Figure 1. Diagram for establishing the patient cohort. To obtain the study cohort, patients who achieved neutrophil engraftment with delayed recovery of erythropoiesis were selected from 2846 recipients of major ABO-incompatible grafts. Patients who died before 100 days or whose underlying malignancies relapsed or progressed within the first 6 months were excluded. Questionnaires were sent to transplant centers, and 48 recipients were identified as having PRCA, whereas 51 patients were not diagnosed with PRCA.

Japan Society for Hematopoietic Cell Transplantation, the Japan Marrow Donor Program, and the Japan Cord Blood Bank Network.

Data Collection

The patient cohort was selected from the registry database of the Transplantation Registry Unified Management Program [35], which covered both adult and pediatric transplantation using all kinds of graft sources in Japan. In an attempt to evaluate the efficacy of treatment for PRCA and the long-term outcome, 145 recipients, who achieved neutrophil engraftment with delayed recovery of erythropoiesis and survived >100 days after transplantation without disease relapse or progression within the first 6 months, were selected from 2846 records of major ABO-incompatible allogeneic transplantation from 2003 to 2007 (Figure 1). Delayed recovery of erythropoiesis was noted when the posttransplantation days to recover >1% reticulocytes exceeded 60 days. Neutrophil recovery was defined by an absolute neutrophil count of at least .5 × 10^9/L for 3 consecutive days.

The first questionnaires were sent to transplantation centers, and the response rate was 68.3% (99 of 145 recipients). Forty-eight recipients were identified as having PRCA. Detailed transplantation data from 46 of those 48 recipients were then collected by sending second questionnaires. Second questionnaires did not collect information on the presence of human parvovirus B19 genome in blood in the present study.

Definition

Posttransplantation PRCA was defined as anemia with low reticulocyte counts (<1%) in peripheral blood for more than 60 days after transplantation in association with neutrophil recovery and a lack of erythroblasts in bone marrow. Diagnosis of PRCA was made by hematologists in each transplantation center. Acute and chronic GVHD was diagnosed and graded according to consensus criteria [36,37]. Calcineurin inhibitors were generally given from day −1 through day 50 of transplantation at the full dose and then tapered at the pace of 5% per week and stopped 6 months after transplantation if recipients did not have active severe GVHD. Rapid tapering of calcineurin inhibitors was defined as a reduction in drug doses exceeding >5% per week.

The definition of a clinical response to treatment for PRCA and the timing of evaluation were previously reported [3-5]. Complete remission, partial remission, and no response were defined as the achievement of normal hemoglobin levels without transfusion, the presence of anemia without transfusion dependence, and the continued need for transfusions, respectively.

Statistical Analysis

All statistical analysis was performed using PASW Statistics 18 (SPSS, Tokyo, Japan). Survival time was estimated by the Kaplan-Meier method, and comparison between the two groups was made by the log-rank test. Comparisons of parametrical data from one group or the other were made with non-paired 2-tailed t-tests after the equality of variances was determined by Levene's test. Comparisons of categorical data were made with Pearson's chi-square test unless otherwise stated. Multivariate analyses were performed using the multiple logistic regression model.

Table 1
Patient Characteristics

Category and Variable	No. of Patients
Total number of patients	46
Age, yr	10-66 (median 49)
Sex	
Male	24
Female	22
Disease	
AML	23 (14 CR, 7 non-CR, 2 unknown)
MDS	10
ALL	2 (2 CR)
CML	1
Unclassified leukemia	1
Aplastic anemia	7
Lymphoma	1
EBV-associated disease	1
Donor	
Related	24
Unrelated	22
Graft	
Bone marrow	33
Peripheral blood stem cell	13
Cord blood	0
Incompatible hemagglutinin	
Anti-A	28
Anti-B	12
Both	6

AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; EBV, Epstein-Barr virus; CR, complete remission.

RESULTS

Patient Characteristics

Characteristics of patients with PRCA after ABO major incompatible HSCT are shown in Table 1. No patient received pretransplantation removal of antidonor hemagglutinins (data not shown). None of the patients with PRCA had cord blood transplantation. Of 1182 patients who received cord blood transplantation from major ABO-mismatched grafts, 17 patients were selected as potential candidates for PRCA. The questionnaires were sent to the transplantation centers where those patients were treated, and responses were obtained from all centers. None of those 17 patients was diagnosed as having PRCA. Incompatible hemagglutinins were anti-A in 28 patients, anti-B in 12, and both in 6 patients.

Initial Treatment of PRCA and Outcome of Anemia

Treatment of PRCA other than transfusion was performed in 22 patients (treatment group) but not in the other 24 patients (nontreatment group) (Tables 2 and 3). Corticosteroids were given as an initial treatment in 12 patients, and rapid tapering of calcineurin inhibitors was performed in 8 patients. Rituximab was given in 1 patient and erythropoietin in 1 patient. In 7 of 12 patients who were given corticosteroids for treatment of PRCA, corticosteroids were started before day 60 because of severe acute GVHD.

Response to the initial treatment was observed in 6 of 12 patients receiving corticosteroids (4 complete response, 2 partial response) and 2 of 8 patients who had rapidly tapered calcineurin inhibitors (2 complete response) (patients 13 and 14). None of the patients receiving rituximab (n = 1; patient 21) or erythropoietin (n = 1; patient 22) responded.

