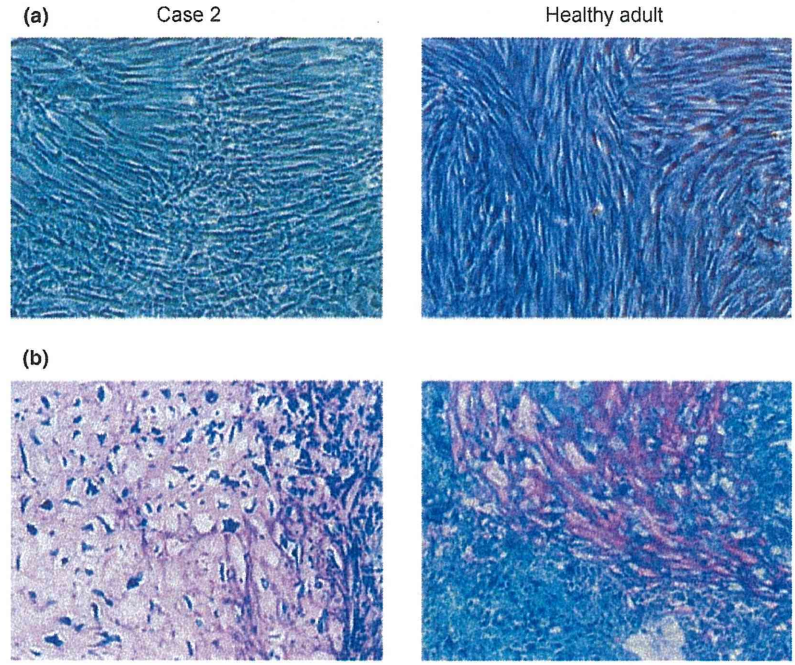

Fig. 1. Mesenchymal stem cells of BM from a haemophilia patient (case 2) and a healthy adult. (a) Morphology of the cultured cells from a haemophilia patient (case 2) and a healthy adult. They were spindle-shape like MSCs (×100). (b) Chondrocyte differentiation of BMMSCs from a haemophilia patient (case 2) and a healthy adult. Cells were stained with toluidine blue, and the stained cells were chondrocytes.

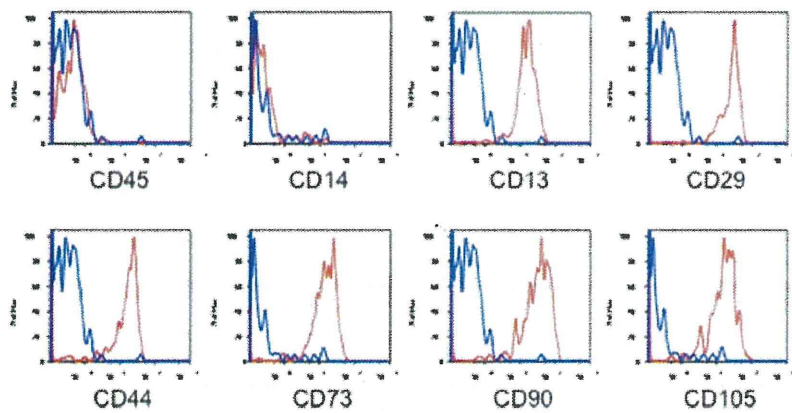

Fig. 2. Flow cytometric profiles of BMMSCs derived from a haemophilia patient (case 3). Cultured cells were stained with phycoerythrin (PE)-conjugated CD45, CD13, CD29, CD44 and CD90, and fluorescein isothiocyanate (FITC)-conjugated CD14, CD73 and CD105. Red and blue lines indicate data from the sample of case 3 and negative control respectively (×200).

All the cell numbers of MSCs derived from 2 mL of BM blood in three haemophilia patients were more than one million as well as a healthy adult (Table 1). Our previous experience demonstrated that 10 million of BMMSCs were enough to repair substantial range of articular cartilage defect in OA patients [3]. Consequently, we need to obtain more than 20 mL of BM blood from each patient to repair articular cartilage defects in haemophilic arthropathy, and it is possible under local anaesthesia.

In addition, chromosomal analysis revealed that the cultured cells from BM cells of three haemophilia patients had normal karyotype (Table 1), suggesting little possibility of the transformation of BMMSCs during the present culture. Thus, MSCs capable of proliferating *in vitro* and differentiating into chondrocytes were safely generated from BM cells of haemophilia patients similarly with those from healthy adult, indicating the feasibility of the regenerative medicine using BMMSCs to repair articular cartilage defects in the patients with haemophilic arthropathy.

Some of adult patients with haemophilia have chronic viral infection. To apply the regenerative medicine using BMMSCs to such patients, it is important to validate that our culture system of BMMSCs does not provoke the reactivation of the viruses. This possibility is now under investigation.

Table 1. Characteristics of patients and a healthy adult, and their BMMSCs.

Case	1	2	3	Healthy adult
Age	23	20	19	55
Type of haemophilia	A	A	A	–
Cell number/2 mL BM blood*	1.5×10^6	1×10^6	1×10^6	1×10^6
Karyotype of MSC†	46, XY (10/10)	46, XY (10/10)	46, XY (3/3)	46, XY (5/5)
Chondrocyte formation	+	+	+	+

*The number indicates the average of two cultures of 2 mL of BM blood.
†The number in each parenthesis indicates the number of cells analyzed.

Besides the transplantation of autologous BMMSCs, autologous chondrocyte implantation (ACI) may be considerable for the repair of articular cartilage defects in the patients with haemophilic

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ORIGINAL ARTICLE

Reduced-intensity allogeneic stem cell transplantation for patients aged 50 years or older with B-cell ALL in remission: a retrospective study by the Adult ALL Working Group of the Japan Society for Hematopoietic Cell Transplantation

This article has been corrected since Advance Online Publication and an erratum is also printed in this issue.

