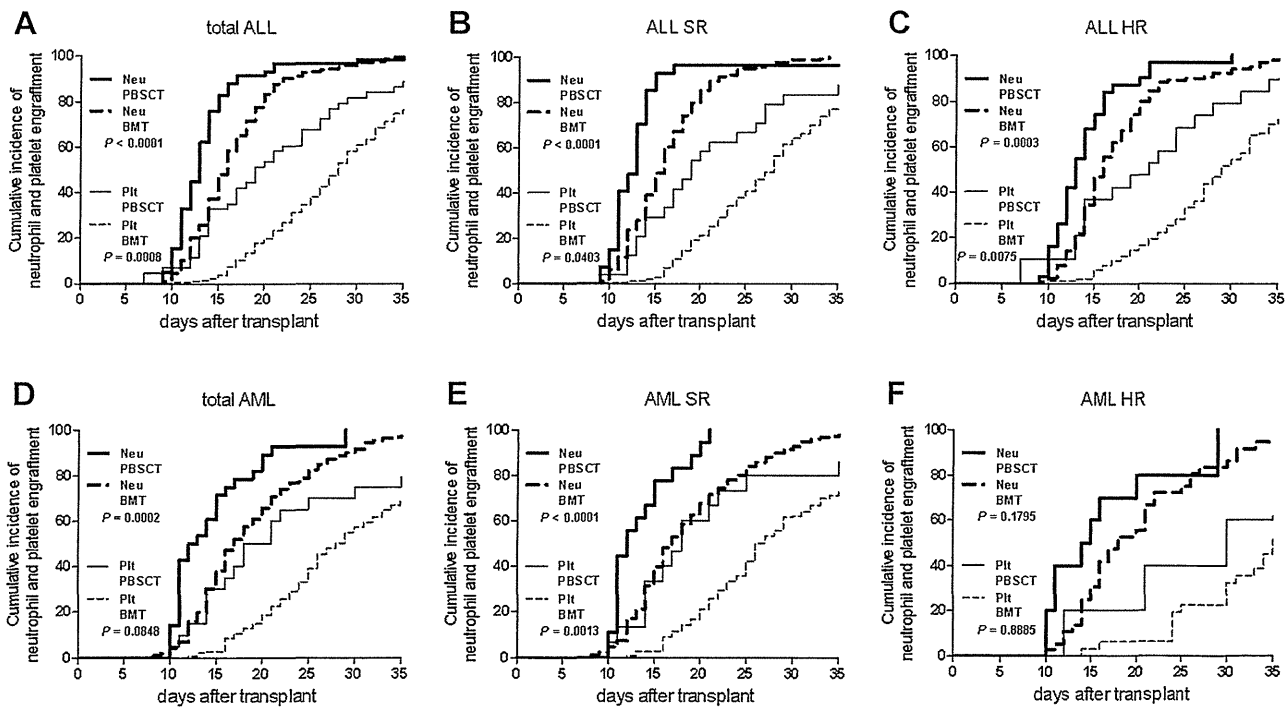

Fig. 1. Engraftment to neutrophil count $>0.5 \times 10^9$/L and engraftment to platelet count $>50 \times 10^9$/L. **A**: total ALL; (**B**) ALL standard-risk; (**C**) ALL high-risk; (**D**) total AML; (**E**) AML standard-risk; and (**F**) AML high-risk.

significantly faster neutrophil recovery than BMT in total ALL, ALL SR, ALL HR, total AML and AML SR.

As shown in Figure 1, for the ALL group, the median times to recovering a platelet count of $>50 \times 10^9$/L after PBSCT and BMT were 19 and 28 days, respectively ($P = 0.0008$). For the ALL SR subgroup, the times after PBSCT and BMT were 19 and 28 days, respectively ($P = 0.0403$), while for the ALL HR subgroup, the times were 21 and 29 days, respectively ($P = 0.0075$). For the AML group, these times after PBSCT and BMT were 19.5 and 28 days, respectively ($P = 0.0848$), while the times for the AML SR subgroup were 18 and 26.5 days, respectively ($P = 0.0013$). For the AML HR subgroup, these times were 30 and 35 days, respectively ($P = 0.8885$). Thus, PBSCT was associated with significantly faster platelet recovery than BMT in the ALL group, ALL SR, ALL HR and AML SR.

Relapse Rate, Disease-Free Survival and Overall Survival Associated With PBSCT and BMT in ALL and AML

As shown in Supplementary Figure 1, the RR after PBSCT and BMT did not differ significantly for ALL and AML or their SR and HR subgroups (total ALL, $P = 0.0663$; ALL SR, $P = 0.0977$; ALL HR, $P = 0.7708$; total AML, $P = 0.1549$; AML SR, $P = 0.4334$; AML HR, $P = 0.9871$).

