OPEN

Citation: Blood Cancer Journal (2013) 3, e164; doi:10.1038/bcj.2013.62

www.nature.com/bcj

ORIGINAL ARTICLE

Effects of KIR ligand incompatibility on clinical outcomes of umbilical cord blood transplantation without ATG for acute leukemia in complete remission

J Tanaka[1], Y Morishima[2], Y Takahashi[3], T Yabe[4], K Oba[5], S Takahashi[6], S Taniguchi[7], H Ogawa[8], Y Onishi[9], K Miyamura[10], H Kanamori[11], N Aotsuka[12], K Kato[13], S Kato[14], Y Atsuta[15] and Y Kanda[16]

To clarify the effect of killer cell immunoglobulin-like receptor (KIR) ligand incompatibility on outcomes of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) patients in complete remission after single cord blood transplantation (CBT), we assessed the outcomes of CBT registered in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) database. A total of 643 acute leukemia (357 AML and 286 ALL) patient and donor pairs were categorized according to their KIR ligand incompatibility by determining whether or not they expressed HLA-C, Bw4 or A3/A11 by DNA typing. A total of 128 patient–donor pairs were KIR ligand-incompatible in the graft-versus-host (GVH) direction and 139 patient–donor pairs were incompatible in the host-versus-graft (HVG) direction. Univariate and multivariate analyses showed no significant differences between the KIR ligand-incompatible and compatible groups in the GVH direction for both AML and ALL patients of overall survival, disease-free survival, relapse incidence, non-relapse mortality and acute GVH disease. However, KIR incompatibility in the HVG direction ameliorated engraftment in ALL patients (hazard ratio 0.66, 95% confidence interval 0.47–0.91, $P = 0.013$). Therefore, there were no effects of KIR ligand incompatibility in the GVH direction on single CBT outcomes for acute leukemia patients without anti-thymocyte globulin use. However, it is necessary to pay attention to KIR incompatibility in the HVG direction for engraftment.

Blood Cancer Journal (2013) **3**, e164; doi:10.1038/bcj.2013.62; published online 29 November 2013

Keywords: CBT; GVHD; HVG; KIR; NK cell

INTRODUCTION

Killer cell immunoglobulin-like receptor (KIR) ligand incompatibility may have some important roles in transplantation outcomes such as leukemia relapse and leukemia-free survival.[1–4] Ruggeri *et al.*[5,6] reported surprisingly good clinical results that indicated no relapse, no rejection and no acute graft-versus-host disease (GVHD) after human leukocyte antigen (HLA) haplotype-mismatched transplantations with KIR ligand incompatibility in the GVH direction for acute myeloid leukemia (AML) patients. They also reported that donor allogeneic natural killer (NK) cells attacked host antigen-presenting cells (APCs), resulting in the suppression of GVHD. However, results of studies regarding the clinical advantage of KIR ligand incompatibility in allogeneic stem cell transplantation (allo SCT) from an unrelated donor are discrepant. Davies *et al.*[7] reported that there was no effect of KIR ligand incompatibility on outcomes of unrelated bone marrow transplantation without using anti-thymocyte globulin (ATG), whereas Giebel *et al.*[8] reported a good effect of KIR ligand incompatibility on the outcomes of unrelated bone marrow transplantation using ATG as part of GVHD prophylaxis. Morishima *et al.*[9] reported that KIR ligand mismatching induced adverse effects on acute GVHD and rejection in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor in Japan. It was reported that cord blood transplantation (CBT) for acute leukemia patients in complete remission (CR) from KIR ligand-incompatible donors in the GVH direction was associated with decreased relapse and improved survival.[10] In another study, it was shown that KIR ligand mismatch was associated with development of severe acute GVHD and risk of death after double CBT with reduced-intensity conditioning (RIC) regimen.[11] Therefore, the role of KIR ligand incompatibility in allo SCT remains controversial. To clarify the effect of KIR ligand incompatibility on the outcomes of AML and acute lymphoblastic leukemia (ALL) patients in CR after single CBT, we assessed the outcomes of CBT registered in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) database between 2001 and 2010 (A Study from the HLA Working Group of the JSHCT).

[1]Depatment of Hematology, Tokyo Women's Medical University, Tokyo, Japan; [2]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan; [3]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [4]Japanese Red Cross Kanto-Koshinetsu Blood Center, Tokyo, Japan; [5]Translational Research and Clinical Trial Center, Hokkaido University Hospital, Hokkaido University, Sapporo, Japan; [6]Division of Molecular Therapy, The Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [7]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [8]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [9]Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai, Japan; [10]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [11]Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan; [12]Division of Hematology-Oncology, Japanese Red Cross Society Narita Hospital, Narita, Japan; [13]Department of Hematology and Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [14]Tokai University Cord Blood Bank, Isehara, Japan; [15]Department of Hematopoietic Stem Cell Transplantation Data Management and Biostatistics, Nagoya University School of Medicine, Nagoya, Japan and [16]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan. Correspondence: Professor J Tanaka, Department of Hematology, Tokyo Women's Medical University, 8-1 Kawada-Cho, Shinjuku-Ku, Tokyo 162-8666, Japan.
E-mail: jutanaka@dh.twmu.ac.jp
Received 25 October 2013; accepted 29 October 2013

MATERIALS AND METHODS

Study design and data collection

This study was a retrospective analysis of data from a Japanese nationwide multicenter survey. Data were provided by the HLA Working Group of the JSHCT. Outcomes of 643 acute leukemia (357 AML nd 286 ALL) patients in CR were analyzed. Informed consent was obtained from patients and donors according to the Declaration of Helsinki, and approval was obtained from the Institutional Review Board of Hokkaido University Hospital.

Patient population

This study included AML and ALL patients who received single CBT in CR and (1) patients and donors whose HLA-A, B, C and DR alleles were determined by DNA typing as described previously,[9] (2) underwent transplantation between 2001 and 2010, (3) received a myeloablative conditioning (MAC) regimen ($n=456$) as high-dose radiation and chemotherapy usually in combination with cyclophosphamide or an RIC regimen ($n=187$) defined basically as the use of fludarabine plus low-dose busulfan or melphalan with or without low-dose total body irradiation[12] and (4) did not receive ATG as a preparative regimen.

Inhibitory KIR ligand assessment

Patients and donors were categorized according to their KIR ligand incompatibility by determining whether or not they expressed HLA-C group 1 or 2, Bw4 or A3/A11 as initially described by Ruggeri *et al.*[5] and Leung.[13] KIR ligand mismatch in the GVH direction was scored when the donor's KIR ligand was not shared by the patient. KIR ligand mismatch in the HVG direction was scored when the patient's KIR ligand was not shared by the donor.

Transplant procedures

Differences among patients, disease and transplantation-related factors according to conditioning regimens, and GVHD prophylaxis are shown in Tables 1a and b.

Endpoints

Primary endpoints included overall survival (OS), disease-free survival (DFS), relapse (cumulative incidence of relapse, CIR), non-relapse mortality (NRM) and engraftment. Relapse was defined as clinical and hematological leukemia recurrence. NRM was defined as death during continuous CR after transplantation. Engraftment was defined as a peripheral granulocyte count of $>500/\mu l$ for three consecutive days after transplantation.

Statistical analysis

Characteristics of patients who received KIR ligand-incompatible CBT in the GVH direction and the compatible group were compared using the χ^2-test for categorical variables and the Wilcoxon two-sample test for continuous variables. To compare the prognosis of the incompatible group with that of the compatible group, univariate survival analyses were conducted for OS, DFS, CIR, NRM, engraftment and acute GVHD (grades II–IV). Survival curves of OS and DFS for each group were depicted using the Kaplan–Meier method and compared using the log-rank test. In the analysis of CIR, NRM, engraftment and acute GVHD, cumulative probabilities were estimated on the basis of cumulative incidence curves to accommodate the following competing events: death for relapse, relapse for transplantation-related mortality, death without GVHD for acute GVHD and death without engraftment for neutrophil engraftment. Groups were compared using the Gray test.[14] To adjust for potential confounders, multivariate analyses were conducted using the Cox proportional hazards model for OS and DFS, and using the Fine–Gray proportional hazards model for CIR and NRM.[15] The variables considered in the multivariate analysis were age at