H Kanamori[1], S Mizuta[2], S Kako[3], H Kato[4], S Nishiwaki[5], K Imai[6], A Shigematsu[7], H Nakamae[8], M Tanaka[1], K Ikegame[9], T Yujiri[10], T Fukuda[11], K Minagawa[12], T Eto[13], T Nagamura-Inoue[14], Y Morishima[15], R Suzuki[16], H Sakamaki[17] and J Tanaka[18]

We retrospectively assessed the outcome and pretransplantation predictors of the outcome in 118 patients aged ⩾50 years who received fludarabine-containing reduced-intensity allo-SCT (RIST) for B-cell ALL in the first or second CR. Eighty patients received transplants from unrelated donors. Seventy-eight patients were positive for the Ph chromosome. The median follow-up period was 18 months and the 2-year OS rate was 56%. The 2-year cumulative incidence of relapse and non-relapse mortality was 28% and 26%, respectively. The incidence of grades II–IV and III–IV acute GVHD was 46% and 24%, respectively. After 2 years, the incidence of chronic GVHD was 37%. Multivariate analysis of pretransplant factors showed that a higher white blood cell count (⩾30×10^9/L) at diagnosis (hazard ratio (HR) = 2.19, $P = 0.007$) and second CR (HR = 2.02, $P = 0.036$) were significantly associated with worse OS, whereas second CR (HR = 3.83, $P < 0.001$) and related donor (HR = 2.34, $P = 0.039$) were associated with a higher incidence of relapse. Fludarabine-containing RIST may be a promising strategy for older patients with B-cell ALL in their first remission.

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Keywords: ALL; elderly; reduced-intensity SCT

INTRODUCTION

The overall CR rate is very high (80–90%) for adult ALL due to the efficacy of induction therapy with relatively low toxicity, which allows many patients to receive postremission therapy. However, adult ALL has a poor long-term outcome, with the 5-year OS rate being only 39–50% despite aggressive chemotherapy[1,2] and declining to 15% for patients over 50 years old.[3] At present, allogeneic hematopoietic SCT (allo-HSCT) is thought to be the most potent therapy for prevention of relapse in adult ALL patients. A recent large-scale prospective study showed that allo-HSCT from matched sibling donors achieved a better outcome compared with chemotherapy or autologous transplantation.[4] However, Goldstone *et al.*[4] reported that TRM is unacceptably high for high-risk older patients and this counteracts the reduced risk of relapse.[4] Therefore, reduced-intensity conditioning allo-HSCT (RIST) is performed in older patients and those who are unsuitable for myeloablative conditioning with the aim of reducing TRM, although its antileukemic efficacy is uncertain.[5–7] In general, the relationship between age and the prognosis of ALL patients aged between 20 and 65 years shows a continuum.[3] Because most older patients are excluded from clinical studies, very few prospective trials have investigated the efficacy of chemotherapy and/or allo-HSCT tailored for older patients. Therefore, more clinical data are needed to establish the optimum transplant strategy for elderly patients with ALL. Accordingly, the objectives of this study were to analyze the outcome and identify pretransplant outcome predictors in older patients with B-cell ALL undergoing RIST.

PATIENTS AND METHODS

Patient selection and data sources

This study enrolled patients aged 50 years or older who received RIST for B-cell ALL in the first or second remission between 2000 and 2009 in Japan.

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Data were provided by the Japan Society of Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP) and the Japan Cord Blood Bank Network (JCBBN). Information on transplantation was collected at 100 days after allo-HSCT, whereas the data concerning survival, disease status and long-term complications, including chronic GVHD and second malignancies, were renewed annually from follow-up forms. This study was approved by the data management committees of the JSHCT, JMDP and JCBBN. Informed consent was obtained from both recipients and donors in accordance with the Declaration of Helsinki Principles.

Graft sources

Peripheral blood stem cell (PBSC) donation from unrelated donor was not permitted until 2009 in Japan. If recipients have no suitable related donors, physicians choose alternative graft sources according to recipient's condition and institutional strategy. HLA matching of related donor–recipient pairs was mainly performed using serologic typing methods. HLA matching of unrelated BM and umbilical cord blood (CB) was performed using low- or high-resolution molecular typing for HLA-A, -B and -C, and high-resolution molecular typing for HLA-DRB1.

Study end points and definitions

The primary endpoints of the study were non-relapse mortality (NRM), relapse, leukemia-free survival (LFS) and OS. NRM was defined as death while in remission, and relapse was defined as hematological recurrence of leukemia. LFS was defined as survival without evidence of relapse or progression and OS was calculated from the date of allo-HSCT. Death from any cause was treated as an event and surviving patients were censored at the date of last contact. The day of engraftment was defined as the first of 3 consecutive days on which the ANC was $\geqslant 0.5 \times 10^9$/L. Acute and chronic GVHD were diagnosed and graded according to established criteria.[8,9] We defined a reduced-intensity regimen as having the following dosage levels: BU <9 mg/kg, melphalan $\leqslant 140$ mg/m^2 and TBI <500 cGy (single or fractionated) or <800 cGy (fractionated).[10]

Statistical analysis

The final date of analysis was 30 November 2010. We compared demographic factors and disease characteristics according to the donor source by using Fisher's exact test for categorical data and the Mann–Whitney *U*-test for continuous variables. LFS and OS were estimated by the Kaplan–Meier method. The Cox proportional hazards model was used for univariate and multivariate analyses. Gray's test was used to compare the cumulative incidence curves for relapse and NRM.[11] Death without acute GVHD was defined as the competing event for acute GVHD, whereas death without neutrophil engraftment and second transplantation without engraftment were the competing events for neutrophil engraftment, NRM and second transplantation without relapse were the competing events for relapse, and relapse and second transplantation were the competing events for NRM. The proportional hazard regression model of Fine and Gray[12] was used for univariate and multivariate analyses of these competing risks. All covariates with $P<0.10$ according to univariate analysis were entered into the multivariate model. All tests were two-sided and $P<0.05$ was considered to indicate significance. Statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R software (The R Foundation for Statistical Computing, version 2.13.0). More precisely, it is a modified version of R commander (version 1.6-3) that includes functions frequently used in biostatistics.[13]