Secondary therapy including rituximab, additional immunosuppressants, or donor lymphocyte infusion was given to 8 patients with a 50% response rate. The overall response rate of treatment for PRCA was 54.5%. Four of 10 patients who did not show any responses to treatment spontaneously became transfusion-independent. Red cell transfusion

Table 2
Response and Outcome of Treatment for Post-Transplant PRCA

UPN	Initial Treatment and Response	Salvage Therapy	Acute GVHD Grades II-IV	Chronic GVHD	Reticulocytes >1%	Outcome of PRCA	Outcome	Cause of Death
1	CS (215-828), CR		No	No	Reached (235)	CR	Alive (2057)	
2	CS (38-718), CR		Yes, grade II	Yes, limited	Reached (187)	CR	Dead (718)	Infection
3	CS (50-309), CR		No	No	Reached (130)	CR	Alive (1364)	
4	CS (51-94), CR		Yes, grade II	No	Reached (73)	CR	Dead (1827)	PD
5	CS (50-418), CR		Yes, grade II	No	Reached (149)	CR	Alive (937)	
6	CS (56-130), CR		Yes, grade II	Yes, limited	Reached (137)	PR	Alive (165)	
7	CS (53-168), PR		Yes, grade II	No	Reached (113)	PR	Dead (291)	Infection
8	CS (140-225), NR	RTX (250), CR	No	No	Reached (350)	CR	Alive (1509)	
9	CS (29-191), NR	MMF (179-333), NR	Yes, grade IV	Not assessable	Reached (83)	NR	Dead (341)	Organ failure
10	CS (36-128), NR		Yes, grade II	No	Not reached	PR	Dead (328)	Infection
11	CS (98-344), NR		No	No	Not reached	NR	Alive (344)	
12	CS (111-184), NR		No	Yes, extensive	Not reached	NR	Alive (184)	
13	CNI rapid tapering, CR		No	No	Reached (144)	CR	Alive (1003)	
14	CNI rapid tapering, CR		Yes, grade II	No	Reached (422)	CR	Alive (1437)	
15	CNI rapid tapering, NR		Yes, grade III	Yes, extensive	Not reached	NR	Dead (743)	Infection
16	CNI rapid tapering, NR	CS (122-239), CR	No	Yes, extensive	Reached (201)	CR	Dead (828)	GVHD
17	CNI rapid tapering, NR	CS (105-991), PR	No	Yes, extensive	Reached (380)	PR	Dead (991)	Infection
18	CNI rapid tapering, NR	DLI (174), NR	No	No	Not reached	Unknown	Dead (220)	PD
19	CNI rapid tapering, NR	EPO (56), NR	No	No	Reached	CR	Alive (1081)	
20	CNI rapid tapering, NR	EPO (95), NR DLI (132), NR RTX (194), NR CsA (530-587), CR	No	Yes, limited	Reached (530)	Unknown	Dead (811)	Infection, organ failure
21	RTX (319), NR	AZT (359-365), NR CS (402-898), NR	No	Yes, limited	Reached (480)	NR	Dead (898)	Infection
22	EPO (64), NR		Yes, grade II	Yes, limited	Reached	PR	Alive (1443)	

CS indicates corticosteroids; CNI, calcineurin inhibitor; FK506, tacrolimus; CsA, cyclosporine A; EPO, erythropoietin; RTX, rituximab; DLI, donor lymphocyte infusion; MMF, mycophenolate mofetil; AZT, azathioprine; CR, complete response; PR, partial response; NR, no response; PD, progression of underlying disease; UPN, unique patient number.
The numbers in parenthesis indicate posttransplantation days when each drug was given.

independence at the last observation was 68% and 96% in the treatment group and nontreatment group, respectively, with a significant difference ($P = .008$, Pearson's chi-square test).

Efficacy of Treatment for PRCA

Days from diagnosis of PRCA to recovery of reticulocytes >1% and cumulative doses of red blood cell transfusion

during this period were not significantly different between the two groups (Figure 2). The average number of red blood cell transfusions in total was 67.6 units (range, 10 to 216) and 65.3 units (range, 12 to 454) in the treatment group and nontreatment group, respectively. One unit of packed red cells is prepared from 200 mL of blood in Japan. No significant difference occurred in the number of red blood cell transfusions between the two groups ($P = .916$; t-test). The

Table 3
Outcome of posttransplantation PRCA Receiving No Treatment

UPN	Acute GVHD Grades II-IV	Chronic GVHD	Reticulocytes >1%	Outcome of PRCA	Outcome	Cause of Death
23	No	No	Reached (67)	CR	Alive (1348)	
24	No	No	Reached (78)	CR	Alive (1580)	
25	No	No	Reached (88)	CR	Alive (2004)	
26	No	No	Reached (99)	CR	Dead (533)	Organ failure
27	No	No	Reached (112)	CR	Alive (505)	
28	No	No	Reached (118)	CR	Alive (1279)	
29	No	Yes, extensive	Reached (146)	CR	Alive (919)	
30	No	No	Reached (164)	CR	Alive (952)	
31	No	No	Reached (178)	CR	Alive (1501)	
32	No	Yes, extensive	Reached (180)	CR	Alive (2326)	
33	No	No	Reached (191)	PR	Dead (701)	PD
34	No	No	Reached (229)	CR	Alive (2244)	
35	No	Yes, limited	Reached (234)	CR	Alive (1786)	
36	Yes, grade II	Yes, limited	Reached (245)	CR	Alive (1789)	
37	No	No	Reached (250)	CR	Alive (1209)	
38	Yes, grade III	Yes, extensive	Reached (253)	CR	Alive (1109)	
39	No	Yes, limited	Reached (322)	CR	Alive (1730)	
40	No	No	Reached (348)	CR	Alive (1911)	
41	No	No	Reached (365)	CR	Alive (1785)	
42	No	Yes, limited	Reached (387)	CR	Alive (731)	
43	No	No	Reached (389)	CR	Alive (498)	
44	No	No	Reached (400)	CR	Alive (723)	
45	Yes, grade II	No	Reached (515)	CR	Alive (1049)	
46	No	Yes, limited	Not reached	NR	Alive (1994)	