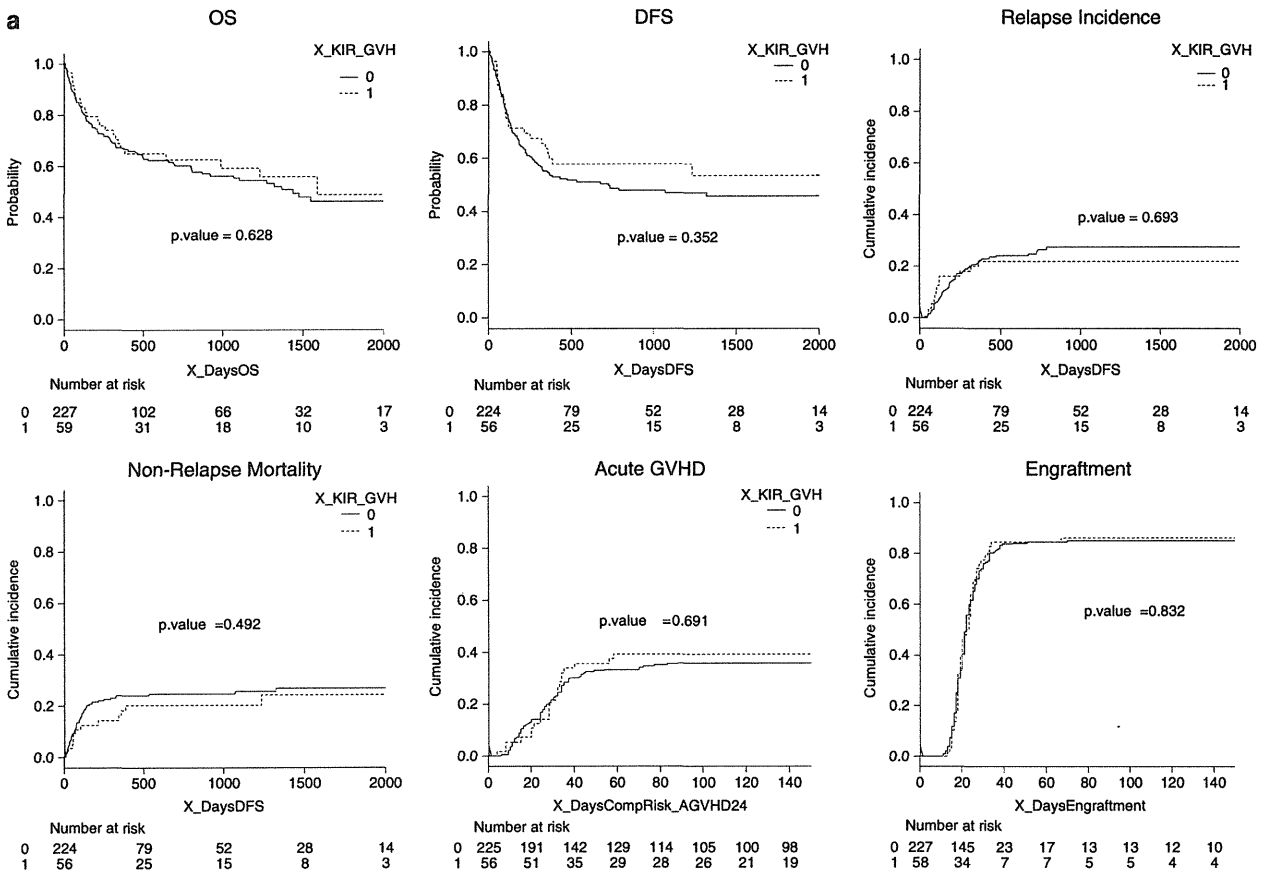

Figure 1. Continued

transplantation (40 years or more, 16–39 years and <15 years), performance status before transplantation (2–4 and 0–1), year of transplantation (2006–2009 and 2001–2005), sex (female and male), disease status (CR2 and CR1), conditioning regimens (RIC and MAC), HLA matching and infused cells ($>2.5 \times 10^7$/kg and $<2.5 \times 10^7$/kg) as a clinically important prognostic factor. All statistical analyses were conducted using SAS ver 9.2 (SAS Institute Inc., Cary, NC, USA) and R (www.r-project.org, last accessed 5 April 2012).

RESULTS

Patients and clinical characteristics

Tables 1a and 1b show clinical and biological characteristics of the 286 ALL and 357 AML patients who received single CBT. One hundred and twenty-eight patient–donor pairs (ALL $n=59$, AML $n=69$) were KIR ligand-incompatible in the GVH direction and 139 patient–donor pairs (ALL $n=65$, AML $n=74$) were incompatible in the HVG direction. Regarding KIR ligand incompatibility in the GVH direction, 59 ALL patients were transplanted with HLA-A, B or C KIR ligand-incompatible cord blood (A3/A11 $n=9$, Bw4 $n=16$, C $n=24$, A+C $n=3$, B+C $n=7$) and 69 AML patients were transplanted with HLA-A, B or C KIR ligand-incompatible cord blood (A3/A11 $n=11$, Bw4 $n=31$, C $n=24$, A+C $n=2$, B+C $n=1$). Regarding KIR ligand incompatibility in the HVG direction, 65 ALL patients were transplanted with HLA-A, B or C KIR ligand-incompatible cord blood (A3/A11 $n=17$, Bw4 $n=13$, C $n=35$, A+B $n=1$, A+C $n=5$) and 74 AML patients were transplanted with HLA-A, B or C KIR ligand-incompatible cord blood (A3/A11 $n=14$, Bw4 $n=14$, C $n=42$, A+C $n=4$). The number of patients mismatched in both the GVH and HVG directions is quite few (15 ALL patients and 18 AML patients). RIC regimens were used in 187 patients (ALL $n=58$ and AML $n=129$). There were no significant differences in other prognostic factors without HLA matching.

Impact of KIR ligand mismatch in the GVH direction on transplantation outcomes

Univariate analysis showed no significant differences between KIR ligand-incompatible and compatible groups in the GVH direction for both AML and ALL patients in OS, DFS, relapse incidence, NRM, acute GVHD and engraftment ($P=0.628$, $P=0.352$, $P=0.693$, $P=0.492$, $P=0.691$, $P=0.832$ for ALL patients and $P=0.674$, $P=0.688$, $P=0.353$, $P=0.766$, $P=0.569$, $P=0474$ for AML patients, respectively; Figures1a and b).

Causes of death are shown in Table 2a. Rates of mortality due to original disease and infections were almost the same in the KIR ligand-compatible and incompatible donor groups.

There were no significant differences in OS, DFS, relapse incidence, NRM, engraftment and acute GVHD between the KIR ligand-incompatible and compatible groups in the GVH direction for both AML and ALL patients by multivariate analysis (hazard ratio (HR) 0.87, $P=0.557$; HR 0.79, $P=0.352$; HR 0.95, $P=0.91$; HR 0.71, $P=0.32$; HR 1.08, $P=0.63$; HR 1.06, $P=0.83$ for ALL patients and HR 0.93, $P=0.752$; HR 1.02, $P=0.945$; HR 0.59, $P=0.12$; HR 0.95, $P=0.86$; HR 0.97, $P=0.89$; HR 0.84, $P=0.51$ for AML patients, respectively; Tables 3a and b). The conditioning regimens (RIC and MAC) did not affect these results.

For ALL patients, age >40 years and CR2 were associated with poor OS (HR 4.25, $P<0.001$ and HR 2.09, $P<0.001$, respectively)