RESULTS

Background of transplantation

The patient and graft characteristics are summarized in Table 1. A total of 187 patients aged $\geqslant 50$ years received RIST for ALL. Of these, 35 patients in non-remission, 32 patients with non-B cell (uncertain in 20, T cell in 10 and null cell in 2) and 2 patients aged $\geqslant 70$ years were excluded in this analysis. There were 118 patients in the study cohort and their median age was 59 years (range: 50–69 years). There were early pre-B-cell type in 6 patients, pre-B-cell type in 34 and common type in 78 according to immunophenotype classification. The median WBC count at diagnosis was 15.6×10^9/L (range: 0.8–1967 $\times 10^9$/L). BM was the most common source of stem cells (55%), followed by cord blood (CB) (24%) and PB (21%). The median time from diagnosis to RIST was 200 days (range: 75–3372 days). TBI was used for 74 patients and its dosages were as follows: 200 cGy in 13 patients, 300 cGy in 9, 400 cGy in 52 and 600 cGy in 1.

Transplantation was carried out on HLA-matched related donors in 33 patients, HLA-mismatched related donors in 5, HLA-matched unrelated donors in 47 and HLA-mismatched unrelated donors in 33. RIST from unrelated donors was significantly more frequent in patients aged 60–69 years compared with those aged 50–59 years. T-cell depletion was performed in six patients (five patients with antithymocyte globulin and 1 with antilymphocyte globulin). The median time from diagnosis to RIST from related and unrelated donors were 154 days (range: 75–617 days) and 229 days (range: 79–3372 days), respectively ($P=0.029$). Furthermore, use of TBI and GVHD prophylaxis showed significant differences among patients with different donor sources (Table 1).

Engraftment

The median time until neutrophil engraftment after transplantation was 16 days (range: 9–39 days). Three patients died before day 35 without achieving neutrophil recovery. Sustained engraftment was achieved in 113 of the remaining 115 patients, whereas primary graft failure was confirmed in two patients who received CB transplantation. One patient died of primary graft failure on day 60, but the other was salvaged by repeat transplantation. The median time to platelet count recovery ($\geqslant 20 \times 10^9$/L) was 26 days (range: 0–154 days). Seven patients died within 60 days after transplantation and stable engraftment of platelets was seen in 102 of the 111 patients who survived beyond day 60.

Acute and chronic GVHD

The incidence of GVHD according to donor type is shown in Table 2. The cumulative incidence of grade II–IV and grade III–IV acute GVHD was 46% and 24%, respectively. Stem cell and donor sources were not associated with the incidence or grade of acute GVHD. The cumulative incidence of chronic GVHD after 2 years was 37%. Limited chronic GVHD was noted in 16 patients (16%), whereas 24 patients (24%) had extensive chronic GVHD. After RIST from related donors, there was a significantly higher incidence of chronic GVHD compared with after RIST from unrelated donors ($P=0.012$). Also, RIST with PB from related donors was associated with a significantly higher incidence of chronic GVHD than when BM or CB was the source (63% vs 36% and 37%, respectively; $P=0.019$).

Outcome

The median follow-up period for the survivors was 18 months (range: 2–77 months). The 2-year LFS, OS, cumulative relapse rate and NRM were 66%, 56%, 28% and 26%, respectively (Figure 1). Detailed results, including the incidence of GVHD, are shown in Table 2, with stratification by donor source. Fifteen patients (14 with NRM and 1 with relapsed leukemia) died within 100 days after transplantation. They included 12 of the 57 patients receiving fludarabine + melphalan, but the conditioning regimen did not have a significant impact on 2-year OS (68% for fludarabine + i.v. BU, 64% for fludarabine + oral BU, 47% for fludarabine + melphalan and 63% for fludarabine + CY; $P=0.472$). When OS at 2 years was stratified according to stem cell source, it was 56%, 55%, 43% and 47% ($P=0.301$) for related BM, related PB, unrelated BM and unrelated CB, respectively. In addition, the 2-year OS of patients with ($n=78$) and without ($n=40$) the Ph chromosome was 58% and 52%, respectively ($P=0.997$). In this study, the information of pre- and post-transplant treatment with tyrosine kinase inhibitors was obtained in only 45 and 9 patients,

Table 1. Patient characteristics

	No. (%)	*Related donors*	*Unrelated donors*	P-*value*
No. of patients (%)	118 (100)	38 (32%)	80 (68%)	
Age (years)				
Median (range)		57 (50–67)	59 (50–68)	0.002
50–59	71 (60)	29	42	0.016
60–69	47 (40)	9	38	
Sex				0.557
Male	55 (47)	16	39	
Female	63 (53)	22	41	
WBC at diagnosis				0.405
$<30\times10^9$/L	77 (65)	23	54	
$\geqslant30\times10^9$/L	39 (33)	15	24	
Missing	2 (2)	0	2	
Ph chromosome				1
Negative	40 (34)	13	27	
Positive	78 (66)	25	53	
Disease status at RIST				0.137
CR1	96 (81)	34	62	
CR2	22 (19)	4	18	
Time from diagnosis to RIST				<0.001
<Median (200 days)	57 (50)	28	29	
⩾Median (200 days)	60 (49)	10	50	
Missing	1 (1)	0	1	
Stem cell				<0.001
BM	65 (55)	13	52	
Peripheral blood	25 (21)	25	0	
Cord blood	28 (24)	0	28	
HLA				0.737
Match	80 (68)	33	47	
Mismatch	38 (32)	5	33	
ABO				0.109
Match	54 (46)	21	33	
Mismatch	58 (49)	11	47	
Missing	6 (5)	6	0	
Female donor/male recipient				0.711
No	102 (86)	33	69	
Yes	14 (12)	5	9	
Missing	2 (2)	0	2	
CMV				0.077
Donor (+)/recipient (−)	4 (3)	3	1	
Others	97 (82)	27	70	
Missing	17 (14)	8	9	
Conditioning regimen				<0.001
Flu + Mel with TBI	37 (31)	5	32	
Flu + Mel wthout TBI	20 (17)	11	9	
Flu + Bu with TBI	27 (23)	8	19	
Flu + Bu without TBI	22 (19)	12	10	
Flu + Cy with TBI	11 (9)	1	10	
Flu + Cy without TBI	1 (1)	1	0	
GVHD prophylaxis				<0.001
CYA based	50 (42)	30	20	
Tacrolimus based	67 (57)	8	59	
Others	1 (1)	0	1	

Abbreviations: Flu = fludarabine; Mel = melphalan; RIST = reduced-intensity SCT; TBI = total body irradiation.