PBSCT and BMT in the ALL group were associated with 5-year DFS values of 40.6% and 57.1%, respectively ($P = 0.0257$; Fig. 2A) and with OS values of 42.4% and 63.7%, respectively ($P = 0.0032$; Fig. 2A). In the AML group, PBSCT and BMT were associated with 5-year DFS values of 52.9% and 67.4%, respectively ($P = 0.0677$; Fig. 2D) and OS values of 49.8% and 71.1%, respectively ($P = 0.0163$; Fig. 2D). In ALL SR, PBSCT and BMT were associated with 5-year DFS values of 59.1% and 70.5%, respectively ($P = 0.2584$; Fig. 2B) and OS values of 58.2% and 76.9%, respectively ($P = 0.0579$; Fig. 2B). In ALL HR, PBSCT and BMT were associated with 5-year DFS values of 23.0% and 34.0%, respectively ($P = 0.2930$; Fig. 2C) and OS values of 28.8% and 40.1%, respectively ($P = 0.2507$; Fig. 2C). In AML SR, PBSCT and BMT were associated with 5-year DFS values of 71.8% and 72.5%, respectively ($P = 0.8519$; Fig. 2E) and OS values of 68.6% and 77.3%, respectively ($P = 0.6297$; Fig. 2E). In AML HR, PBSCT and BMT were associated with 5-year DFS values of 25.0% and 40.0%, respectively ($P = 0.4275$; Fig. 2F) and OS values of 22.2% and 43.2%, respectively ($P = 0.1339$; Fig. 2F).

As shown in Table II, multivariate Cox analysis revealed that PBSCT was significant risk factor for a poorer DFS (HR = 1.37; 95% CI, 1.01–1.88; $P = 0.044$) and OS (HR = 1.51; 95% CI, 1.09–2.09; $P = 0.013$). In addition, having HR disease was found to be a significant adverse risk factor for DFS (HR = 3.32; 95% CI, 2.59–4.26; $P < 0.001$) and OS (HR = 3.58; 95% CI, 2.73–4.70; $P < 0.001$).

Acute GvHD Associated With PBSCT and BMT in ALL and AML

As shown in Supplementary Figure 2, PBSCT was associated with higher cumulative incidences of grades II–IV acute GvHD than BMT only in AML HR (57.6% vs. 23.2%, $P = 0.0264$) but not in the ALL group (31.2% vs. 21.8%, $P = 0.0826$), ALL SR (26.0% vs. 19.7%, $P = 0.4255$), ALL HR (34.9% vs. 25.4%, $P = 0.1784$), the AML group (33.9% vs. 18.0%, $P = 0.0506$) and AML SR (15.4% vs. 17.1%, $P = 0.8503$). Six patients died of grade IV acute GvHD: one patient with ALL after PBSCT, two patients with ALL after BMT and three patients with AML after BMT.

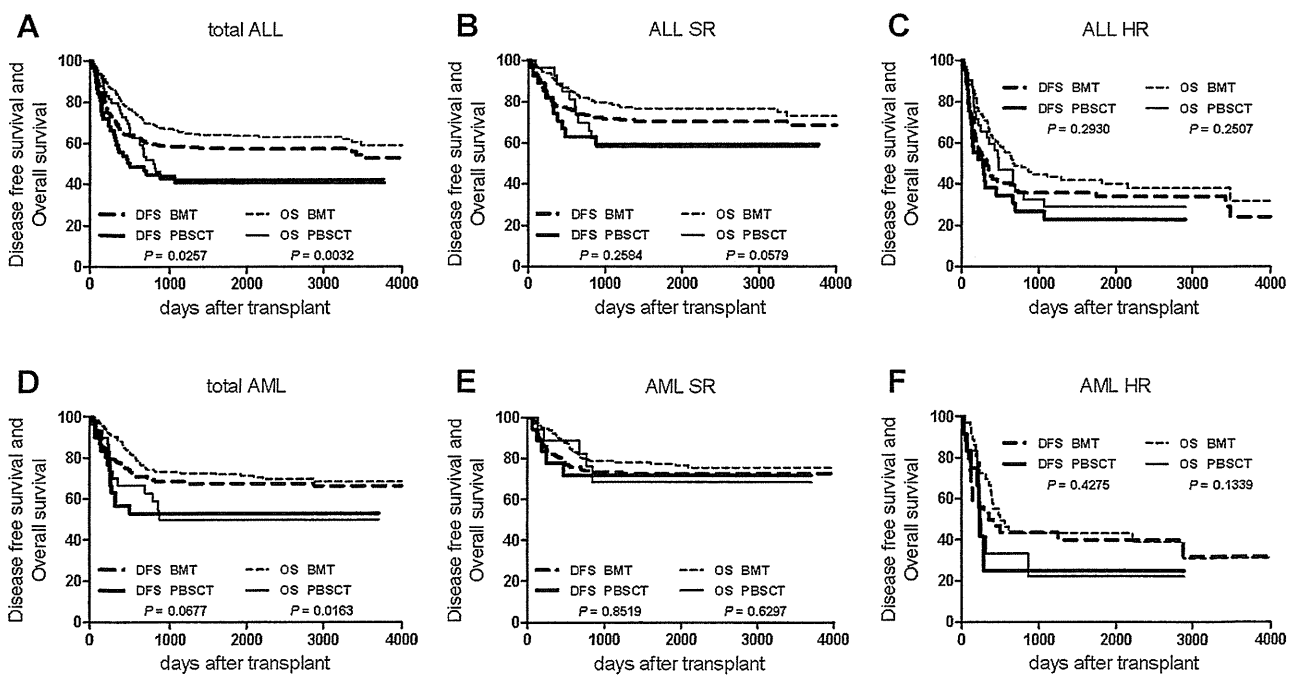

Fig. 2. Disease free survival and overall survival. **A**: total ALL; (**B**) ALL standard-risk; (**C**) ALL high-risk; (**D**) total AML; (**E**) AML standard-risk; and (**F**) AML high-risk.