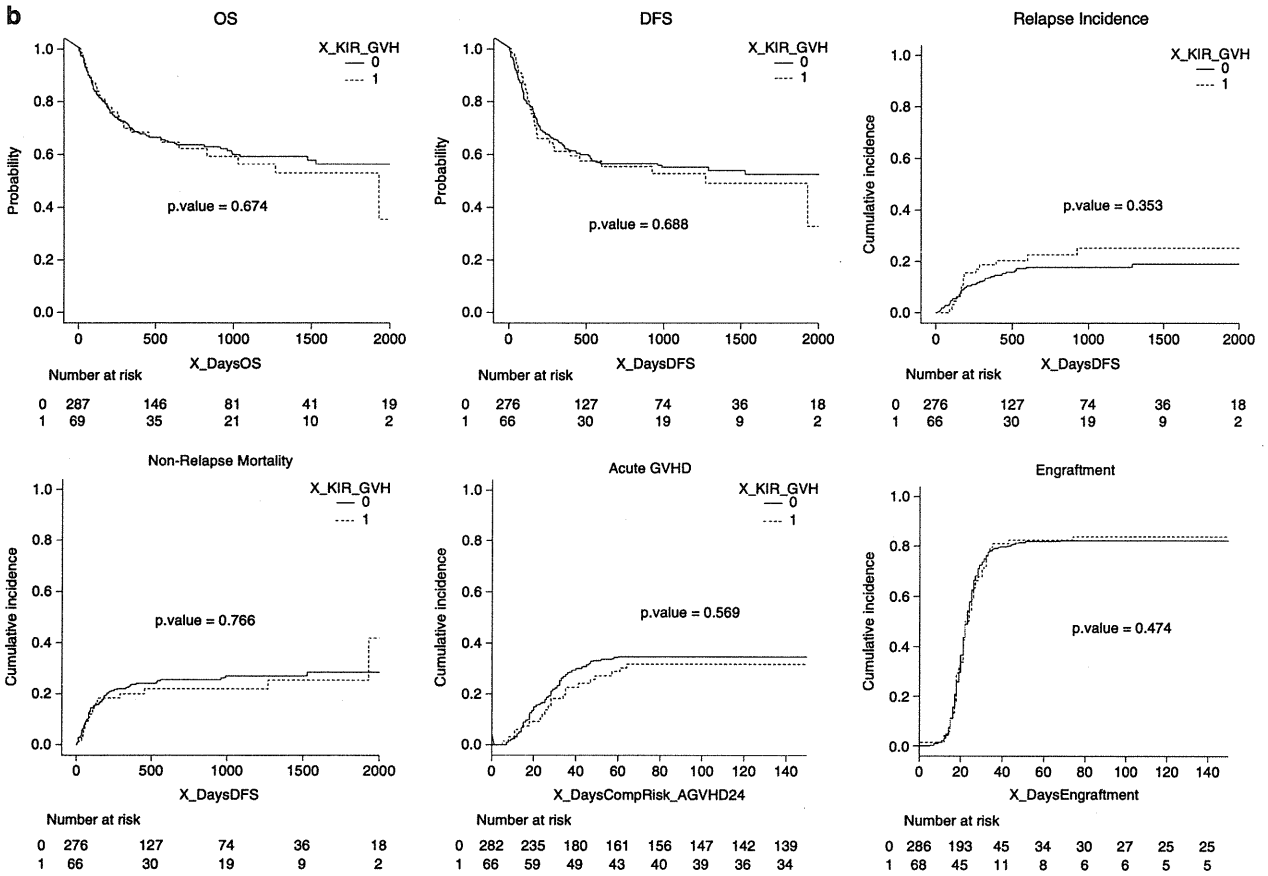

Figure 1. Continued

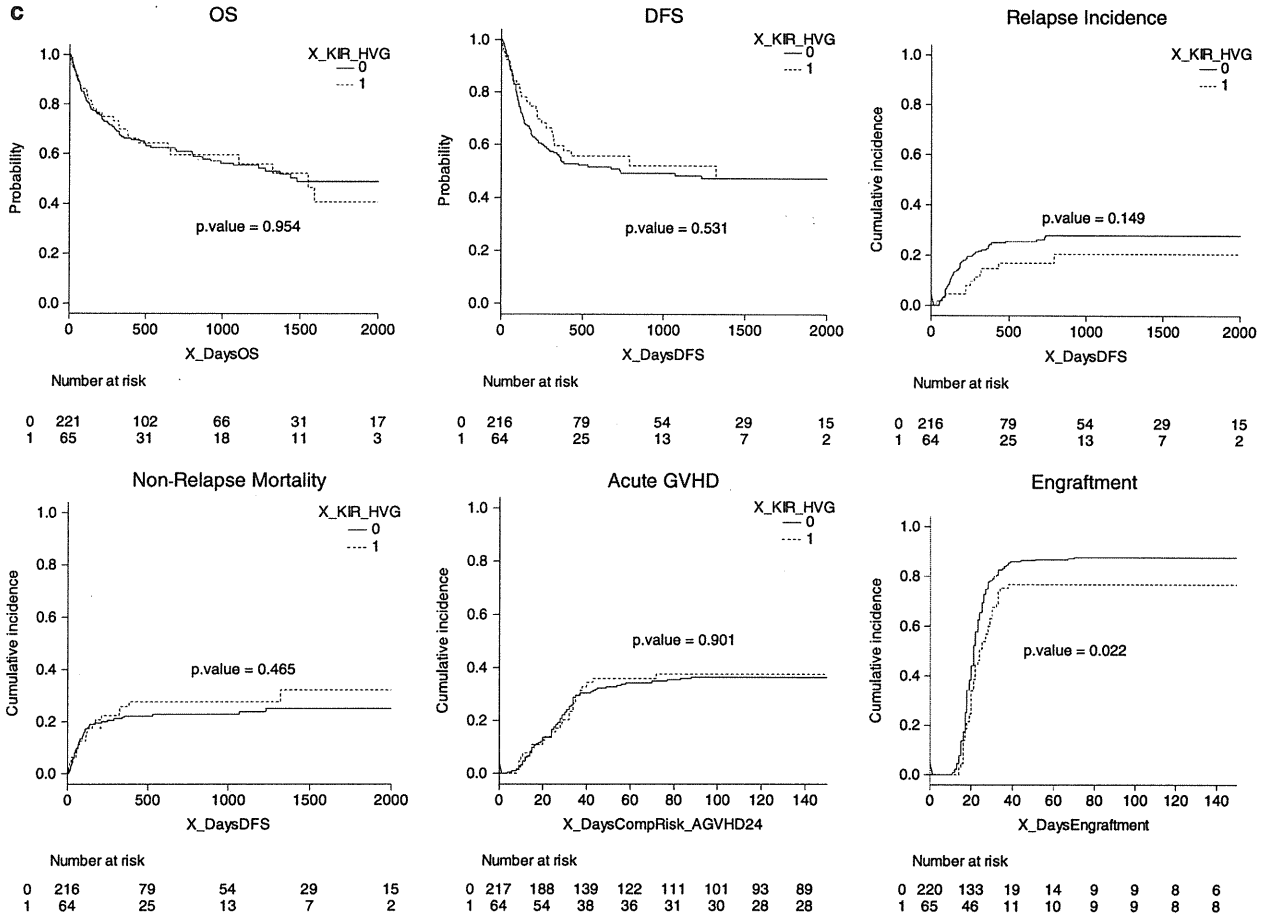

Figure 1. Continued

and also with poor DFS (HR 2.41, $P=0.002$ and HR 1.67, $P=0.011$, respectively). Also, age >40 years was associated with higher NRM and lower engraftment rate (HR 6.96, $P<0.001$ and HR 0.55, $P<0.001$, respectively). For AML patients, age >40 years and male gender were associated with poor OS (HR 1.93, $P=0.057$ and HR 1.78, $P=0.003$, respectively) and also with higher NRM (HR 2.59, $P=0.052$ and HR 1.71, $P=0.031$, respectively). Also, male gender was associated with poor DFS (HR 1.48, $P=0.033$). Infused cell number of $>2.5\times10^7$/kg was associated with higher engraftment rate and MAC regimen was associated with lower engraftment rate (HR 1.369, $P=0.018$ and HR 0.686, $P=0.007$, respectively). Age >40 years was associated with lower incidence of GVHD (HR 0.50, $P=0.031$) and HLA mismatch was associated with higher incidence of GVHD (HR 1.58, $P=0.058$).

Impact of KIR ligand mismatch in the HVG direction on transplantation outcomes

Univariate analysis showed no significant differences between the KIR ligand-incompatible and compatible groups in the HVG direction for both AML and ALL patients in OS, DFS, relapse incidence, NRM and acute GVHD ($P=0.954$, $P=0.531$, $P=0.149$, $P=0.465$, $P=0.901$ for ALL patients and $P=0.264$, $P=0.383$, $P=0.654$, $P=0.598$, $P=0.628$ for AML patients, respectively; Figures1c and d). However, there was a significant difference in engraftment between the KIR ligand-incompatible and compatible groups in the HVG direction for ALL patients ($P=0.022$ for ALL patients and $P=0.151$ for AML patients).

Causes of death are shown in Table 2b. Rates of mortality owing to original disease were almost the same in the KIR ligand-compatible and incompatible donor groups. Rate of mortality owing to infection was higher in the KIR ligand-incompatible donor group with ALL.