Table 2. Transplant outcomes according to donor

	Related donors (n = 38)	*Unrelated donors (n = 80)*	*P*-value
	% (95% CI)	*% (95% CI)*	
Acute GVHD (grade II–IV) at 100 days	31 (15–49)	53 (39–66)	0.099
Acute GVHD (grade III–IV) at 100 days	29 (13–47)	21 (12–32)	0.602
Chronic GVHD at 2 years	57 (39–72)	34 (22–46)	0.012
OS			0.521
1 year			
2 years	60 (41–74)	53 (40–65)	
3 years	56 (38–71)	44 (29–58)	
5 years	40 (21–59)	44 (29–58)	
Relapse			0.030
100 days	8 (2–19)	5 (2–11)	
1 year	36 (20–51)	12 (6–21)	
2 years	39 (23–55)	22 (13–34)	
3 years	46 (28–63)	27 (15–42)	
5 years	46 (28–63)	27 (15–42)	
Non-relapse mortality			0.106
100 days	5 (1–16)	15 (8–24)	
1 year	16 (6–29)	24 (16–35)	
2 years	16 (6–29)	31 (20–43)	
3 years	20 (8–35)	39 (24–53)	
5 years	24 (11–40)	39 (24–53)	

Abbreviation: CI = confidence interval.

respectively. Therefore, we could not do further analyses in the viewpoint of tyrosine kinase inhibitors treatment for Ph chromosome-positive ALL. The cumulative relapse rate in patients with unrelated donors was significantly low compared to those with related donors (22% vs 39% at 2 years, $P=0.030$). In subgroup analysis according to disease status at RIST, the difference of relapse rate at 2 years was significant in CR1 (13% vs 35%, $P=0.019$) but not in CR2 (54% vs 74%, $P=0.140$). In the patients transplanted from unrelated donors, there was no difference of relapse rate at 2 years between unrelated BM ($n=52$, 22%) and unrelated CB ($n=28$, 23%) ($P=0.976$). Patients who developed grade III and IV acute GVHD had significantly worse OS at 2 years than those with grade 0–II acute GVHD (20% vs 59%, $P<0.001$). Patients with severe acute GVHD also had a high NRM (grade 0–II: 22%; III–IV: 64%, $P<0.001$). In contrast, chronic GVHD did not influence the outcome.

The causes of death are shown in Table 3. Eighteen of the 38 patients with related donors died as did 34 of 80 with unrelated donors. Relapse and infection were the main causes of death. Infection was more common in patients with unrelated donors (35% vs 6%, $P=0.021$), whereas relapse was the most common cause of death in patients with related donors.

Prognostic factors

Using pretransplantation variables, the prognostic factors for OS, relapse and NRM were assessed by univariate analysis (Table 4). The WBC count at diagnosis ($<30\times10^9$/L: 63%; $\geqslant30\times10^9$/L: 42%, $P=0.012$) and the disease status at transplantation (CR1: 60%; CR2: 36%, $P=0.039$) were associated with the 2-year OS (Figure 2). Disease risk at transplantation (CR1: 21%; CR2: 56%, $P=0.016$), type of donor (related: 39%; unrelated: 22%, $P=0.030$) and TBI (no: 39%; yes: 21%, $P=0.031$) had an influence on relapse, whereas the WBC count at diagnosis was a significant predictor of NRM ($<30\times10^9$/L: 19%; $\geqslant30\times10^9$/L: 39%, $P=0.017$). According to multivariate analysis (Table 4), a high WBC count (HR = 2.19; 95% confidence interval (CI): 1.24–3.89, $P=0.007$) and CR2 (HR = 2.02; 95% CI: 1.05–3.89, $P=0.036$) were significant predictors of worse OS, while CR2 (HR = 3.83; 95% CI: 1.73–8.48, $P<0.001$) and related donor (HR = 2.34; 95% CI: 1.05–5.23, $P=0.039$) were significantly associated with a higher cumulative relapse rate. No risk factors for a higher cumulative NRM were identified. Other variables (including recipient age, sex, Ph chromosome, time from diagnosis to transplantation, conditioning

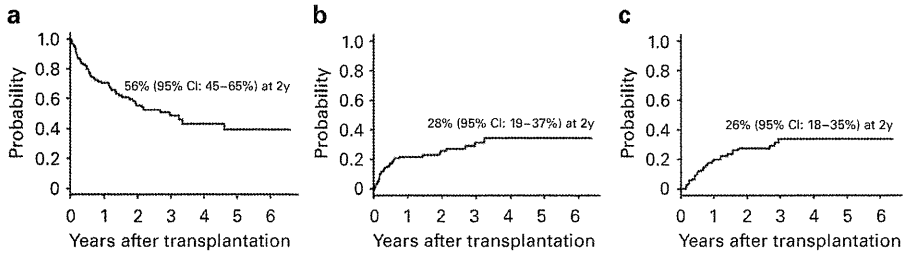

Figure 1. (**a**) OS, (**b**) cumulative incidence of relapse and (**c**) cumulative incidence of non-relapse mortality.