CR indicates complete response; PR, partial response; NR, no response; PD, progression of underlying disease.
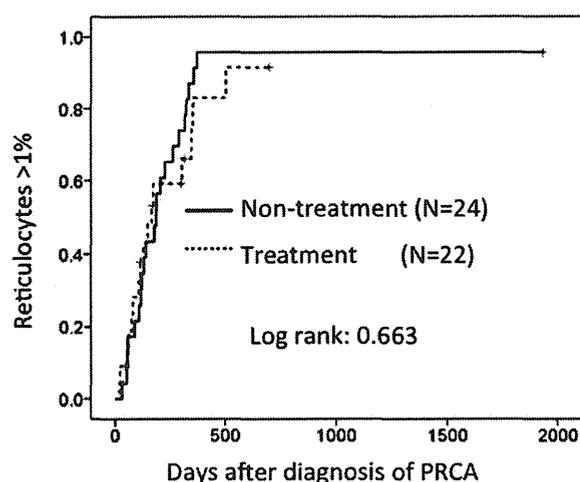Numbers in parenthesis indicate posttransplantation days.

Figure 2. Recovery of erythropoiesis. Days from diagnosis of PRCA to recovery of reticulocytes >1% were compared between the intervention and nonintervention groups, showing no significant difference (log rank .663).

numbers of red blood cell transfusions per day, which was calculated as the total units of red blood cell transfusion divided by the posttransplantation days until the last red blood cell transfusion, were .24 (.09 to .64) and .22 (.10 to 032) units per day in the treatment and nontreatment groups, respectively, with no significant difference ($P = .484$).

Comparison of the Profiles Between the Treatment and Nontreatment Groups

We compared the background profiles of patients receiving treatment of PRCA with those not receiving treatment (Table 4). Hemoglobin levels were lower in the treatment group than in the nontreatment group, although incompatible hemagglutinin titers at diagnosis of PRCA were not different. There were no significant differences in age, underlying diseases, type of donor and graft, HLA compatibility, conditioning regimen, immunosuppressive drugs, acute GVHD, and chronic GVHD between the 2 groups, although grades II to IV acute GVHD was more frequently found in the treatment group ($P = .013$).

Survival of PRCA Patients

The estimated median overall survival of all 46 PRCA patients has not been reached (Figure 3, left). The median overall survival time of patients receiving the intervention was 991 days, whereas estimated median overall survival of the nonintervention group has not been reached (Figure 3, middle and right). Two and 11 deaths were observed in the nontreatment group and treatment group, respectively. Causes of death in the treatment group were infections in 6 patients, infection plus organ failure in 1, organ failure in 1, GVHD in 1, and disease progression in 2 patients (Table 2). Thus, infections accounted for the death of 7 patients in the treatment group. Causes of death in the nontreatment group included organ failure and disease progression in each patient, respectively (Table 3).

Univariate analysis identified 5 variables attributable to the death of PRCA patients, including male gender, grades II to IV acute GVHD, chronic GVHD, disease progression, and treatment of PRCA (Table 5). These 5 variables were then incorporated into the multiple logistic regression model. Multivariate analysis revealed that progression of the

Table 4
Comparison of Backgrounds between Treatment and Nontreatment Groups

Category and Variable	Treatment (n = 22)	Nontreatment (n = 24)	P*
Age, yr	10-66 (42.7)	11-62 (42.8)	.982
Sex			.369
Male	13	11	
Female	9	13	
Severity of anemia†			
Red blood cell, ×10⁴/μL	143-347 (242)	196-350 (271)	.038
Hemoglobin, g/dL	4.1-10.1 (7.2)	5.9-10.3 (8.2)	.024
Hematocrit, %	12.2-30.2 (21.1)	17.4-31.0 (24.0)	.025
Underlying disease			.487
AML	10	13	
ALL	2	0	
MDS	5	5	
CML	1	0	
Unclassified leukemia	0	1	
Lymphoma	1	0	
Aplastic anemia	3	4	
EBV-associated disease	0	1	
Donor			.382
Related	10	14	
Unrelated	12	10	
Graft			.243
Bone marrow	14	19	
Peripheral blood stem cell	8	5	
Serological HLA compatibility			.339
Matched	21	21	
Mismatched	1	3	
Genotypical HLA compatibility			.821
Matched	18	19	
Mismatched	4	5	
Conditioning			.536
Myeloablative	9	12	
Nonmyeloablative	13	12	
Calcineurin inhibitor			.758
Cyclosporine	12	12	
FK506	10	12	
MTX			.255
Yes	19	23	
No	3	1	
Acute GVHD grades II-IV			.013
No	12	21	
Yes	10	3	
Chronic GVHD			.687
No	12	16	
Yes	9	8	
Not assessable	1	0	
Incompatible hemagglutinin titer‡			
IgG, mg/dL, range (median)	2-1024 (128)	2-1024 (64)	.252
IgM, mg/dL, range (median)	4-256 (16)	2-64 (16)	1.000

AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; EBV, Epstein-Barr virus; FK506, tacrolimus.
* Comparison was made with Pearson's chi-square test unless otherwise stated.
† Student's t-test.
‡ Mann-Whitney's U-test.

disease remained as the only factor negatively affecting overall survival ($P = .028$) (Table 6). Likewise, univariate analysis for risk factors for nonrelapse mortality was performed showing that 3 variables, including male gender, chronic GVHD, and treatment of PRCA, were risk factors. With these 3 variables, multiple logistic regression analysis was done and treatment of PRCA remained as the significant risk factor ($P = .036$).