Also, there were no significant differences in OS, DFS, relapse incidence, NRM and acute GVHD between the KIR ligand-incompatible and compatible groups in the HVG direction for both AML and ALL patients by multivariate analysis (HR 0.84, $P=0.457$; HR 0.76, $P=0.225$; HR 1.12, $P=0.76$; HR 1.06, $P=0.85$; HR 1.08, $P=0.75$ for ALL patients and HR 0.73, $P=0.197$; HR 0.83, $P=0.414$; HR 0.86, $P=0.68$; HR 0.88, $P=0.66$; HR 1.20, $P=0.42$ for AML patients, respectively; Tables 3c and d). However, there was a significant difference in engraftment between the KIR ligand-incompatible and compatible groups in the HVG direction for ALL patients (HR 0.66, $P=0.013$). The conditioning regimens (RIC and MAC) did not affect these results.

For ALL patients, age >40 years and CR2 were associated with poor OS (HR 4.33, $P<0.001$ and HR 2.11, $P<0.001$, respectively) and also with poor DFS (HR 2.49, $P=0.001$ and HR 1.70, $P=0.009$, respectively). Also, age >40 years was associated with higher NRM and lower engraftment rate (HR 6.87, $P<0.001$ and HR 0.56, $P<0.001$, respectively). For AML patients, age >40 years and male gender were associated with poor OS (HR 2.00, $P=0.045$ and HR 1.76, $P=0.003$, respectively) and also with higher NRM (HR 2.62, $P=0.051$ and HR 1.69, $P=0.032$, respectively). Also, male gender was associated with poor DFS (HR 1.48, $P=0.032$). Infused cell number of $>2.5\times10^7$/kg was

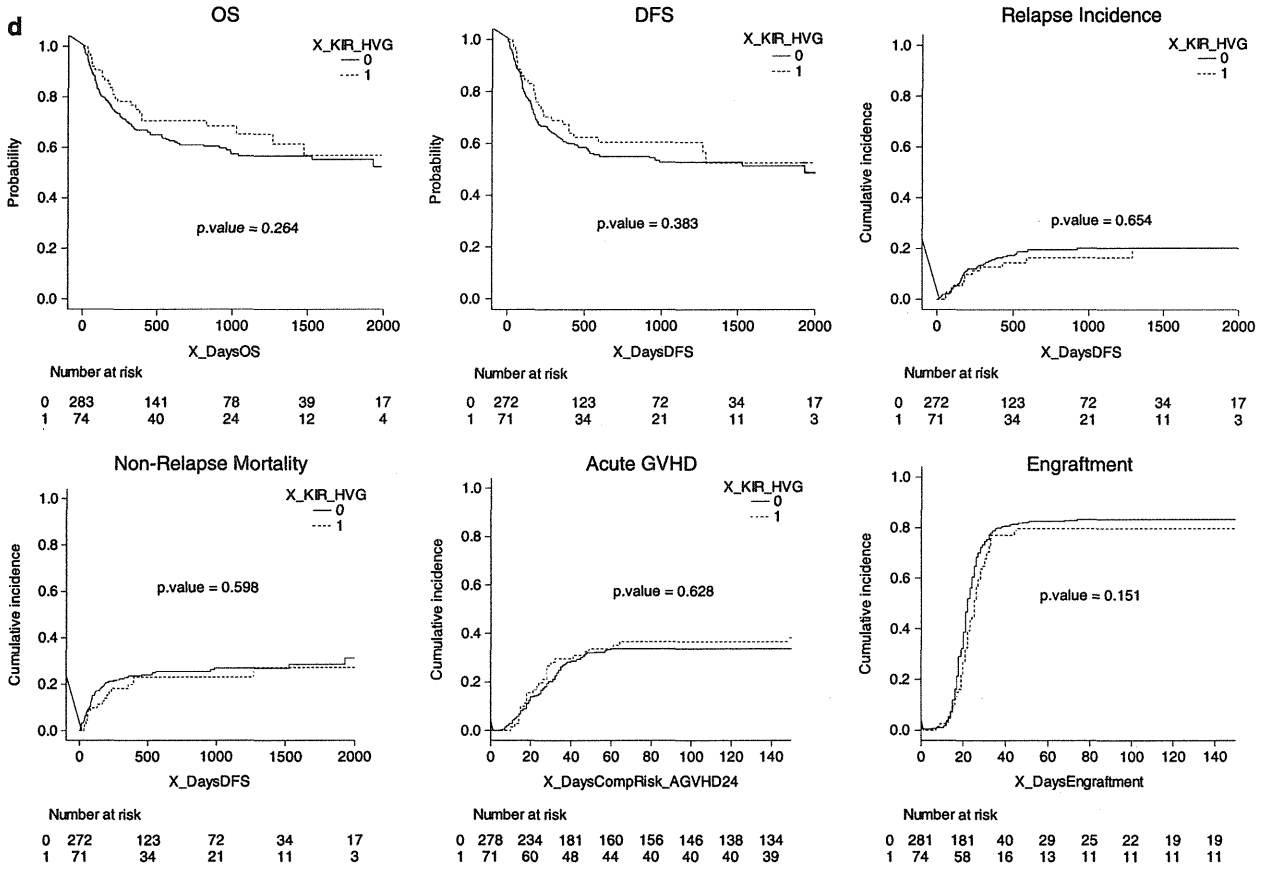

Figure 1. Kaplan–Meier curves forOS, DFS, CIR, NRM, acute GVHD and engraftment in (**a**) ALL and (**b**) AML patients transplanted from KIR-compatible and incompatible donors in the GVH direction and in (**c**) ALL and (**d**) AML patients transplanted from KIR-compatible and incompatible donors in the HVG direction.

associated with higher engraftment rate and MAC regimen was associated with lower engraftment rate (HR 1.387, $P=0.014$ and HR 0.694, $P=0.009$, respectively). Age >40 years was associated with lower incidence of GVHD (HR 0.51, $P=0.035$) and HLA mismatch was associated with higher incidence of GVHD (HR 1.49, $P=0.086$).

DISCUSSION

The role of KIR ligand incompatibility in allo SCT is controversial with various diseases and conditionings.[16,17] It has been suggested that NK cell alloreactivity is associated with better outcome after allo SCT when a high stem cell dose, extensive T-cell depletion and ATG are used.[18,19] NK cell engraftment is earlier and more robust and T-cell engraftment is delayed after CBT.[20,21] Therefore, CBT may represent a setting in which KIR ligand incompatibility is associated with protection from leukemia relapse. Willemze *et al.*[22] reported transplantation outcomes after single-unit CBT for AML patients ($n=94$) and ALL patients ($n=124$). Among those patients, KIR ligand incompatibility was associated with reduced relapse of AML and increased OS. In their study, >80% of the patients were administered ATG or antilymphocyte globulin under MAC. Brunstein *et al.*[23] reported results for 257 patients with single-unit CBT ($n=91$) and double-unit CBT ($n=166$) after myeloablative ($n=155$) and reduced intensity ($n=102$) conditioning. KIR ligand incompatibility was associated with higher rate of acute GVHD and decreased OS under RIC. In their study, only 30% of the patients were administered ATG. Garfall *et al.*[24] reported outcomes of double-unit CBT for 80 patients with various hematological malignancies including 31 AML patients. Among those patients, KIR ligand incompatibility was not associated with relapse reduction. In their study, >70% of the patients were administered ATG with RIC (Flu/Mel/ATG). Those studies that included different transplantation protocols with different disease distributions after single-unit and double-unit CBT showed conflicting results.[25,26]

Lowe *et al.*[27] investigated the relative significance of NK cell and T-cell alloreactivity in 105 pediatric patients who received minimally T-cell-depleted HLA-non-identical bone marrow transplantation. They showed that donor NK cell incompatibility did not improve patient outcome. In contrast, donor T-cell incompatibility was a risk factor for acute GVHD, chronic GVHD and death. Thus, T-cell alloreactivity dominated that of NK cells in minimally T-cell-depleted grafts. It was reported that KIR ligand mismatching induced adverse effects on acute GVHD and rejection and brought no survival benefits to leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor in Japan.[9] Also, Yabe *et al.*[28] reported that KIR ligand incompatibility had potent adverse effects with a higher incidence of acute GVHD and lower OS without ATG, whereas ATG administration ameliorated most of the adverse effects. Therefore, administration of ATG extensively depletes patient's and donor's T cells and becomes a critical factor in attenuating the adverse effects of KIR ligand-incompatible transplantation predominating alloreactive NK cells to induce an antileukemic effect. NK cell cytotoxicity toward a particular target cell is regulated by a

Table 1a. Patients characteristics with or without KIR incompatibility in the GVH direction