Table 3. Causes of death

	Related donors, no. (%)	*Unrelated donors, no. (%)*	P-*value*
No. of patients	38	80	
No. of deaths	18	34	
Relapse	7 (39)	7 (21)	0.197
Acute GVHD	2 (11)	4 (12)	1
Infection	1 (6)	12 (35)	0.021
Bleeding	1 (6)	3 (9)	1
Idiopathic interstitial pneumonia	1 (6)	1 (3)	1
Organ failure	4 (22)	2 (6)	0.166
Engraftment failure	1 (6)	1 (3)	1
Second malignancy	0	1 (3)	1
Others	1 (6)	3 (9)	1

regimen, donor source and TBI) were not identified as prognostic factors.

DISCUSSION

In the present study, we analyzed the outcome of elderly patients with B-cell ALL who underwent fludarabine-containing RIST and investigated potential prognostic factors. After transplantation in CR1 or CR2, 2-year OS was 56%, which was comparable to the results of previous large-scale retrospective studies of ALL patients in remission.[14,15] It was reported that the 3-year OS was 38% in patients receiving RIST in a study by the Center for International Blood and Marrow Transplant Research (CIBMTR), although the cohort included 93 Ph chromosome-negative ALL patients with a median age of 45 years (range: 17–66 years).[14] According to a study by the European Group for Blood and Marrow Transplantation, the 2-year OS was 48% for 127 patients with a median age of 56 years (range: 45–73 years), including those with Ph chromosome-positive ALL.[15] The 2-year cumulative incidence of relapse (28%) and NRM (26%) in the present study were also similar to the results of the above two studies. Those studies mainly involved comparison of RIST and myeloablative conditioning allo-HSCT, but we focused on the outcome stratified according to the donor source or conditioning regimen and pretransplant factors to identify predictors of survival in this study.

The optimum regimen of reduced-intensity conditioning for elderly ALL patients has not yet been defined. Cho *et al.*[16] reported an excellent outcome for high-risk ALL patients in remission when they used conditioning with fludarabine and melphalan followed by transplantation from a matched sibling. We compared three fludarabine-containing regimens in this study. The 2-year OS achieved when BU, melphalan or CY was combined with fludarabine was 64%, 47% and 63%, respectively ($P = 0.287$). In addition, 2-year OS showed no difference between i.v. BU (68%) and oral BU (64%). Unexpectedly, fludarabine plus BU achieved a better outcome than use of melphalan, although a significant difference was not confirmed. Owing to the small number of patients in each group and the short duration of follow-up, we could not find any differences of relapse and NRM among the regimens. To clarify the most suitable conditioning regimen for older patients with ALL, a prospective randomized study of fludarabine plus BU or melphalan seems to be warranted on the basis of our results.

The other main aim of this study was to identify factors associated with the outcome of fludarabine-containing RIST for elderly B-cell ALL. According to previous studies, factors such as age, immunophenotype, WBC and cytogenetic abnormalities are associated with the outcome of chemotherapy and/or transplantation for ALL.[17] The Ph chromosome is the most frequent and clinically significant abnormality in adult ALL, with an incidence ranging from 15 to 50% among older patients with B-cell ALL.[18] Use of tyrosine kinase inhibitors combined with chemotherapy has altered the prognosis of these patients.[19] We could not obtain detailed information about tyrosine kinase inhibitor treatment before or after transplantation and minimal residual disease from the registry data, but the Ph chromosome was not a risk factor in the present retrospective study. Taken together with previous reports,[7,20] RIST for Ph chromosome-positive ALL in CR1 is thought to be a hopeful strategy from the viewpoint of curability.

The better outcome of patients who underwent RIST in CR1 compared with CR2 was confirmed in this study, as demonstrated in previous reports.[7,14–16,20] The European Group for Blood and Marrow Transplantation reported that patients in CR1 had a lower NRM (18% vs 44%, $P = 0.01$) and higher OS (52% vs 20%, $P = 0.003$) at 2 years after transplantation than patients beyond CR1, which strongly supports the importance of RIST for patients with a favorable disease status.[7] Cho *et al.*[16] also reported that RIST achieved a better outcome in CR1 patients than in CR2 patients with respect to relapse (14.8% vs 55.6%, $P = 0.07$) and OS (74.7% vs 21.7%, $P = 0.01$).[16] They identified a GVL effect of chronic GVHD, because chronic GVHD was associated with a significantly lower incidence of relapse (4.8% vs 45.5%, $P = 0.02$). In our study, patients with unrelated donors had a lower relapse rate than those with related donors, but chronic GVHD was conversely seen in the patients with related donors. These results may have been influenced by the unique situation in Japan that PB as a stem cell source is only available from related donors. As one of the possible explanations, the longer time from diagnosis to RIST in patients from unrelated donors might influence on patient's selection with favorable prognosis.

The WBC count at diagnosis and the disease status were two important prognostic factors in this analysis. A cutoff value of 30×10^9/L has often been used in clinical studies of B-cell ALL and its significance has been shown in previous reports.[3,17] Marks reported that a WBC $>25 \times 10^9$/L at diagnosis predicted a worse outcome for adult patients with ALL in CR1/2 who received

Table 4. Pretransplant prognostic factors

Variables	No.	*OS at 2 years*				*Relapse at 2 years*				*Non-relapse mortality at 2 years*			
		Univariate		*Multivariate*		*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
		%	P-*value*	*HR (95% CI)*	P-*value*	%	P-*value*	*HR (95% CI)*	P-*value*	%	P-*value*	*HR (95% CI)*	P-*value*
Age (years)			0.147				0.729				0.069		0.180
50–59	71	59				27				21		1	
60–69	47	52				31				34		1.66 (0.79–3.46)	
Sex			0.280				0.919				0.086		0.310
Male	55	52				30				31		1	
Female	63	59				26				21		0.65 (0.29–1.48)	
WBC at diagnosis			0.012		0.007		0.729				0.017		0.064
$<30 \times 10^9$/L	77	63		1		30				19		1	
$\geqslant 30 \times 10^9$/L	39	42		2.19 (1.24–3.89)		25				39		1.99 (0.96–4.12)	
Disease status at RIST			0.039		0.036		0.016		<0.001		0.733		
CR1	96	60		1		21		1		27			
CR2	22	36		2.02 (1.05–3.89)		56		3.83 (1.73–8.48)		23			
Donor			0.521				0.030		0.039		0.106		
Unrelated	80	53				22		1		31			
Related	38	60				39		2.34 (1.05–5.23)		16			
TBI			0.403				0.031		0.068		0.270		
No	43	54				39		1		23			
Yes	75	57				21		0.49 (0.23–1.06)		27			

Abbreviations: CI = confidence interval; HR = hazard ratio; RIST = reduced-intensity SCT.