TABLE II. Multivariate Model of Prognostic Risk Factors for Disease Free Survival and Overall Survival

	Disease free survival			Overall survival		
Variable	Hazard ratio	95% conf. interval	*P*-value	Hazard ratio	95% conf. interval	*P*-value
Diagnosis: AML vs. ALL	0.91	0.70–1.18	0.470	1.03	0.78–1.36	0.849
Risk group: HR vs. SR	3.32	2.59–4.26	<0.001	3.60	2.74–4.72	<0.001
Stem cell source: peripheral blood vs. bone marrow	1.38	1.01–1.88	0.044	1.51	1.09–2.09	0.013

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; HR, high-risk; SR, standard-risk; HLA, human leucocyte antigen.

As shown in Supplementary Figure 2, the cumulative incidences of grades III–IV acute GvHD was similar for PBSCT and BMT in the ALL group (9.1% vs. 7.7%, $P = 0.7741$), ALL SR (0% vs. 4.8%, $P = 0.3027$), ALL HR (16.2% vs. 12.6%, $P = 0.5896$), the AML group (15.7% vs. 8.5%, $P = 0.3268$), AML SR (9.1% vs. 8.3%, $P = 0.9534$) and AML HR (27.1% vs. 9.8%, $P = 0.1729$).

Chronic GvHD Associated With PBSCT and BMT in ALL and AML

Shown in Figure 3, PBSCT was associated with a higher cumulative incidence of any grade of chronic GvHD than BMT for the ALL group (52.6% vs. 19.6%, $P = 0.0002$), ALL SR (39.7% vs. 18.3%, $P = 0.0007$), ALL HR (48.8% vs. 21.6%, $P = 0.0949$), the AML group (56.3% vs. 23.1%, $P = 0.0002$), AML SR (40.2% vs. 21.0%, $P = 0.0905$) and AML HR (81.8% vs. 39.7%, $P = 0.0027$). Similarly, as shown in Figure 3, the cumulative incidence of extensive chronic GvHD was significantly higher in PBSCT than BMT for the ALL group (26.0% vs. 9.9%, $P = 0.0066$), ALL SR (24.3% vs. 8.3%, $P = 0.0059$), the AML group (41.6% vs. 11.1%, $P < 0.0001$), AML SR (30.6% vs. 9.9%, $P = 0.0215$) and AML HR (56.4% vs. 23.5%, $P = 0.0046$). However, the difference observed in ALL HR did not achieve statistical significance (36.4% vs. 12.7%, $P = 0.3225$). In addition, as shown in Figure 3, the cumulative incidence of limited chronic GvHD was higher after PBSCT than after BMT for the ALL group (34.6% vs. 10.6%, $P = 0.0172$) and ALL SR (39.0% vs. 10.8%, $P = 0.0419$), but it was similar for ALL HR (18.1% vs. 9.9%, $P = 0.1812$), the AML group (23.1% vs. 13.2%, $P = 0.3273$), AML SR (12.8% vs. 12.0%, $P = 0.9658$) and AML HR (54.5% vs. 20.6%, $P = 0.2462$).

Transplantation-Related Mortality Associated With PBSCT and BMT in ALL and AML

As indicated by Figure 4, for the ALL group, cumulative incidences of TRM in PBSCT and in BMT were 5.1% and 4.0% at