Factor	*ALL, n (%)*			*AML, n (%)*		
	KIR compatible	*KIR incompatible*	P	*KIR compatible*	*KIR incompatible*	P
Number of patients	227	59		288	69	
Year of transplant			0.621			0.639
2001–2005 (%)	49 (22)	11 (19)		44 (15)	9 (13)	
2006–	178 (78)	48 (81)		244 (85)	60 (87)	
Median age (years)	27	33	0.895	47	50	0.195
0–15	83 (37)	16 (27)	0.355	41 (14)	9 (13)	0.926
16–39	58 (26)	19 (32)		79 (27)	18 (26)	
>40	86 (38)	24 (41)		168 (59)	42 (61)	
Male	108 (48)	38 (64)	0.021	145 (50)	44 (64)	0.045
Disease status			0.741			0.077
CR1	153 (68)	43 (73)		182 (63)	37 (54)	
CR2	69 (30)	15 (25)		95 (33)	25 (36)	
>CR2	4 (2)	1 (2)		9 (3)	6 (9)	
TNC infused $\times 10^7$/kg	3.04 (1.61–24.77)	2.81 (1.45–24.91)	0.461	2.70 (1.46–38.70)	2.60 (1.59–10.84)	0.103
Conditioning						
RIC	47 (21)	11 (19)	0.703	101 (35)	28 (41)	0.392
TBI	187 (82)	52 (86)	0.457	237 (82)	60 (87)	0.38
ATG	0	0		0	0	
HLA allele matching			<0.001			0.013
0 miss	16 (7)	1 (2)		14 (5)	0	
1 miss	25 (11)	2 (3)		19 (7)	3 (4)	
2 miss	37 (16)	3 (5)		36 (13)	3 (4)	
3 miss	75 (33)	12 (20)		92 (32)	22 (32)	
4 miss	46 (20)	23 (39)		73 (25)	18 (26)	
>4 miss	28 (12)	18 (31)		54 (19)	23 (33)	
GVHD prophylaxis			0.202			0.687
CsA ± MTX	96 (42)	31 (53)		133 (46)	30 (44)	
FK ± MTX	126 (56)	28 (47)		151 (53)	38 (55)	

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; CR, complete remission; CsA, cyclosporine; FK, tacrolimus; GVH, graft-versus-host; GVHD, GVH disease; HLA, human leukocyte antigen; KIR, killer cell immunoglobulin-like receptor; MTX, methotrexate; RIC, reduced-intensity conditioning; TBI, total body irradiation; TNC, total nucleated cells.

Table 1b. Patients characteristics with or without KIR incompatibility in the HVG direction

Factor	*ALL, n (%)*			*AML, n (%)*		
	KIR compatible	*KIR incompatible*	P	*KIR compatible*	*KIR incompatible*	P
Number of patients	221	65		283	74	
Year of transplant			0.413			0.717
2001–2005	44 (20)	16 (25)		43 (15)	10 (14)	
2006–	177 (80)	49 (75)		240 (85)	64 (86)	
Median age (years)	24	35	0.134	48	47	0.976
0–15	83 (38)	16 (25)	0.149	45 (16)	5 (7)	0.038
16–39	56 (25)	21 (32)		70 (25)	27 (36)	
>40	82 (37)	28 (43)		168 (59)	42 (57)	
Male	112 (51)	34 (52)	0.817	152 (54)	37 (50)	0.569
Disease status			0.435			0.372
CR1	149 (67)	47 (72)		171 (60)	48 (65)	
CR2	68 (31)	16 (25)		95 (34)	25 (34)	
>CR2	3 (1)	2 (3)		14 (5)	1 (1)	
TNC infused $\times 10^7$/kg	3.06 (1.50–24.91)	2.89 (1.45–17.25)	0.133	2.71 (1.46–18.17)	2.58 (1.77–38.7)	0.065
Conditioning						
RIC	46 (21)	12 (18)	0.655	107 (38)	22 (30)	0.198
TBI	179 (81)	59 (91)	0.064	231 (82)	66 (89)	0.134
ATG	0	0				
HLA allele matching			<0.001			0.017
0 miss	17 (8)	0		14 (5)	0	
1 miss	26 (12)	1 (2)		21 (7)	1 (1)	
2 miss	33 (15)	7 (11)		31 (11)	8 (11)	
3 miss	67 (30)	20 (31)		96 (34)	18 (24)	
4 miss	50 (23)	19 (29)		69 (24)	22 (30)	
>4 miss	28 (12)	18 (27)		52 (19)	25 (34)	
GVHD prophylaxis			0.645			0.171
CsA ± MTX	96 (43)	31 (48)		124 (44)	39 (53)	
FK ± MTX	120 (54)	34 (52)		155 (56)	34 (47)	

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; CR, complete remission; CsA, cyclosporine; FK, tacrolimus; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HVG, host-versus-graft; KIR, killer cell immunoglobulin-like receptor; MTX, methotrexate; RIC, reduced-intensity conditioning; TBI, total body irradiation; TNC, total nucleated cells.

Table 2a. Cause of death for patients after single CBT with KIR incompatibility in the GVH direction

	ALL, n (%)		*AML, n (%)*	
	KIR compatible	*KIR incompatible*	*KIR compatible*	*KIR incompatible*
Original disease	29 (30)	11 (46)	29 (27)	8 (30)
Acute GVHD	3 (3)	0 (0)	5 (5)	0 (0)
Chronic GVHD	0 (0)	0 (0)	1 (1)	0 (0)
Graft failure	7 (7)	1 (4)	4 (4)	4 (15)
Infection	16 (16)	5 (21)	22 (20)	6 (22)
Hemorrhage	6 (6)	0 (0)	2 (2)	4 (15)
Interstitial pneumonitis	10 (10)	1 (4)	9 (8)	2 (7)
ARDS	4 (4)	0 (0)	4 (4)	0 (0)
Organ failure	7 (7)	3 (13)	14 (13)	2 (7)
Others	15 (15)	3 (13)	18 (17)	1 (4)

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CBT, cord blood transplantation; GVH, graft-versus-host; GVHD, GVH disease; KIR, killer cell immunoglobulin-like receptor; ARDS, acute respiratory distress syndrome.

Table 2b. Cause of death for patients after single CBT with KIR incompatibility in the HVG direction

	ALL, n (%)		*AML, n (%)*	
	KIR compatible	*KIR incompatible*	*KIR compatible*	*KIR incompatible*
Original disease	32 (34)	8 (29)	31 (28)	6 (25)
Acute GVHD	2 (2)	1 (4)	4 (4)	1 (4)
Chronic GVHD	0 (0)	0 (0)	1 (1)	0 (0)
Graft failure	7 (8)	1 (4)	7 (6)	1 (4)
Infection	13 (14)	8 (29)	24 (21)	4 (17)
Hemorrhage	6 (6)	0 (0)	4 (4)	2 (8)
Interstitial pneumonitis	8 (9)	3 (11)	9 (8)	2 (8)
ARDS	3 (3)	1 (4)	1 (1)	3 (13)
Organ failure	10 (11)	0 (0)	15 (13)	1 (4)
Others	12 (13)	6 (21)	16 (14)	4 (17)

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CBT, cord blood transplantation; GVHD, graft-versus-host disease; HVG, host-versus-graft; KIR, killer cell immunoglobulin-like receptor; ARDS, acute respiratory distress syndrome.