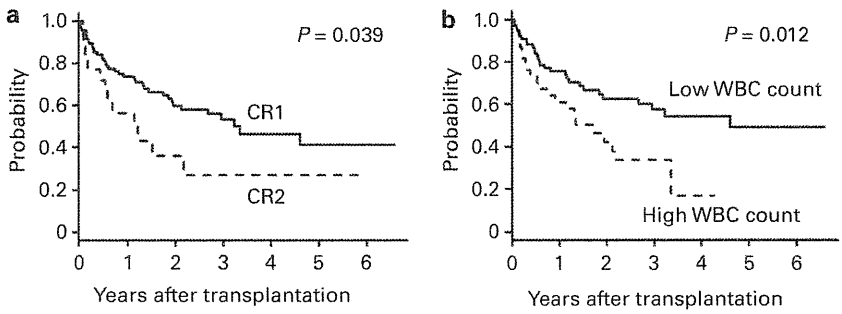

Figure 2. (**a**) OS of patients in their first (CR1) vs those in their second CR (CR2). (**b**) OS of patients with a low WBC count vs high WBC count at diagnosis.

transplantation after either full conditioning ($n = 1421$) or reduced-intensity conditioning ($n = 92$).[14] However, there has been no report about the clinical impact of WBC at diagnosis on survival among patients receiving RIST alone. Therefore, our results support the WBC count at diagnosis as one of the useful parameters for stratifying patients in studies of RIST.

Although there are limitations because this was a retrospective study based on registry data from multiple centers, our cohort consisted of a relatively large number of B-cell ALL patients aged 50 years or older and this study had the unique characteristic of investigating elderly B-cell ALL patients with 66% being Ph chromosome-positive. In contrast with the stem cell sources available in Western countries, only BM or CB was transplanted from unrelated donors in this study because transplantation with unrelated PB was not approved in Japan until 2009.

In conclusion, the results of this study support further investigation of fludarabine-containing RIST for ALL in the elderly, especially for patients in CR1, although longer follow-up is needed to confirm the durability of remission and the quality of life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

HK (Kanamori) designed the study, analyzed the data and wrote the draft version of this manuscript. HN, MT, KI, TY, TF, KM and TE submitted and cleaned the data; T-NI, YM, RS and HS collected and reviewed the data; And SM, SK, HK (Kato), SN, KI, AS and JT interpreted the results and critically revised the manuscript.

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Clinical Factors Predicting the Response of Acute Graft-versus-Host Disease to Corticosteroid Therapy: An Analysis from the GVHD Working Group of the Japan Society for Hematopoietic Cell Transplantation

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A B S T R A C T

Systemic corticosteroid therapy is recommended as a first-line treatment for acute graft-versus-host disease (GVHD). We performed a retrospective study to identify the factors affecting the response of grade II to IV acute GVHD to systemic corticosteroid therapy using the Japanese national registry data for patients who received first allogeneic hematopoietic cell transplantation with bone marrow (BM) (n = 1955), peripheral blood stem cells (PBSCs) (n = 642), or umbilical cord blood (UCB) (n = 839). Of 3436 patients, 2190 (63.7%) showed improvement of acute GVHD to first-line therapy with corticosteroids. Various factors were identified to predict corticosteroid response. Interestingly, UCB (versus HLA-matched related BM) transplantation was significantly associated with a higher probability of improvement, whereas HLA-matched unrelated BM and HLA-mismatched stem cell sources other than UCB were significantly associated with a lower probability of improvement. HLA-matched related PBSC transplantation was not significantly different from HLA-matched related BM transplantation. Patients without improvement from corticosteroid therapy had a 2.5-times higher nonrelapse mortality and a .6-times lower overall survival rate. The present study demonstrated, for the first time, a higher probability of improvement in grade II to IV acute GVHD with systemic corticosteroid therapy in patients after UCB transplantation than in those after BM and PBSC transplantation. A prospective study is warranted.

INTRODUCTION

Despite prophylactic treatment with immunosuppressive agents, acute graft-versus-host disease (GVHD) remains a major problem after allogeneic hematopoietic cell transplantation (HCT). Several studies have evaluated a variety of agents added to prednisone [1-7], but the use of prednisone or methylprednisolone alone is recommended as a standard first-line treatment for acute GVHD [8]. The response rate is approximately 40% to 60%, and patients unresponsive or resistant to corticosteroid therapy have an increased risk of mortality related to uncontrolled GVHD [2,9-16]. Some clinical factors are reported to be statistically predictive of a response to systemic corticosteroid therapy: HLA-mismatched donor transplantation, unrelated donor transplantation, combination of male recipient and female donor, early onset of GVHD, higher grade of GVHD, and liver or gut involvement of GVHD have lower response rates [2,9,10,14].

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These significant factors were identified in retrospective studies in which most or all patients underwent bone marrow (BM) transplantation. However, stem cell sources for allogeneic HCT have changed dramatically with the frequent use of peripheral blood stem cells (PBSCs) and umbilical cord blood (UCB), and no study has compared the response rates of corticosteroid therapy among stem cell sources.

To identify the factors affecting the response to systemic corticosteroid therapy as a first-line treatment for patients with grade II to IV acute GVHD, a retrospective study was conducted using the national registry data on 3436 patients who received first allogeneic HCT in Japan with BM (n = 1955), PBSCs (n = 642), or UCB (n = 839).