DISCUSSION

In the present retrospective cohort study, we demonstrated for the first time that treatment of PRCA after
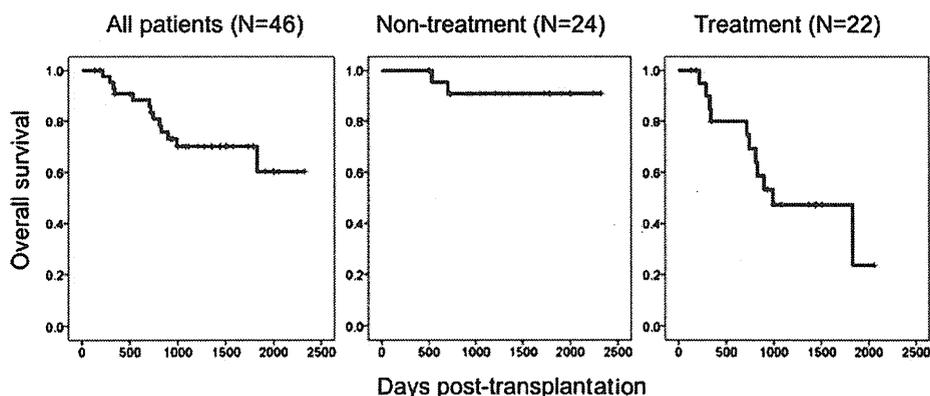
Figure 3. Overall survival of PRCA patients. Kaplan-Meyer estimates of survival for PRCA patients are shown.

allogeneic HSCT from major ABO-incompatible donors did not improve the recovery of erythropoiesis or decrease the red blood cell transfusion requirement. Although the results

Table 5
Univariate Analysis of Categorical Factors Influencing Survival

Category and Variable	n	Death	P*
Gender			.035
Female	22	3	
Male	24	10	
Underlying disease			.327
AML	23	6	
ALL	2	0	
MDS	10	6	
CML	1	0	
Unclassified leukemia	1	0	
Lymphoma	1	0	
Aplastic anemia	7	1	
EBV-associated disease	1	0	
Donor			.608
Related	24	6	
Unrelated	22	7	
Graft			.335
Bone marrow	33	8	
Peripheral blood stem cell	13	5	
Serological HLA compatibility			.880
Matched	42	12	
Mismatched	4	1	
Genotypical HLA compatibility			.203
Matched	37	12	
Mismatched	9	1	
Conditioning			.539
Myeloablative	21	5	
Nonmyeloablative	25	8	
GVHD prophylaxis			
Cyclosporine	22	7	.608
FK506	24	6	.608
MTX	42	11	.312
Acute GVHD grades II-IV			.091
No	33	7	
Yes	13	6	
Chronic GVHD			.197
No	28	6	
Yes	17	6	
Not assessable	1	1	
Disease progression			.003
No	36	6	
Yes	9	6	
Not assessable	1	1	
Treatment of PRCA			.002
No	24	2	
Yes	22	11	

AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; EBV, Epstein-Barr virus; FK506, tacrolimus; MTX, methotrexate.
* P values were calculated by Pearson's chi-square test.

have to be interpreted very carefully because of the retrospective nature of the study and the relatively small number of PRCA patients, our findings have important clinical implications.

Failure to demonstrate the beneficial effects of treatment on recovery of erythropoiesis in these PRCA patients may be debated by raising the possibility that the treatment group included more patients with severe anemia. This is partly true because average hemoglobin levels at diagnosis of PRCA were significantly lower in the treatment group than in the nontreatment group (Table 2). However, because the days from diagnosis of PRCA to the recovery of reticulocytes >1% were not different between the 2 groups seems to support the hypothesis that treatment of PRCA does not improve the recovery of erythropoiesis in bone marrow.

There are several potential arguments for apparently inferior survival of PRCA patients receiving treatment of PRCA. Severe acute GVHD and chronic GVHD may be confounding variables for treatment in PRCA and survival of post-transplantation patients because corticosteroids could be used for treatment of severe acute GVHD and chronic GVHD, and both are well-known adverse risk factors on survival. There were more patients with grades II to IV acute GVHD in the treatment group than in the nontreatment group (Table 2). However, multiple logistic regression analysis revealed that neither acute GVHD nor chronic GVHD was a significant hazard in the present patient cohort (Table 3). We are not ready to provide a reasonable explanation for

Table 6
Multiple Logistic Regression Analysis of Risk Factors for Survival of Patients with posttransplantation PRCA

Covariate	OR	95% CI	P
Sex			.114
Female	1.00		
Male	1.81	−.42 to 4.04	
Acute GVHD grades II-IV			.349
No	1.00		
Yes	1.00	−1.10 to 3.10	
Chronic GVHD			.934
No	1.00		
Yes	.08	−1.86 to 2.02	
Treatment of PRCA			.066
No	1.00		
Yes	1.93	−.13 to 3.99	
Disease progression			.028
No	1.00		
Yes	2.31	.25 to 4.37	

OR indicates odds ratio.

why male gender was selected as an adverse risk factor for overall survival, although this factor was eventually judged as an insignificant hazard by multivariate analysis.