Table 3a. Multivariate analysis for each event KIR ligand incompatibility in the GVH direction with ALL patients

Variables	*Reference*	*HR*	*95% CI*		*P-value*
Overall survival					
KIR incompatible	Compatible	0.87	0.53	1.40	0.557
Age >40	Age 0–15	4.25	2.31	7.83	<0.001
Male	Female	1.08	0.72	1.62	0.718
CR2–	CR1	2.09	1.39	3.16	<0.001
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.93	0.59	1.45	0.739
Disease-free survival					
KIR incompatible	Compatible	0.79	0.49	1.29	0.352
Age >40	Age 0–15	2.41	1.39	4.18	0.002
Male	Female	1.00	0.68	1.47	0.995
CR2–	CR1	1.67	1.12	2.47	0.011
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.85	0.56	1.30	0.465
Relapse incidence					
KIR incompatible	Compatible	0.95	0.43	2.10	0.91
Age >40	Age 0–15	0.59	0.26	1.32	0.2
Male	Female	0.65	0.39	1.10	0.11
CR2–	CR1	1.37	0.80	2.35	0.250
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.69	0.35	1.35	0.280
Non-relapse mortality					
KIR incompatible	Compatible	0.71	0.37	1.39	0.32
Age >40	Age 0–15	6.96	2.93	16.57	<0.001
Male	Female	1.44	0.79	2.64	0.24
CR2–	CR1	1.62	0.90	2.92	0.100
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.13	0.61	2.10	0.700
Engraftment					
KIR incompatible	Compatible	1.08	0.78	1.50	0.63
Age >40	Age 0–15	0.55	0.39	0.78	<0.001
Male	Female	0.77	0.58	1.02	0.066
CR2–	CR1	0.76	0.56	1.02	0.067
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.08	0.82	1.43	0.590
Infused cell $>2.5 \times 10^7$/kg	≦2.5	1.02	0.76	1.36	0.910
MAC	RIC	0.79	0.58	1.09	0.15
Acute GVHD					
KIR-incompatible	Compatible	1.06	0.64	1.74	0.83
Age >40	Age 0–15	0.95	0.53	1.71	0.87
Male	Female	1.16	0.75	1.79	0.52
CR2–	CR1	1.34	0.89	2.02	0.170
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.40	0.86	2.28	0.180

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; CR, complete remission; GVH, graft-versus-host; GVHD, GVH disease; HLA, human leukocyte antigen; HR, hazard ratio; KIR, killer cell immunoglobulin-like receptor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

balance of activating and inhibitory cell–cell contacts. The absence of HLA class I on a target cell allows other activating signals to dominate.[29,30] Inhibitory NK receptors protect self-HLA-expressing normal tissue from NK cells. The second property of an inhibitory NK receptor is to educate or license NK cells to acquire function. NK cells acquire function following engagement of inhibitory receptors with self-ligands after their differentiation from hematopoietic progenitors. Therefore, allo SCT provides a unique environment for NK cell education and NK cell development from hematopoietic stem cells in a short period.[31]

We analyzed the effects of KIR ligand incompatibility in both GVH and HVG directions on single CBT outcomes in 643 acute leukemia patients in CR (ALL $n=286$ and AML $n=357$) without ATG in Japan. In contrast to the results of previous studies indicating that KIR ligand mismatching induced adverse effects on GVHD and survival in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor in Japan,[27,28] our study did not show any positive or negative effects of KIR ligand incompatibility in either the GHV or HVG direction on OS, DFS, CIR, NRM and acute GVHD after single CBT without ATG. CBT may be tolerable to KIR ligand incompatibility in terms of transplantation outcomes such as GVHD, OS and DFS. Therefore, the source of stem cell may also be important to determine the clinical advantage of NK cell alloreactivity after unrelated SCT. We also analyzed transplantation outcomes for only patients with engraftment; however, there were no differences in OS and DFS between patients who received KIR ligand-compatible and incompatible transplantations (data not shown). There was also no difference in outcomes of KIR ligand-compatible and incompatible transplantations in acute leukemia patients combined with ALL and AML in CR. However, multivariate analysis showed a significantly lower rate of engraftment in ALL patients who were KIR ligand incompatible in the HVG direction than compatible patients (HR 0.66, 95% confidence interval 0.47–0.91, $P=0.013$). Also, AML patients who were KIR ligand incompatible in the HVG direction tended to have a lower rate of engraftment (HR 0.799, 95% confidence interval 0.59–1.084, $P=0.15$). It has been reported that NK epitope mismatching in

Table 3b. Multivariate analysis for each event KIR ligand incompatibility in the GVH direction with AML patients

Variables	*Reference*	*HR*	*95% CI*		*P-value*
Overall survival					
KIR incompatible	Compatible	0.93	0.58	1.49	0.752
Age >40	Age 0–15	1.93	0.98	3.79	0.057
Male	Female	1.78	1.21	2.60	0.003
CR2–	CR1	0.76	0.52	1.11	0.160
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.08	0.71	1.65	0.725
Disease-free survival					
KIR incompatible	Compatible	1.02	0.65	1.59	0.945
Age >40	Age 0–15	1.31	0.71	2.42	0.380
Male	Female	1.48	1.03	2.12	0.033
CR2–	CR1	0.77	0.54	1.10	0.152
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.01	0.68	1.50	0.959
Relapse incidence					
KIR incompatible	Compatible	0.59	0.31	1.14	0.12
Age >40	Age 0–15	0.61	0.27	1.38	0.24
Male	Female	0.65	0.39	1.09	0.1
CR2–	CR1	1.39	0.82	2.34	0.220
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.71	0.36	1.38	0.310
Non-relapse mortality					
KIR incompatible	Compatible	0.95	0.52	1.72	0.86
Age >40	Age 0–15	2.59	0.99	6.76	0.052
Male	Female	1.71	1.05	2.77	0.031
CR2–	CR1	0.85	0.54	1.36	0.510
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.08	0.63	1.84	0.780
Engraftment					
KIR incompatible	Compatible	0.97	0.71	1.339	0.89
Age >40	Age 0–15	0.94	0.67	1.332	0.74
Male	Female	0.92	0.73	1.181	0.53
CR2–	CR1	1.00	0.79	1.287	0.96
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.97	0.75	1.27	0.840
Infused cell $>2.5 \times 10^7$/kg	≦2.5	1.36	1.06	1.776	0.018
MAC	RIC	0.68	0.52	0.904	0.007
Acute GVHD					
KIR incompatible	Compatible	0.84	0.51	1.40	0.51
Age >40	Age 0–15	0.50	0.27	0.94	0.031
Male	Female	1.10	0.75	1.61	0.62
CR2–	CR1	0.98	0.66	1.44	0.900
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.58	0.98	2.54	0.058

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; CR, complete remission; GVH, graft-versus-host; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; KIR, killer cell immunoglobulin-like receptor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

Table 3c. Multivariate analysis for each event KIR ligand incompatibility in the HVG direction with ALL patients

Variables	*Reference*	*HR*	*95% CI*		*P-value*
Overall survival					
KIR incompatible	Compatible	0.84	0.54	1.33	0.457
Age >40	Age 0–15	4.33	2.35	7.97	<0.001
Male	Female	1.08	0.72	1.62	0.718
CR2–	CR1	2.11	1.40	3.18	<0.001
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.91	0.59	1.41	0.671
Disease-free survival					
KIR incompatible	Compatible	0.76	0.49	1.18	0.225
Age >40	Age 0–15	2.49	1.44	4.32	0.001
Male	Female	1.00	0.68	1.47	0.999
CR2–	CR1	1.70	1.14	2.51	0.009
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.84	0.55	1.26	0.394
Relapse incidence					
KIR incompatible	Compatible	1.12	0.55	2.28	0.76
Age >40	Age 0–15	0.67	0.29	1.55	0.35
Male	Female	1.09	0.62	1.91	0.76
CR2–	CR1	0.75	0.42	1.34	0.330
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.95	0.52	1.74	0.870
Non-relapse mortality					
KIR incompatible	Compatible	1.06	0.59	1.89	0.85
Age >40	Age 0–15	6.87	2.87	16.42	<0.001
Male	Female	1.43	0.77	2.64	0.26
CR2–	CR1	1.62	0.90	2.90	0.110
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.08	0.58	2.00	0.800
Engraftment					
KIR incompatible	Compatible	0.66	0.47	0.91	0.013
Age >40	Age 0–15	0.56	0.4	0.78	<0.001
Male	Female	0.78	0.59	1.02	0.065
CR2–	CR1	0.71	0.52	0.96	0.026
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.14	0.86	1.5	0.370
Infused cell $>2.5 \times 10^7$/kg	≦2.5	1.04	0.78	1.39	0.800
MAC	RIC	0.80	0.58	1.09	0.160
Acute GVHD					
KIR incompatible	Compatible	1.08	0.67	1.76	0.75
Age >40	Age 0–15	0.95	0.52	1.71	0.85
Male	Female	1.16	0.75	1.79	0.49
CR2–	CR1	1.35	0.88	2.07	0.170
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.41	0.87	2.29	0.160

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; CR, complete remission; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; HVG, host-versus-graft; KIR, killer cell immunoglobulin-like receptor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

the rejection direction was associated with an increased probability of rejection after unrelated bone marrow transplantation.[9,32] Signaling lymphocytic activation molecule (SLAM)-associated protein-related adaptors and SLAM family receptors were reported to act together in a mechanism that was essential for the elimination of hematopoietic cells but not non-hematopoietic cells by NK cells.[33] Therefore, alloreactive NK cells induced by KIR ligand incompatibility in the HVG direction may attack donor hematopoietic cells to ameliorate donor cell engraftment after CBT with blood containing a relatively small number of hematopoietic stem cells. Administration of ATG as a preparative regimen may be important to obtain some positive effects of KIR ligand incompatibility in the GVH direction on CBT outcomes such as survival and relapse. The present study suggests that it is not necessary to consider KIR ligand compatibility in the GVH direction at CBT without ATG for transplantation outcomes. Also, there is the possibility that KIR ligand incompatibility in the GVH direction induces a graft-versus-leukemia effect for acute leukemia if patients receive ATG as a preparative regimen. On the other hand, it may be necessary to pay attention to KIR ligand compatibility in the HVG direction for engraftment after CBT.