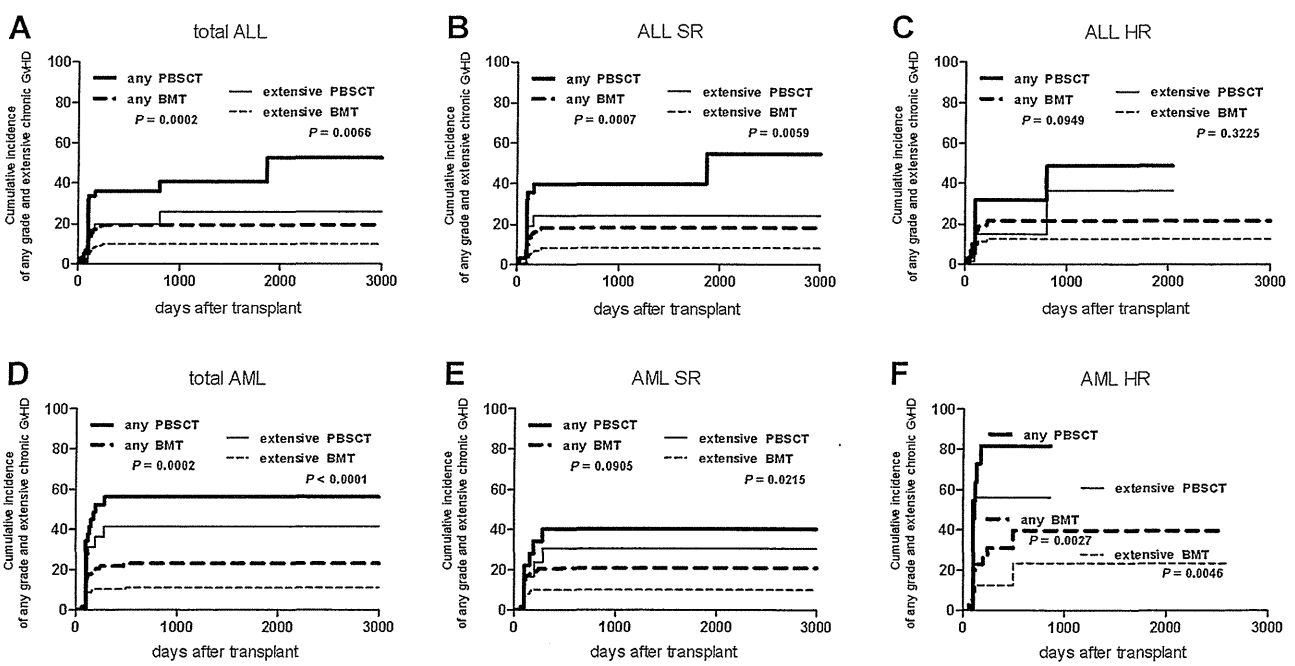

Fig. 3. Cumulative incidence of any grade and extensive chronic GvHD. **A**: total ALL; (**B**) ALL standard-risk; (**C**) ALL high-risk; (**D**) total AML; (**E**) AML standard-risk; and (**F**) AML high-risk.

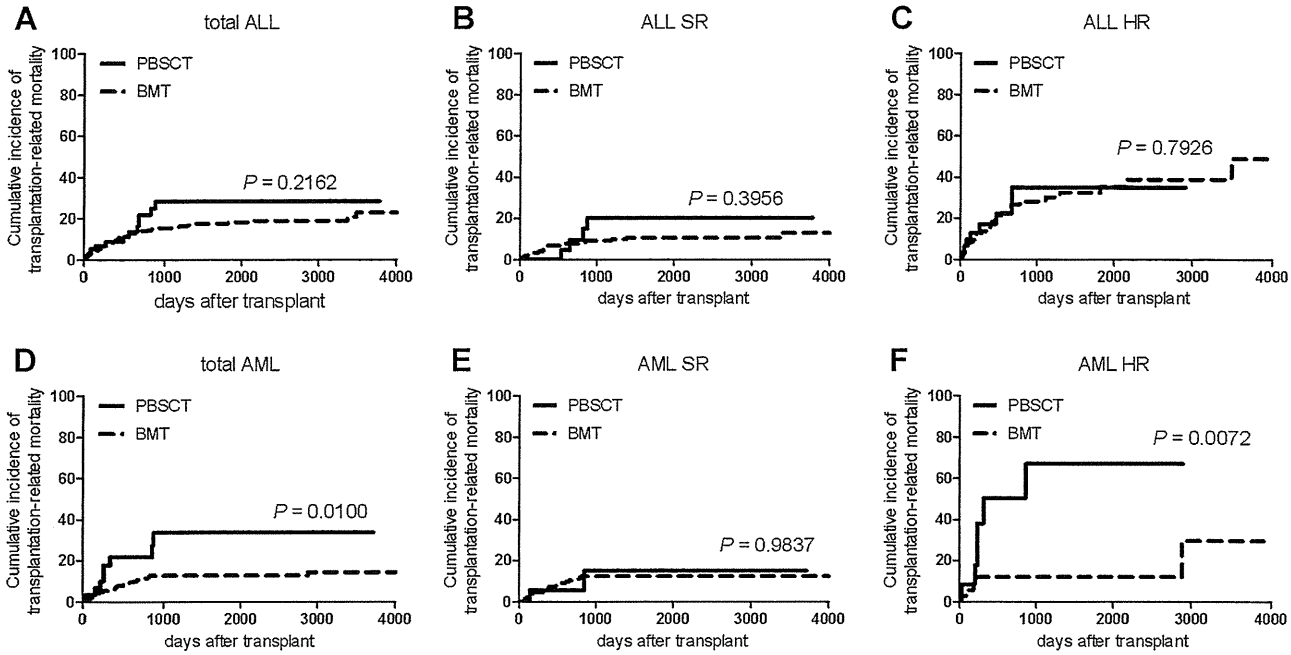

Fig. 4. Cumulative incidence of transplantation-related mortality. **A**: total ALL; (**B**) ALL standard-risk; (**C**) ALL high-risk; (**D**) total AML; (**E**) AML standard-risk; and (**F**) AML high-risk.

100 days, 8.8% and 9.6% at 1 year and 28.2% and 15.3% at 3 years, respectively ($P = 0.2162$). For the AML group, cumulative incidences of TRM in PBSCT and in BMT were 3.3% and 1.8% at 100 days, 21.6% and 6.0% at 1 year and 33.7% and 12.7% at 3 years, respectively ($P = 0.0100$). Thus, for both ALL and AML, PBSCT and BMT were associated with similar TRM incidences early after transplantation, PBSCT is associated with higher TRM incidences later after transplantation, only for the AML group and AML HR (ALL SR, $P = 0.3956$; (ALL SR, $P = 0.3956$; ALL HR, $P = 0.7926$; AML SR, $P = 0.9837$; AML HR, $P = 0.0072$; Supplementary Table I).

DISCUSSION

This retrospective study compared the outcomes of allogeneic HSCT using two stem cell sources, namely BM or PBSC, in 661 Japanese children with acute leukaemia. The stem cell donors were HLA-matched sibling donors and the study was based on data acquired between January 1996 and December 2007. It was observed that PBSCT was associated with more rapid haematopoietic recovery than BMT, as has also been shown in most previous studies with adult patients [3,4,9,11–15] and paediatric patients [20]. The OS and DFS were significantly lower after PBSCT than after BMT.