PATIENTS AND METHODS

Patients

Clinical data for patients who received the first allogeneic HCT in Japan, achieved neutrophil engraftment (>.5 × 10^9/L), developed grade II to IV acute GVHD, and received systemic corticosteroid therapy as a first-line treatment for acute GVHD were extracted from the Transplant Registry Unified Management Program system, which is a registry of the outcomes of Japanese transplantation patients [17]. Patients who relapsed before GVHD development were excluded, as were patients who received other agents as initial therapy in addition to systemic corticosteroid therapy. This study was approved by the Data Management Committee of the Japan Society for Hematopoietic Cell Transplantation and by the ethical committee of the Nagoya University School of Medicine.

Definitions

Acute GVHD was diagnosed and graded according to established criteria [18]. Persistent nausea with histologic evidence of GVHD but no diarrhea was included as stage 1 gut GVHD. Responses of acute GVHD to corticosteroid therapy were defined as *improved* if the grade was improved without additional systemic treatment. Responses were evaluated without time limitation, and therefore were considered improved even if the GVHD was improved later than day 28 of corticosteroid therapy, although response by day 28 is proposed as the best endpoint to define need for second-line treatment [16]. Responses were also considered improved even if acute GVHD was improved and then a new immunosuppressant was added to treat chronic GVHD. Responses were defined as *stable or progressive* if the grade was unchanged or worsened after first-line corticosteroid therapy or if second-line systemic treatment for acute GVHD was added regardless of responsiveness to first-line corticosteroid therapy. Thus, all patients who received second-line treatment for acute GVHD were considered stable or progressive even if the GVHD was improved temporarily after corticosteroid therapy.

Acute myeloid leukemia in the first or second remission, acute lymphoblastic leukemia in the first remission, chronic myelogenous leukemia in the first chronic phase, and myelodysplastic syndromes with refractory anemia or refractory anemia with ringed sideroblasts were defined as *standard-risk malignancies*, and other malignant diseases were defined as *high-risk malignancies*.

BM transplantation from serological HLA-A, B, and DR 6/6 matched related donors was defined as *MRD-BM*, and BM transplantation from serological HLA-A, B, and DR at least 3/6 matched, but not 6/6 matched related donors, was defined as *MMRD-BM*. PBSC transplantation from serological HLA-A, B, and DR 6/6 matched related donors was defined as *MRD-PB*, and PBSC transplantation from serological HLA-A, B, and DR at least 3/6 matched, but not 6/6 matched related donors, was defined as *MMRD-PB*. For unrelated BM transplantation, all patient–donor pairs were HLA-typed to allele level for at least 3 loci (HLA-A, B, and DRB1) during the coordination process. BM transplantation from HLA-A, B, and DRB1 alleles 6/6 matched unrelated donors was defined as *MUD-BM*, and BM transplantation from HLA-A, B, and DRB1 alleles 5/6 or 4/6 matched unrelated donors was defined as *MMUD-BM*. UCB transplantation from serological HLA-A, B, and DR at least 4/6 matched donors was defined as *UCB*.

Based on the report by the Center for International Blood and Marrow Transplant Research [19], the conditioning regimens were classified as *myeloablative* if total body irradiation >8 Gy, oral busulfan ≥9 mg/kg, intravenous busulfan ≥7.2 mg/kg, or melphalan >140 mg/m^2 was included in the conditioning regimen, whereas other conditioning regimens were classified as *nonmyeloablative*.

Onset of acute GVHD was classified into 3 groups: day ≤28, day ≥29, and unknown; however, acute GVHD that occurred earlier than day 4, which might be an error at the time of registration, was classified into unknown.

Endpoints

The primary endpoint of this study was to identify the factors affecting the response to systemic corticosteroid therapy as a first-line treatment for grade II to IV acute GVHD. The secondary endpoints were to identify factors associated with nonrelapse mortality (NRM) after corticosteroid therapy and to evaluate the impact of response to corticosteroid therapy on the overall survival (OS) rate after corticosteroid therapy.

Statistical Analysis

Univariate and multivariate logistic regression analyses were used to identify factors associated with the response to corticosteroid therapy. The probability of NRM after systemic corticosteroid therapy stratified by response to corticosteroid therapy was estimated on the basis of cumulative incidence curves in which relapse was treated as a competing event [20]. The probability of OS after systemic corticosteroid therapy stratified by response to corticosteroid therapy was estimated according to the Kaplan-Meier method [21]. The groups were compared using the log-rank test. Competing risk regression analysis was used to identify factors associated with NRM after corticosteroid therapy. The adjusted probability of OS after corticosteroid therapy was estimated using the Cox proportional hazards model, with consideration of other significant clinical variables in the final multivariate models [22]. P values were 2 sided, and $P < .05$ was considered significant. The following covariates were considered for the multivariate models: patient age, patient sex, sex mismatch between patient and donor, disease, stem cell source, cytomegalovirus serostatus, preconditioning, GVHD prophylaxis, in vivo T cell depletion, year of transplantation, onset of acute GVHD, grade of acute GVHD, organ involvement of acute GVHD, and response to systemic corticosteroid therapy (improved or stable/progressive). The data were analyzed by STATA version 12 statistical software (StataCorp, TX).

RESULTS

Patient, Transplantation, and GVHD Characteristics

A total of 3436 patients met the inclusion criteria. Patient and transplantation characteristics are shown in Table 1. Patient age at transplantation ranged from 0 to 82 years (median, 40 years); the number of patients age <18, 18 to 49, and ≥50 years was 672, 1626, and 1138, respectively. Stem cell sources were BM (n = 1955), PBSC (n = 642), and UCB (n = 839). All UCB transplantation was performed with a single unit. In vivo T cell depletion was performed in 168 (5%) patients by either antithymocyte globulin or antilymphocyte globulin. No other drugs, such as alemtuzumab, were used for in vivo T cell depletion, nor was ex vivo T cell depletion used in any patients. The year of transplantation ranged from 1984 to 2009; the majority of cases (94%) were performed in 2000 or later.

Characteristics of acute GVHD cases are shown in Table 2. The numbers of patients who developed acute GVHD at day ≤28 and day ≥29 were 2344 and 994, respectively. Of 3436 patients who received systemic corticosteroid therapy as the first-line treatment for grade II to IV acute GVHD, 2190 (63.7%) showed improvement of acute GVHD.