We did not perform KIR genotyping in our cohort study; however, recent data have suggested an important role of KIR polymorphisms and KIR genotype in transplantation outcomes of allo SCT.[34,35] NK cell alloreactivity is regulated by a balance of activating and inhibitory cell–cell contacts. Although phenotypes of the KIR repertoire are personalized by various conditions,[36] however, not only simple algorithm on ligands for inhibitory KIR but also KIR genotypes may be useful for predicting clinically relevant NK cell alloreactivity in a future study.

Table 3d. Multivariate analysis for each event KIR ligand incompatibility in the HVG direction with AML patients

Variables	*Reference*	*HR*	*95% CI*		*P-value*
Overall survival					
KIR incompatible	Compatible	0.73	0.46	1.18	0.197
Age >40	Age 0–15	2.00	1.02	3.93	0.045
Male	Female	1.76	1.21	2.58	0.003
CR2–	CR1	0.74	0.50	1.08	0.120
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.09	0.72	1.65	0.681
Disease-free survival					
KIR incompatible	Compatible	0.83	0.53	1.30	0.414
Age >40	Age 0–15	1.33	0.72	2.45	0.357
Male	Female	1.48	1.03	2.11	0.032
CR2–	CR1	0.76	0.53	1.09	0.131
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.03	0.70	1.51	0.893
Relapse incidence					
KIR incompatible	Compatible	0.86	0.42	1.75	0.68
Age >40	Age 0–15	0.67	0.29	1.58	0.36
Male	Female	1.09	0.62	1.91	0.76
CR2–	CR1	0.75	0.42	1.34	0.330
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.98	0.55	1.76	0.950
Non-relapse mortality					
KIR incompatible	Compatible	0.88	0.49	1.57	0.66
Age >40	Age 0–15	2.62	1	6.88	0.051
Male	Female	1.69	1.05	2.74	0.032
CR2–	CR1	0.84	0.53	1.35	0.480
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.08	0.64	1.83	0.770
Engraftment					
KIR-incompatible	Compatible	0.799	0.59	1.084	0.15
Age >40	Age 0–15	0.958	0.68	1.352	0.81
Male	Female	0.918	0.72	1.17	0.49
CR2–	CR1	0.994	0.78	1.264	0.96
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	0.997	0.77	1.291	0.98
Infused cell >2.5 × 10^7/kg	≦2.5	1.387	1.07	1.8	0.014
MAC	RIC	0.694	0.53	0.914	0.009
Acute GVHD					
KIR-incompatible	Compatible	1.20	0.76	1.90	0.42
Age >40	Age 0–15	0.51	0.28	0.96	0.035
Male	Female	1.09	0.75	1.59	0.64
CR2–	CR1	0.98	0.66	1.45	0.910
HLA mismatching (>5/6)	HLA mismatching (6/6, 5/6)	1.49	0.95	2.34	0.086

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; CR, complete remission; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; HVG, host-versus-graft; KIR, killer cell immunoglobulin-like receptor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan and a Japanese Grant-in-Aid for Scientific Research.

REFERENCES

1 Ruggeri L, Capanni M, Mancusi A, Urbani E, Perruccio K, Burchielli E *et al.* Alloreactive natural killer cells in mismatched hematopoietic stem cell transplantation. *Blood Cells Mol Dis* 2004; **33**: 216–221.
2 Moretta L, Locatelli F, Pende D, Marcenaro E, Mingari MC, Moretta A. Killer Ig-like receptor-mediated control of natural killer cell alloreactivity in haploidentical hematopoietic stem cell transplantation. *Blood* 2011; **117**: 764–771.
3 Velardi A, Ruggeri L, Mancusi A. Killer-cell immunoglobulin-like receptors reactivity and outcome of stem cell transplant. *Curr Opin Hematol* 2012; **19**: 319–323.
4 Tsirigotis PD, Resnick IB, Shapira M. The role of natural killer cells in hematopoietic stem cell transplantation. *Ann Med* 2012; **44**: 130–145.
5 Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002; **295**: 2097–2100.
6 Ruggeri L, Mancusi A, Capanni M, Urbani E, Carotti A, Aloisi T *et al.* Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. *Blood* 2007; **110**: 433–440.
7 Davies SM, Ruggieri L, DeFor T, Wagner JE, Weisdorf DJ, Miller JS *et al.* Evaluation of KIR ligand incompatibility in mismatched unrelated donor hematopoietic transplants. Killer immunoglobulin-like receptor. *Blood* 2002; **100**: 3825–3827.
8 Giebel S, Locatelli F, Lamparelli T, Velardi A, Davies S, Frumento G *et al.* Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. *Blood* 2003; **102**: 814–819.
9 Morishima Y, Yabe T, Matsuo K, Kashiwase K, Inoko H, Saji H *et al.* Effects of HLA allele and killer immunoglobulinlike receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell–replete marrow from an unrelated donor. *Biol Blood Marrow Transplant* 2007; **13**: 315–328.
10 Farag SS, Bacigalupo A, Eapen M, Hurley C, Dupont B, Caligiuri MA *et al.* The effect of KIR ligand incompatibility on the outcome of unrelated donor transplantation: a report from the center for international blood and marrow transplant research, the european blood and marrow transplant registry, and the Dutch registry. *Biol Blood Marrow Transplant* 2006; **12**: 876–884.
11 Hsu K, Gooley T, Malkki M, Pinto-Agnello C, Dupont B, Bignon J-D *et al.* KIR ligands and prediction of relapse after unrelated donor hematopoietic cell transplantation for hematologic malignancy. *Biol Blood Marrow Transplant* 2006; **12**: 828–836.
12 Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M *et al.* Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant* 2009; **15**: 367–369.
13 Leung W. Use of NK cell activity in cure by transplant. *Br J Haematol* 2011; **155**: 14–29.
14 Pepe MS, Mori M. Kaplan-Meier, marginal or conditional probability curves in summarizing competing risks failure time data? *Stat Med* 1993; **12**: 737–751.
15 Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; **94**: 496–509.
16 Farag SS, Fehniger TA, Ruggeri L, Velardi A, Caligiuri MA. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. *Blood* 2002; **100**: 1935–1947.
17 Parham P. MHC class I molecules and KIRs in human history, health and survival. *Nat Rev Immunol* 2005; **5**: 201–214.
18 Miller JS, Cooley S, Parham P, Farag SS, Verneris M, McQueen K *et al.* Missing KIR ligands are associated with less relapse and increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT. *Blood* 2007; **109**: 5058–5861.
19 Hsu KC, Keever-Taylor CA, Wilton A, Pinto C, Heller G, Arkun K *et al.* Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. *Blood* 2005; **105**: 4878–4884.
20 Tanaka J, Sugita J, Asanuma S, Arita K, Shono Y, Kikutchi M *et al.* Increased number of $CD16^+CD56^{dim}$ NK cells in peripheral blood mononuclear cells after allogeneic cord blood transplantation. *Hum Immunol* 2009; **70**: 701–705.
21 Jacobson CA, Turki AT, McDonough SM, Stevenson KE, Kim HT, Kao G *et al.* Immune reconstitution after double umbilical cord blood stem cell transplantation: comparison with unrelated peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 2012; **18**: 565–574.
22 Willemze R, Rodrigues CA, Labopin M, Sanz G, Michel G, Socieål G *et al.* KIR-ligand incompatibility in the graft-versus-host direction improves outcomes after umbilical cord blood transplantation for acute leukemia. *Leukemia* 2009; **23**: 492–500.
23 Brunstein CG, Wagner JE, Weisdorf DJ, Cooley S, Noreen H, Barker JN *et al.* Negative effect of KIR alloreactivity in recipients of umbilical cord blood transplant depends on transplantation conditioning intensity. *Blood* 2009; **113**: 5628–5634.
24 Garfall A, Kim HT, Sun L, Ho VT, Armand P, Koreth J *et al.* KIR ligand incompatibility is not associated with relapse reduction after double umbilical cord blood transplantation. *Bone Marrow Transplant* 2013; **48**: 1000–1002.
25 Willemze R, Ruggeri A, Purtill D, Rodrigues CA, Gluckman E, Rocha V *et al.* Is there an impact of killer cell immunoglobulin-like receptors and KIR-ligand incompatibilities on outcomes after unrelated cord blood stem cell transplantation? *Best Pract Res Clin Haematol* 2010; **23**: 283–290.
26 Sideri A, Neokleous N, Brunet De La Grange P, Guerton B, Le Bousse Kerdilles MC, Uzan G *et al.* An overview of the progress on double umbilical cord blood transplantation. *Haematologica* 2011; **96**: 1213–1220.