Multivariate analysis revealed that the use of peripheral blood allografts was an adverse risk factor for OS and DFS. Similarly, while previous studies of adults with acute leukaemia suggest that PBSCT and BMT were associated with equivalent survival rates [9,11,15], the IBMRT study of children and adolescents reported that PBSCT was associated with lower survival and higher TRM [21]. The higher mortality observed in children after PBSCT is likely to be due to the higher incidences of chronic GvHD. This has been reported in younger patients with acquired severe aplastic anemia [22]. Thus, peripheral blood allografts in younger recipients may be associated with a higher risk of mortality and chronic GvHD, which suggests that caution should be exercised when considering the use of PBSC in paediatric patients.

It should be noted that this is a retrospective study, which has a number of limitations. In particular, we could not exclude the possibility that unidentified confounding variables could affect the transplant outcomes and could not adjust the data for unknown or unmeasured factors. Thus, the results presented here should be interpreted with caution. Nevertheless, these observations suggest that BM should be the preferred stem cell source for children with acute leukaemia who have HLA-matched sibling donors. It remains to be seen whether these conclusions can be extrapolated to alternative donors, namely HLA-mismatched related donors and HLA-identical unrelated donors. At present, there are no guidelines regarding the preferred source of stem cells (PBSC or BM) from unrelated volunteer donors. However, the biggest risk associated with selecting PBSC over BM concerns the fact that higher numbers of T cells are infused, which increases the risk of chronic GvHD and transplantation-related mortality. This risk may be greater with unrelated volunteer donors than with sibling donors. Indeed, it has been shown that when using unrelated donors, a single HLA mismatch increases the risk of GvHD more significantly if the transplant involves PBSC than if it involves BM [23]. Since a bank that stores PBSC from unrelated volunteer donors is now available in Japan, it is likely that peripheral blood allografts will be used more frequently for allogeneic transplantation in children. However, before such widespread clinical changes take place, the risks and benefits of the various allografts that are available should be considered. In cases of PBSC will be used more frequently such as active infections at SCT, mycophenolate

mofeti of GvHD prophylaxisis. More detailed analyses and future trials may reveal that BM stem cells and PBSC are suitable for different situations.

PBSCT did not differ from BMT in terms of the incidence of grades III–IV acute GvHD for the total ALL and total AML groups (and all of their SR and HR subgroups). This has also been observed in several studies with adult patients [3,12–14] and paediatric patients [20], even though there are 10-fold more T cells in the peripheral blood than in the BM. This may relate to the use of G-CSF [24,25]. However, the AML HR subgroup was observed to develop grades II–IV acute GvHD significantly more often after PBSCT than after BMT. It is possible that the acute GvHD experienced by the PBSC recipients in the AML HR subgroup reflects the induction of a graft-versus-leukemia effect. However, arguing against this is that the RR after PBSCT and BMT in the AML HR subgroup was similar. The observation that allogeneic PBSCT is not associated with a higher incidence of acute GvHD does not appear to extend to chronic GvHD. As observed in other studies with adult patients [3,9,11,13,26,27], PBSCT was associated with a significantly higher incidence of chronic GvHD than BMT in the total ALL and total AML groups (and the ALL SR and AML HR subgroups). In particular, PBSCT was associated with a significantly higher incidence of extensive chronic GvHD in the total ALL and total AML groups along with all SR and HR subgroups apart from ALL HR. Although we were not able to investigate organ injury in detail, another study has shown that chronic GvHD after PBSCT involves higher numbers of organs and requires longer and multiple courses of immunosuppressive therapy [28].

While a meta-analysis has demonstrated that allogeneic PBSCT is associated with a significant decrease in relapse in both early and late stage patients [29], our study found that the RR after PBSCT and BMT did not differ significantly for ALL and AML. This was also observed by a study analysing the IBMTR/EBRT registry data of adult patients with leukaemia [10], as well as by the IBMTR study of children and adolescents with acute leukaemia [21]. The allogeneic graft-versus-leukaemia effect varies from one disease to another and depends on the stage of the disease and donor histocompatibility. In our study, which only assessed paediatric patients with acute leukaemia, increases in the incidence of extensive chronic GvHD did not lead to a concomitant decrease in the RR. It will be necessary to examine the relationship between GvHD and relapse in the future.

In summary, our study demonstrates that while the use of peripheral blood allografts from HLA-matched sibling donors in Japanese paediatric patients with ALL or AML leads to faster engraftment, it is also associated with poorer survival and quality of life due to chronic GvHD.

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Rabbit antithymocyte globulin and cyclosporine as first-line therapy for children with acquired aplastic anemia

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To the editor:

Rabbit antithymocyte globulin and cyclosporine as first-line therapy for children with acquired aplastic anemia

Horse antithymocyte globulin (hATG) and cyclosporine have been used as standard therapy for children with acquired aplastic anemia (AA) for whom an HLA-matched family donor is unavailable. However, in 2009, hATG (lymphoglobulin; Genzyme) was withdrawn and replaced by rabbit ATG (rATG; thymoglobulin; Genzyme) in Japan. Many other countries in Europe and Asia are facing the same situation.[1] Marsh et al recently reported outcomes for 35 adult patients with AA who were treated with rATG and cyclosporine as a first-line therapy.[2] Although the hematologic response rate was 40% at 6 months, several patients subsequently achieved late responses. The best response rate was 60% compared with 67% in a matched-pair control group of 105 patients treated with hATG. The overall and transplantation-free survival rates appeared to be significantly inferior with rATG compared with hATG at 68% versus 86% ($P = .009$) and 52% versus 76% ($P = .002$), respectively. These results are comparable to those from a prospective randomized study reported by Scheinberg et al comparing hATG and rATG.[3] Both studies showed the superiority of hATG over rATG.[2,3]