Infections accounted for the death of 7 of 11 patients in the treatment group, whereas 2 deaths were observed in the nontreatment group, neither of which was attributed to infection. In particular, all 4 patients who had developed chronic GVHD after rapid tapering of calcineurin inhibitors died of infection or GVHD (Table 2). The use of additional immunosuppressive agents might be associated with the death of PRCA patients.

Corticosteroids were given to 12 patients, and 7 of those patients had severe acute GVHD. In these patients, it is difficult to define the purpose of corticosteroid therapy given for PRCA or GVHD. Questionnaires were designed to ask physicians whether any intervention was given to treat PRCA and what type of therapy was given. Therefore, the use of corticosteroids was counted as an intervention for PRCA based on the physician's intention, but corticosteroid therapy could also have been given to treat concurrent acute GVHD.

Absence of acute GVHD has been reported as 1 risk factor for PRCA after major ABO-mismatched HSCT, suggesting the presence of graft-versus-plasma cell effect [20]. Corticosteroid therapy has been reported to be effective to ameliorate anemia in some cases of PRCA after transplantation, whereas corticosteroids could also suppress graft-versus-plasma cell effect resulting in the survival of host-derived incompatible hemagglutinins. Thus, the efficacy of corticosteroids for anemia is not predictable in PRCA patients with acute GVHD.

In the present study, different interventions for PRCA were included together in the treatment group for analyzing the data. Although various treatment options have been reported for patients with PRCA after major ABO-incompatible HSCT, there is no consensus regarding which one is better than the other, because of the small number of patients in most studies and absence of prospective studies. Also, there is no recommendation whether any treatment should be given to PRCA patients in this clinical setting. Any previously reported treatment for PRCA has a potential risk for adverse events, including opportunistic infections or exacerbation of GVHD. The principal aim of the present study was to learn the efficacy of treatment for PRCA in general, and thus all treatment modalities were included in the treatment group for data analysis.

Previous studies have shown that the validity of the logistic model becomes problematic when the number of events per variable analyzed becomes small. Peduzzi et al. [38] reported that for event per variable less than 10 the regression coefficients are biased. This appears to be the case with our study, suggesting the requirement of a large number of patients in future studies.

In this patient cohort, no PRCA patient was reported with cord blood stem cell transplantation from major ABO-incompatible donors. Tomonari et al. [39] reported that none of the 39 patients receiving major ABO-incompatible cord blood cells developed PRCA. Although the reason recipients of cord blood cells did not develop PRCA is unknown, susceptibility of erythroid precursor cells to incompatible isohemagglutinin-induced cytotoxicity may be different between cord blood stem cells and other stem cell grafts. Reduction of incompatible isohemagglutinin titers such as plasma exchange before transplantation has previously been tried with various results, but there has been no specific

recommendation so far [40,41]. In our patient cohort, no patient with PRCA received this practice.

In summary, we could not demonstrate any beneficial effects of treatment on the recovery of PRCA after allogeneic HSCT from major ABO-incompatible donors. However, extracorporeal treatment was not included in the treatment options of our patient cohort [32]. The present study has several limitations, including a small sample size, reporting bias resulting from the retrospective study, the lack of parvovirus B19 data, heterogeneity of underlying diseases and its status, the treatment received, and so on. Thus, a well-designed prospective study should be performed to clarify the role of interventions for PRCA, including rapid tapering of calcineurin inhibitors after major ABO-mismatched hematopoietic cell transplantation.

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Comparison of Intravenous with Oral Busulfan in Allogeneic Hematopoietic Stem Cell Transplantation with Myeloablative Conditioning Regimens for Pediatric Acute Leukemia
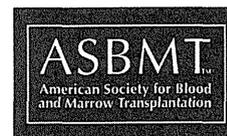
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A B S T R A C T

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.

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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 anti-leukemic 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

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.
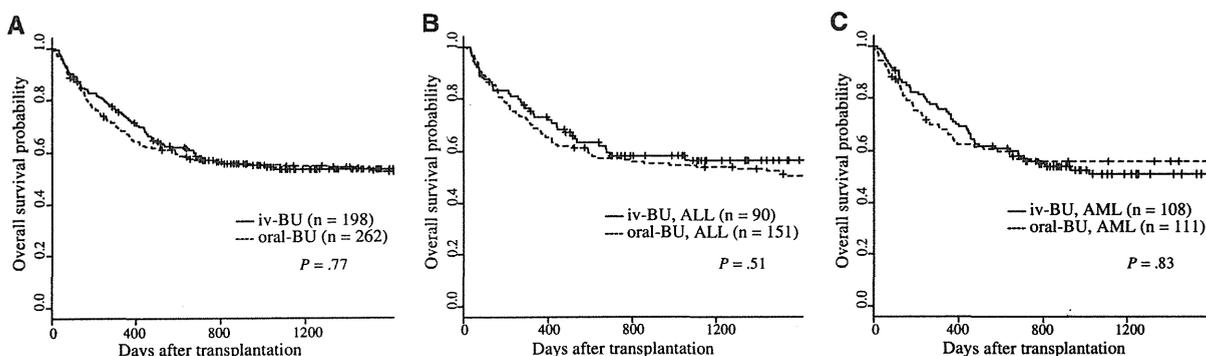

Figure 1. Overall survival probability of transplantation. Overall survival probability are shown according to busulfan form, (A) in all patients, (B) in patients with ALL, and (C) in patients with AML.