Factors Associated with Improvement of GVHD by Corticosteroid Therapy

MUD-BM, HLA-mismatched stem cell source other than UCB (MMRD-BM, MMRD-PB, and MMUD-BM), more severe acute GVHD, and multiple organ involvement of acute GVHD, including gut, were significantly associated with a lower probability of improvement by corticosteroid therapy (Table 3). On the other hand, adult patient (ages 18 to 49 years) and UCB were significantly associated with a higher probability of improvement by corticosteroid therapy (Table 3). Although some factors, such as disease, cytomegalovirus serostatus, and preconditioning, were significant for corticosteroid response in univariate analysis, they were not significant in multivariate analysis. Additional analysis in which onset of acute GVHD was modeled as a continuous variable could not detect a significant association between

Table 1
Patient and Transplantation Characteristics (N = 3436)

Characteristic	Total (N = 3246)	MRD-BM/PB (n = 926)	MUD-BM + mm* (n = 1671)	UCB (n = 839)
Patient age at transplantation				
<18 yr	672 (20)	99 (11)	310 (19)	263 (31)
18 to 49 yr	1626 (47)	520 (56)	836 (50)	270 (32)
≥50 yr	1138 (33)	307 (33)	525 (31)	306 (37)
Patient sex				
Female	1393 (41)	380 (41)	668 (40)	345 (41)
Male	2043 (59)	546 (59)	1003 (60)	494 (59)
Sex mismatch between patient and donor				
Female donor to male patient	815 (24)	251 (27)	348 (21)	216 (26)
Other combinations	2525 (73)	662 (72)	1321 (79)	542 (64)
Unknown	96 (3)	13 (1)	2 (0)	81 (10)
Disease				
Standard-risk malignancies	1320 (38)	372 (40)	686 (41)	262 (31)
High-risk malignancies	1926 (57)	509 (55)	900 (54)	517 (62)
Nonmalignancies	154 (4)	40 (4)	80 (5)	34 (4)
Unknown	36 (1)	5 (1)	5 (0)	26 (3)
Stem cell source				
MRD-BM	445 (13)	445 (48)	0 (0)	0 (0)
MRD-PB	481 (14)	481 (52)	0 (0)	0 (0)
MUD-BM	783 (23)	0 (0)	783 (47)	0 (0)
UCB	839 (24)	0 (0)	0 (0)	839 (100)
MMRD-BM	155 (4)	0 (0)	155 (9)	0 (0)
MMRD-PB	161 (5)	0 (0)	161 (10)	0 (0)
MMUD-BM	572 (17)	0 (0)	572 (34)	0 (0)
Cytomegalovirus serostatus				
Negative donor to negative patient	322 (9)	53 (6)	112 (7)	159 (19)
Positive donor to negative patient	215 (6)	64 (7)	149 (9)	0 (0)
Negative donor to positive patient	899 (26)	107 (12)	290 (17)	509 (61)
Positive donor to positive patient	1541 (46)	574 (61)	960 (57)	0 (0)
Unknown	459 (13)	128 (14)	160 (10)	171 (20)
Preconditioning				
Myeloablative	2094 (61)	578 (62)	1030 (62)	486 (58)
Nonmyeloablative	1307 (38)	323 (35)	636 (38)	348 (41)
Unknown	35 (1)	25 (3)	5 (0)	5 (1)
GVHD prophylaxis				
Cyclosporine A–based	1676 (49)	800 (87)	417 (25)	459 (55)
Tacrolimus-based	1691 (49)	103 (11)	1227 (73)	361 (43)
Others	56 (2)	20 (2)	26 (2)	10 (1)
Unknown	13 (0)	3 (0)	1 (0)	9 (1)
In vivo T cell depletion				
No	3251 (95)	876 (94)	1556 (93)	819 (98)
Yes	168 (5)	34 (4)	115 (7)	19 (2)
Unknown	17 (0)	16 (2)	0 (0)	1 (0)
Year of transplantation				
1984 to 1999	200 (6)	103 (11)	63 (4)	34 (4)
2000 to 2004	721 (21)	182 (20)	221 (13)	318 (38)
2005 to 2009	2515 (73)	641 (69)	1387 (83)	487 (58)

MRD-BM indicates HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow; GVHD, graft-versus-host disease.
Data presented are n (%).
* mm indicates MMRD-BM, MMRD-PB, and MMUD-BM.

onset of acute GVHD and response to corticosteroid therapy. Response rates to corticosteroid therapy in each stem cell source are summarized in Table 4.

Impact of the Response to Corticosteroid Therapy on NRM

The cumulative incidence rates of NRM after systemic corticosteroid therapy for grade II to IV acute GVHD are shown in Figure 1. Patients who did not achieve improvement of acute GVHD by corticosteroid therapy had a significantly higher NRM compared with those who achieved improvement ($P < .0001$).

To identify factors associated with NRM after corticosteroid therapy for grade II to IV acute GVHD, competing risk regression analysis was performed. The patients with a stable or progressive response to corticosteroid therapy were approximately 2.5 times more likely to have NRM than patients with an improved response to corticosteroid therapy (Table 5). Other factors associated with significantly worse NRM included older patient age (18 to 49 years and ≥50 years), higher grades of acute GVHD (grades III and IV), and liver or multiple organ involvement including liver of acute GVHD (Table 5). Although some factors such as patient sex, disease, and preconditioning were significant for NRM in univariate analysis, they were not significant in multivariate analysis. Additional analysis in which onset of acute GVHD was modeled as a continuous variable could not detect a significant association between onset of acute GVHD and NRM.

Impact of the Response to Corticosteroid Therapy on the OS Rate

The Kaplan-Meier estimates of OS rates after systemic corticosteroid therapy for grade II to IV acute GVHD are shown in Figure 2. Patients who did not achieve improvement of acute GVHD by corticosteroid therapy had