We recently analyzed outcomes for 40 Japanese children (median age, 9 years; range, 1-15) with AA treated using rATG and cyclosporine. The median interval from diagnosis to treatment was 22 days (range, 1-203). The numbers of patients with very severe, severe, and nonsevere disease were 14, 10, and 16, respectively. The ATG dose was 3.5 mg/kg/day for 5 days. The median follow-up time for all patients was 22 months (range, 6-38). At 3 months, no patients had achieved a complete response (CR) and partial response (PR) was seen in only 8 patients (20.0%). At 6 months, the numbers of patients with CR and PR were 2 (5.0%) and 17 (42.5%), respectively. After 6 months, 5 patients with PR at 6 months had achieved CR and 4 patients with no response at 6 months had achieved PR, offering a total best response rate of 57.5%. Two patients relapsed at 16 and 19 months without receiving any second-line treatments. Two patients with no response received a second course of rATG at 13 and 17 months, but neither responded. Sixteen patients underwent hematopoietic stem cell transplantation (HSCT) from alternative donors (HLA-matched unrelated donors, n = 13; HLA-mismatched family donors, n = 3). Two deaths occurred after rATG therapy, but no patients died after HSCT. Causes of death were intracranial hemorrhage at 6 months and acute respiratory distress syndrome at 17 months. The overall 2-year survival rate was 93.8% and the 2-year transplantation-free survival rate was 50.3% (Figure 1).

In our previous prospective studies with hATG, the response rates after 6 months were 68% and 70%, respectively, with no increases in response rates observed after 6 months.[4,5] Our results support the notion that rATG is inferior to hATG for the treatment of AA in children. First-line HSCT from an alternative donor may be justified, considering the excellent outcomes in children who received salvage therapies using alternative donor HSCT.

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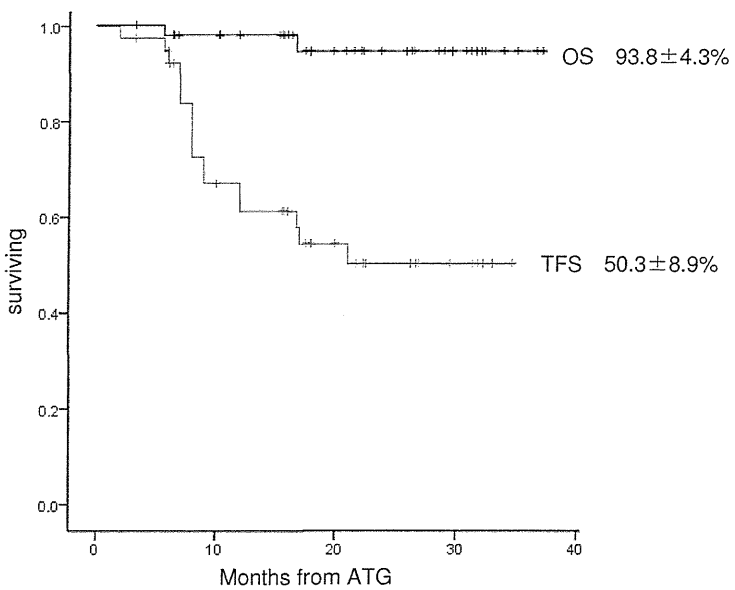

Figure 1. Kaplan-Meier estimates of overall survival (OS) and transplantation-free survival (TFS) in 40 Japanese children with AA. Survival was investigated using Kaplan-Meier methods. OS for all patients with AA after rATG and cyclosporine as first-line therapy included patients who later received HSCT for nonresponse to rATG. In the analysis of TFS for all patients treated with rATG and CSA, transplantation was considered an event.

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To the editor:

Peripheral blood stem cells versus bone marrow in pediatric unrelated donor stem cell transplantation

The relative benefits and risks of peripheral blood stem cells (PBSCs) versus bone marrow (BM) for allogeneic hematopoietic stem cell transplantation (SCT) are still a matter of highly controversial debates.[1-3] The first randomized study comparing the 2 stem cell sources in unrelated donor SCT recently documented comparable overall and event-free survival, but indicated a higher risk for chronic graft-versus-host disease (GVHD) with PBSCs.[4] Only a few pediatric patients were included in this study even though the long-term sequelae of chronic GVHD are of particular concern in this patient group.

We retrospectively compared the long-term outcome of contemporaneous unrelated donor SCT in 220 children transplanted with BM (n = 102) or PBSCs (n = 118) for hematologic malignancies and reported to the German/Austrian pediatric registry for SCT. All patients had received myeloablative conditioning followed by unmanipulated SCT from HLA-matched unrelated donors. The PBSC and BM groups were comparable with regard to patient and donor age, sex, cytomegalovirus (CMV) serostatus, disease status at transplantation, GVHD prophylaxis, growth factor use, and degree of HLA matching. The groups differed with regard to disease category with slightly more myelodysplastic syndrome patients ($P = .02$) and a higher CD34-cell dose ($P = .001$) in the PBSC group.