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.
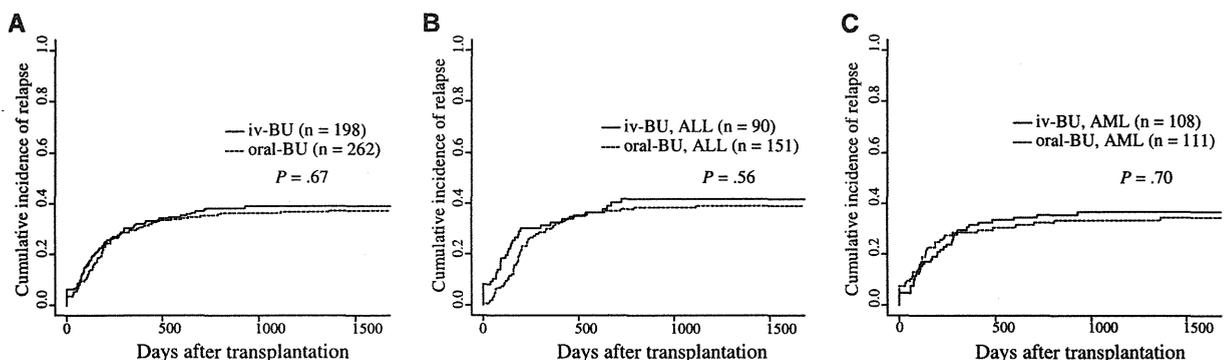

Figure 2. Cumulative incidences of relapse. Cumulative incidences of relapse are shown according to busulfan form, (A) in all patients, (B) in patients with ALL, and (C) in patients with AML.
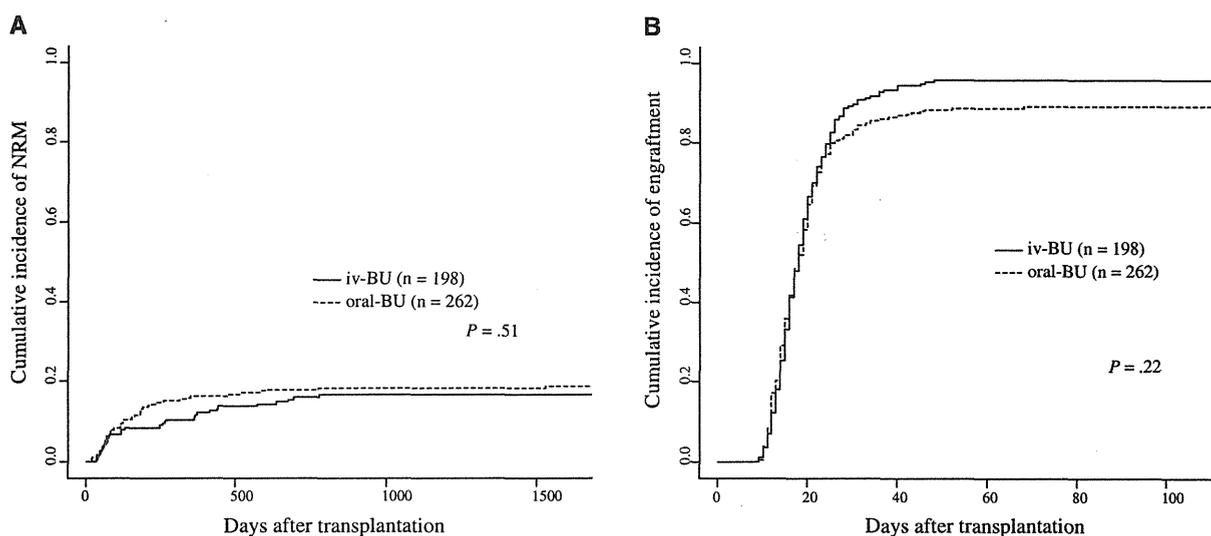
A

B

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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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. © 2013 Wiley Periodicals, Inc.

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 $0.5 \times 10^9/L$ occurring on the first of three consecutive days. Platelet engraftment was defined as the first day with a platelet count greater than $50 \times 10^9/L$ 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 (%).
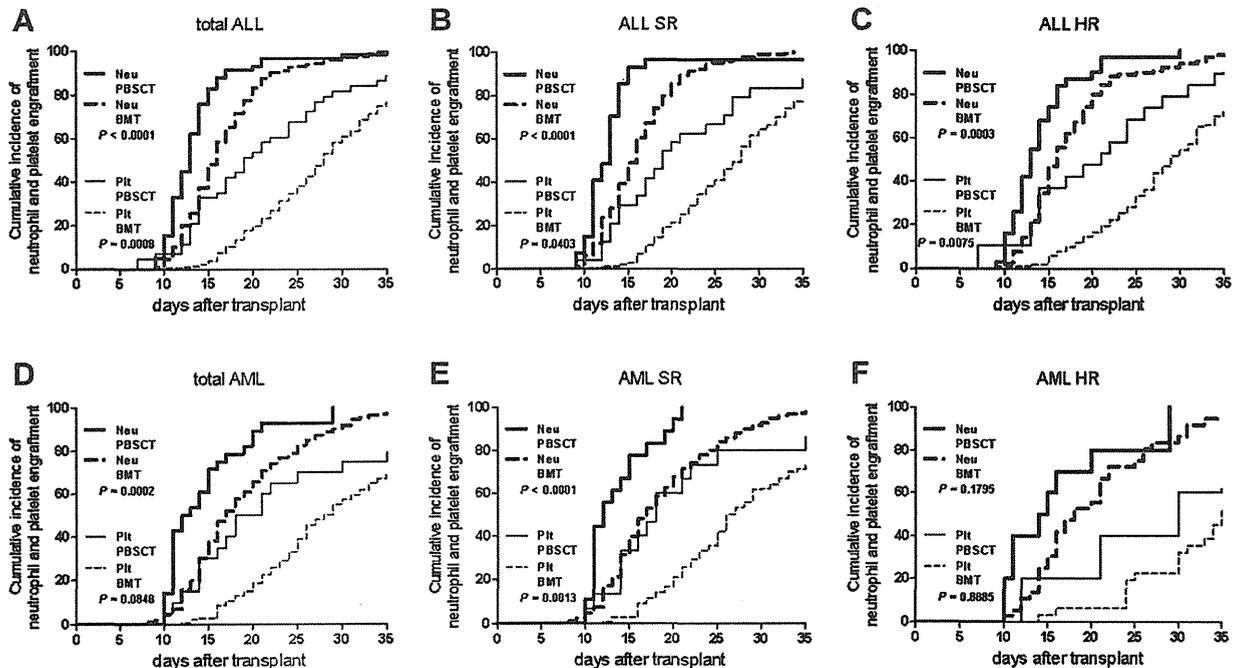