Neutrophil and platelet engraftment were achieved significantly faster after PBSC than BM transplantation (Figure 1A-B). In this entirely pediatric cohort, the incidence of clinically relevant grade II-IV acute GVHD (Figure 1C) did not differ. Most importantly, the incidence of chronic GVHD (PBSCs vs BM: 35% vs 33%, respectively; $P = .9$) and extensive chronic GVHD (Figure 1D) proved low and was virtually identical in the 2 groups. With a median follow-up time of 3 years, overall survival (PBSCs vs BM: 50% ± 5% vs 46% ± 6%, respectively; $P = .63$) and event-free survival (PBSCs vs BM: 45% ± 5% vs 44% ± 6%, respectively; $P = .59$) were comparable (Figure 1E-F). In multivariable analysis, taking into account all parameters with $P < .2$ in univariate analysis, the only significant independent risk factor for treatment failure was advanced disease status at the time of transplantation (relative risk = 2.4, 95% confidence interval, 1.5-3.8; $P = .001$). In contrast, stem cell source (PBSCs vs BM) had no effect (relative risk = 1.1, 95% confidence interval, 0.7-1.6; $P = .8$).

Our registry-based analysis provides evidence that in pediatric recipients of HLA-matched unrelated-donor transplantation with consistent antithymocyte globulin (ATG) use during conditioning, transplantation with PBSCs and BM results in comparable clinical outcomes without detectable differences in the risk of acute or, more importantly, chronic GVHD. Consistent with a recent study underscoring the role of ATG for the prevention of acute and chronic GVHD,[5] the use of ATG in 96% of our transplantation procedures compared with only 27% in the above-mentioned randomized study by Anasetti et al[4] might be one of the key factors responsible for the overall low and comparable incidence of

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CASE REPORT

Detection of donor-derived CMV-specific T cells in cerebrospinal fluid in a case of CMV meningoencephalitis after cord blood stem cell transplantation

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Abstract Cytomegalovirus (CMV) meningoencephalitis is a rather rare complication after allogeneic stem cell transplantation. We describe here the case of a 59-year-old man with acute myeloid leukemia who developed CMV meningoencephalitis after cord blood transplantation. The patient presented with a sudden onset of neurological symptoms, such as convulsion, on day 37. The analysis of cerebrospinal fluid (CSF) sample revealed an increase in the number of cells, which were of donor (cord blood) origin, consisting mainly of T cells. No bacteria were detected in the CSF sample. Real-time PCR analysis revealed that the CSF sample was positive for CMV, but was negative for HHV-6, adenovirus, or BK virus. The patient was diagnosed with CMV meningoencephalitis and received cidofovir. His neurological symptoms were gradually improved and completely disappeared by day 60. CMV-specific dextramer-positive $CD8^+$ T cells were detected in the peripheral blood and CSF samples, with the frequency being much higher in the CSF. To our knowledge, this is the first report on the appearance of CMV-specific T cells in CSF samples from a patient with CMV meningoencephalitis. Cord blood-derived CMV-specific T cells may develop early after transplantation, enter the intrathecal compartment, and likely contribute to the regulation of CMV-meningoencephalitis.

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Introduction

Cytomegarovirus (CMV) meningoencephalitis (CMV-ME) is a rather rare complication, occurring in 6 % of patients with viral encephalitis after allogeneic stem cell transplantation (SCT) [1]. Recently, we had a patient with acute myeloid leukemia (AML) who developed CMV-ME after cord blood stem cell transplantation (CBT), and could, for the first time, confirm the presence of donor-derived CMV-specific CD8 T cells in the cerebrospinal fluid (CSF), using the dextramer staining procedure.

Case report

A 59-year-old Japanese man with AML (M6) evolving from myelodysplastic syndrome received a chemotherapy consisting of aclarubicin and cytarabine, and achieved complete remission morphologically, which was, however, considered as returning to refractory anemia because of the existence of deletion chromosome 20 in 17 of 20 cells in the karyotype analysis of bone marrow (BM) samples. The patient therefore was referred to our hospital for allogeneic SCT. Since there was no suitable donor in related and unrelated donor pools, we decided to perform CBT using reduced-intensity conditioning regimen, which consisted of fludarabine (FLU) 30 mg/m^2/day (day −6 to −2, total 150 mg), cyclophosphamide (CY) 50 mg/kg/day on day −6, and total body irradiation (TBI) 3 Gy on day −7 [2]. Graft-versus-host disease (GVHD) prophylaxis consisted of continuous infusion of cyclosporine (CsA) (target blood

concentration was 300–400 ng/ml), and mycophenolate mofetil (MMF) 30 mg/kg, both of which started from day −3. Cord blood (CB) graft was female and contained 6.66×10^7 nucleated cells/kg and 4.77×10^5 $CD34^+$ cells/kg. The HLA profiles of the patient and CB unit were as follows: patient HLA A*24:02 *02:01, B*35:01 *56:01, DRB1*04:10 *09:01, and CB HLA A*24:02 *02:01, B*35:01 *40:01, DRB1*04:03 *09:01, which means mutual HLA 1 antigen mismatch in B locus. The clinical course of the patient is shown in Fig. 1. Hematopoietic engraftment was rapidly achieved, with absolute neutrophil count $>0.5 \times 10^9$/l on day 15 and platelet count $>50 \times 10^9$/l on day 29. On day 17, complete donor chimerism was confirmed in both $CD3^+$ and myeloid fractions of peripheral blood (PB) using informative short tandem repeat-PCR technique. The patient started having spiking fever >39 °C on day 7, which was considered as pre-engraftment immune reaction (PIR) [3]. Prednisolone was started at a dose of 5 mg/day on day 9 and increased to 10 mg/day on day 12. On day 12, the patient developed acute cutaneous GVHD (stage 1), which subsided a few days later without the need for any additional treatment. Foscarnet (FCN) 80 mg/kg/day was also started on day 8 as the prophylaxis of CMV or human herpes virus 6 (HHV6) infection. The fever subsided by day 20, but reappeared on day 32. CMV pp65 antigenemia, which was monitored weekly, continued to be negative from day −4 to day 87. Reactivation status for CMV, HHV6, adenovirus (ADV), and BK virus (BKV) was also monitored weekly using real-time PCR analysis of plasma samples. No viral reactivation was observed, except for a positive result for CMV (7.7×10^7 copy/μg DNA) on day 42.