Fig. 1. Engraftment to neutrophil count >0.5 × 10⁹/L and engraftment to platelet count >50 × 10⁹/L. A: total ALL; (B) ALL standard-risk; (C) ALL high-risk; (D) total AML; (E) AML standard-risk; and (F) AML high-risk.

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 × 10⁹/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.
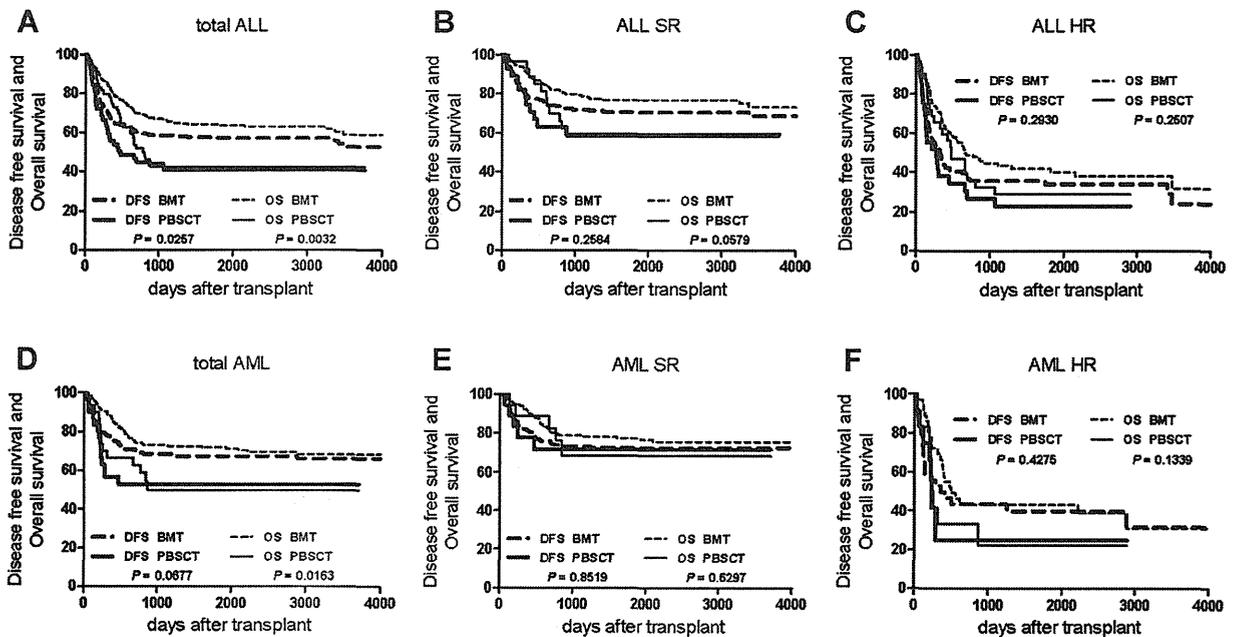
Fig. 2. Disease free survival and overall survival. A: total ALL; (B) ALL standard-risk; (C) ALL high-risk; (D) total AML; (E) AML standard-risk; and (F) AML high-risk.

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

| Variable | Disease free survival | | | Overall survival | | |
	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