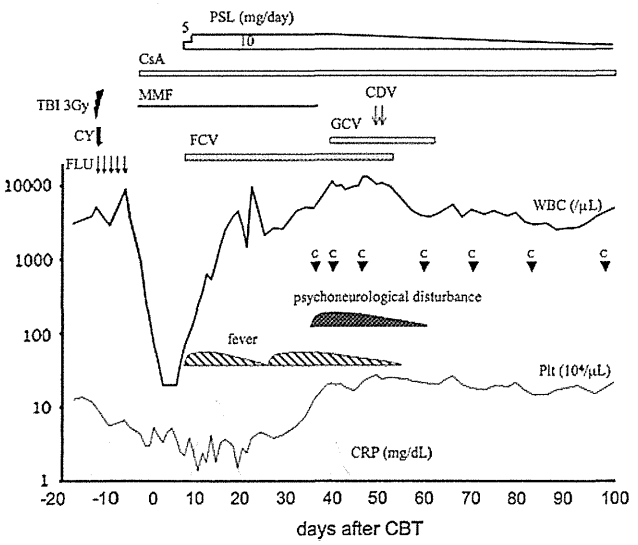

Fig. 1 Clinical course. *Bold*, *thin*, and *dotted lines* denote white blood cells (WBC) (/μL), platelets (10^4/μL), and CRP (mg/dL), respectively. *Flu* fludarabine, *CsA* cyclosporine, *MMF* mycophenolate mofetil, *PSL* prednisolone, *CY* cyclophosphamide, *TBI* total body irradiation, *FCN* foscarnet, *GCV* ganciclovir, *CDV* cidofovir, *C* CSF sample analysis

On the night of day 37, the patient's behavior suddenly became abnormal, such as cutting the infusion line with a pair of scissors or urinating on the bed board. Next morning, the patient was barely able to make even a simple conversation and his consciousness level was decreased, with the occurrence of general convulsion for a few minutes. An analysis of CSF sample on day 38 revealed that the cell number was 3464/3 μl (normal range 0–15/3 μl) consisting of polymorphonuclear leukocytes (PMN) 83 % and mononuclear cells (NMC) 17 %. The biochemical data of the CSF sample were: protein 261 mg/dl (normal range 40–75 mg/dl), Cl 116 mmol/l (normal range 120–130 mmol/l), and LDH 95 U/l (normal range 8–50 U/l). These results suggested that the patient had bacterial meningitis, but no bacterium was cultured in the CSF sample. MRI of the brain on day 39 showed no abnormal findings. Meropenem 3 g/day and ganciclovir (GCV) 3 mg/kg/day were started, with the administration of an increased dose of immunoglobulin. Real-time PCR data revealed that the CSF sample on day 38 was positive for CMV and negative for HHV6, while the PB sample was negative for the 2 viruses. There was no sign of CMV disease in other organs [4]. Follow-up data of the CSF are shown in Table 1. The CSF cell components turned to an MNC-dominant status after day 42. Although the reason why PMN was dominant in the CSF sample on day 38 is unknown, we speculate that PMN might have reflected a hyperacute inflammatory response in the central nervous system (CNS). Cidofovir 1 mg/kg/day was administered on days 50 and 52, and discontinued due to the elevation of serum creatinine level. His psychological and neurological symptoms gradually improved and completely disappeared on day 60. Follow-up brain MRI showed also normal results. He was discharged without any sequelae on day 104.

We performed the immunological characterization of CSF cells. The CSF cells on day 61 were of 100 % donor (CB) origin on chimerism analysis using STR-PCR. MNCs in the CSF mainly consisted of T cells: $CD3^+$ $CD4^+$ T cells 48.2 % and $CD3^+$ $CD8^+$ T cells 23.7 %, NK cells ($NKp46^+$ cells) 21.4 %, and B cells 0.8 %. The patient and CB shared HLA A*24:02 and A*02:01. We tested the presence of CMV-specific CD8 T cells in the PB and CSF samples on day 70 using CMV-specific HLA A*24:02-restricted and HLA A*02:01-restricted dextramers (Immudex, Copenhagen, Denmark). Dextramer staining was performed according to the manufacturer's protocol. Cells were stained with phycoerythrin-Cy7-conjugated anti-CD8, phycoerythrin-Cy5-conjugated anti-CD3 (Beckman Coulter Inc., Fullerton, CA, USA), and phycoerythrin- conjugated dextrameric-HLA A*02:01-restricted NLVPMVATV peptide complex or fluorescein isothiocyanate-conjugated