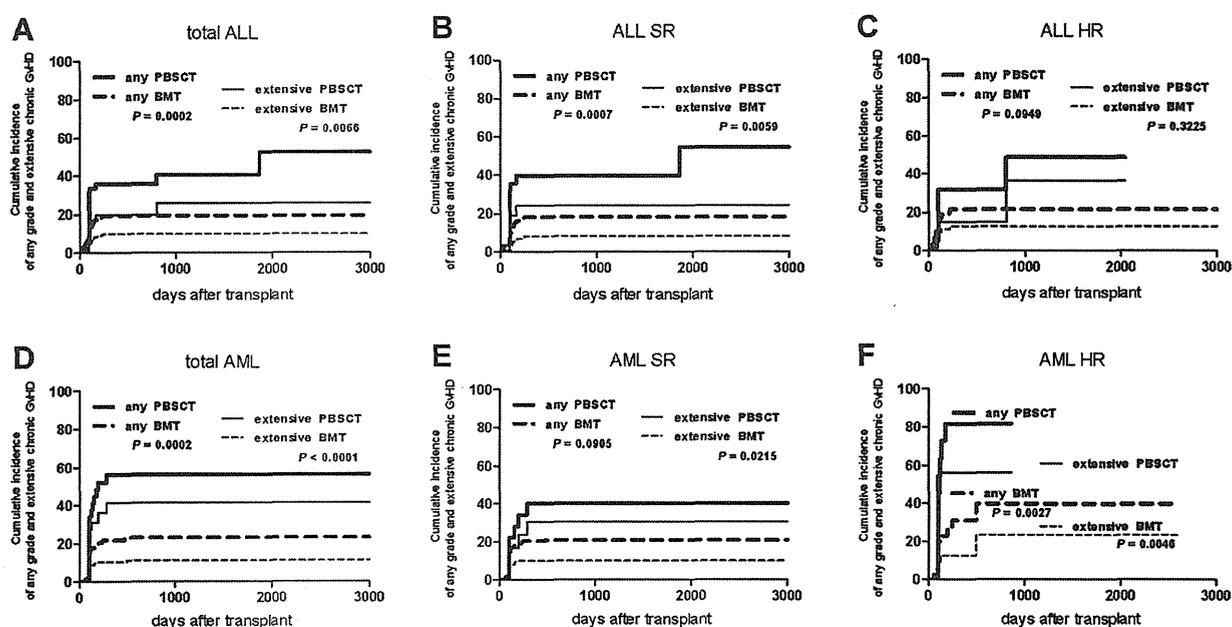As indicated by Figure 4, for the ALL group, cumulative incidences of TRM in PBSCT and in BMT were 5.1% and 4.0% at

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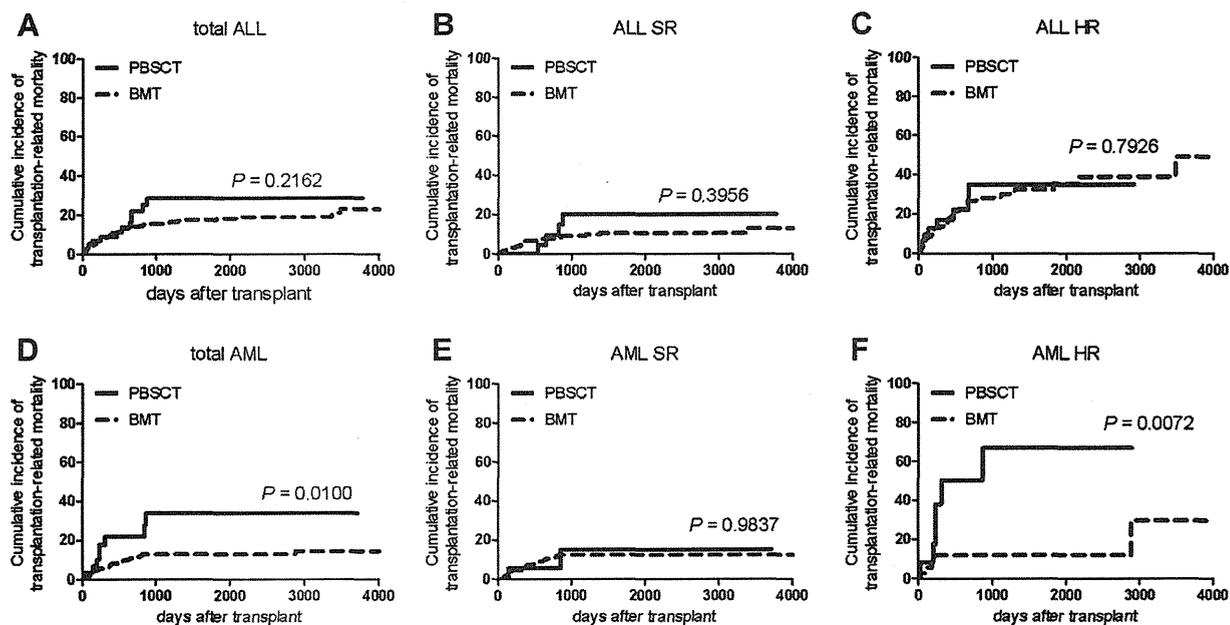
Fig. 4. Cumulative incidence of transplantation-related mortality. A: total ALL; (B) ALL standard-risk; (C) ALL high-risk; (D) total AML; (E) AML standard-risk; and (F) AML high-risk.

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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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 re-

sponse 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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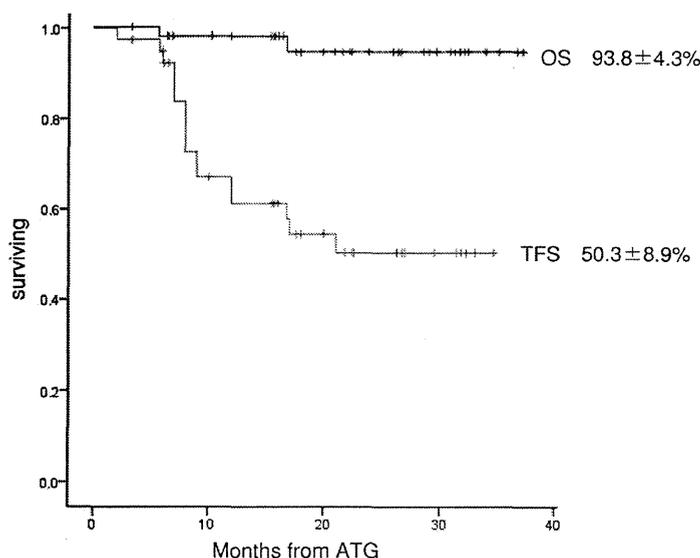*Department of Pediatrics, Hiroshima Red Cross Hospital, Hiroshima, Japan*

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