27 Lowe EJ, Turner V, Handgretinger R, Horwitz EM, Benaim E, Hale GA *et al.* T-cell alloreactivity dominates natural killer cell alloreactivity in minimally T-cell-depleted HLA-non-identical paediatric bone marrow transplantation. *Br J Haematol* 2003; **123**: 323–326.

28 Yabe T, Matsuo K, Hirayasu K, Kashiwase K, Kawamura-Ishii S, Tanaka H *et al.* Donor killer immunoglobulin-like receptor (KIR) genotype-patient cognate KIR ligand combination and antithymocyte globulin preadministration are critical factors in outcome of HLA-C-KIR ligand-mismatched T cell-replete unrelated bone marrow transplantation. *Biol Blood Marrow Transplant* 2008; **14**: 75–87.

29 Krzewski K, Strominger JL. The killer's kiss: the many functions of NK cell immunological synapses. *Curr Opin Cell Biol* 2008; **20**: 597–605.

30 Cheent K, Khakoo SI. Natural killer cells: integrating diversity with function. *Immunology* 2009; **126**: 449–457.

31 Murphy WJ, Parham P, Miller JS. NK cells--from bench to clinic. *Biol Blood Marrow Transplant* 2012; **18**: S2–S7.

32 De Santis D, Bishara A, Witt CS, Nagler A, Brautbar C, Slavin S *et al.* Natural killer cell HLA-C epitopes and killer cell immunoglobulin-like receptors both influence outcome of mismatched unrelated donor bone marrow transplants. *Tissue Antigens* 2005; **65**: 519–528.

33 Dong Z, Cruz-Munoz ME, Zhong MC, Chen R, Latour S, Veillette A. Essential function for SAP family adaptors in the surveillance of hematopoietic cells by natural killer cells. *Nat Immunol* 2009; **10**: 973–980.

34 Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT *et al.* Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. *Blood* 2010; **116**: 2411–2419.

35 Venstrom JM, Pittari G, Gooley TA, Chewning JH, Spellman S, Haagenson M *et al.* HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. *N Engl J Med* 2012; **367**: 805–816.

36 Yawata M, Yawata N, Draghi M, Partheniou F, Little AM, Parham P. MHC class I-specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires toward a balance of missing self-response. *Blood* 2008; **112**: 2369–2380.

Bone Marrow Transplantation (2013) **48,** 1389–1394

www.nature.com/bmt

ORIGINAL ARTICLE

Reduced-intensity vs myeloablative conditioning allogeneic hematopoietic SCT for patients aged over 45 years with ALL in remission: a study from the Adult ALL Working Group of the Japan Society for Hematopoietic Cell Transplantation (JSHCT)

J Tanaka[1,16], H Kanamori[2], S Nishiwaki[3], K Ohashi[4], S Taniguchi[5], T Eto[6], H Nakamae[7], K Minagawa[8], K Miyamura[9], H Sakamaki[4], Y Morishima[10], K Kato[11], R Suzuki[12], N Nishimoto[13], K Oba[14] and N Masauzi[15]

In this study, outcomes for 575 adult ALL patients aged ⩾45 years who underwent first allo-SCT in CR were analyzed according to the type of conditioning regimen (myeloablative conditioning (MAC) for 369 patients vs reduced-intensity conditioning (RIC) for 206 patients). Patients in the RIC group were older (median age, 58 vs 51 years, $P<0.0001$). There were no statistically significant differences in 3-year OS, disease-free survival (DFS) and non-relapse mortality (NRM): 51% vs 53%, 47% vs 39% and 38% vs 36%, respectively. Multivariate analysis showed that CR2 and HLA mismatching were associated with poor OS ($P=0.002$ and $P=0.019$, respectively). HLA mismatching was associated with lower rate of relapse ($P=0.016$), but was associated with higher rate of NRM ($P=0.001$). RIC was associated with good OS and DFS in patients who received HLA-mismatch transplantation and were aged ⩾55 years compared with MAC by multivariate analysis for each event with interaction (hazard ratio (HR) and 95% confidence interval 0.35 and 0.15–0.81, $P=0.014$ for OS and 0.36 and 0.16–0.81, $P=0.013$ for DFS). Therefore, patients ⩾55 years of age with HLA-mismatch transplantation should be candidates for RIC rather than MAC.

Bone Marrow Transplantation (2013) **48,** 1389–1394; doi:10.1038/bmt.2013.68; published online 13 May 2013

Keywords: ALL; reduced-intensity conditioning; myeloablative conditioning; allogeneic hematopoietic SCT

INTRODUCTION

Although 80–90% of patients with adult ALL achieve CR, most patients relapse and die from the disease.[1] Chemotherapy has resulted in long-term leukemia-free survival in 30 to 40% of ALL patients, but much higher rates of leukemia-free survival have been obtained with conventional myeloablative conditioning (MAC) allo-SCT. Recent large-scale prospective donor vs no donor studies have revealed that outcomes of matched sibling allografts were better than those of chemotherapy.[2–6] Moreover, allo-SCT can provide better disease-free survival (DFS) not only for ALL patients in first CR (CR1) but also for those in second CR (CR2).[7–9] Most conditioning regimens have included TBI, sometimes exceeding 13 Gy for patients in CR2.[10,11] We have reported excellent outcomes of allo-SCT using a conditioning regimen with medium-dose VP-16, CY and TBI (12 Gy) for adult patients with ALL.[12,13] However, non-relapse mortality (NRM) may cause a worse overall outcome of MAC allo-SCT for elderly patients and patients with comorbidities. Therefore, allo-SCT using reduced-intensity conditioning (RIC) may provide opportunities to obtain a significant GVL effect, without the adverse effects of intense myeloablative preparative regimens.[14–17] Marks *et al.*[18] reported no effect of conditioning intensity on TRM or relapse risk after RIC and MAC in 93 and 1428 Ph chromosome-negative ALL patients, respectively, in first or second CR and in patients >16 years of age who received allografts from siblings and unrelated donors. Mohty *et al.*[19] reported no effect of conditioning intensity on leukemia-free survival after RIC and after MAC in 127 and 449 ALL patients, respectively, in first or second CR and in patients >45 years of age who received allografts from HLA-identical sibling donors and were followed up for a median period of 16 months. A Japanese nationwide survey of 77 patients with hematological malignancies (aged 25–68 years) who recieved BMT after RIC from unrelated donors showed 50% OS with a median follow-up period of 439 days.[20]

In the current study, outcomes for 575 adult ALL patients aged ⩾45 years at transplantation who underwent allo-SCT in CR were

[1]Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [2]Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan; [3]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [4]Division of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [5]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [6]Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; [7]Department of Hematology, Osaka City University Graduate School of Medicine, Osaka, Japan; [8]Department of Hematology, Kobe University Graduate School of Medicine, Kobe, Japan; [9]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [10]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan; [11]Department of Pediatric Hematology/Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [12]Department of Hematopoietic Stem Cell Transplantation Data Management/Biostatistics, Nagoya University School of Medicine, Nagoya, Japan; [13]Center for Translational Research, Hokkaido University, Sapporo, Japan; [14]Translational Research and Clinical Trial Center, Hokkaido University Hospital, Hokkaido University, Sapporo, Japan and [15]Department of Medical Laboratory Science, Faculty of Health Sciences, Hokkaido University, Sapporo, Japan. Correspondence: Dr J Tanaka, Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-Ku, Sapporo 060-8638, Japan.
E-mail: jutanaka@med.hokudai.ac.jp or jutanaka@dh.twmu.ac.jp
[16]Current address: Department of Hematology, Tokyo Women's Medical University, Tokyo, Japan.
Received 26 November 2012; revised 5 March 2013; accepted 8 April 2013; published online 13 May 2013

analyzed according to the type of conditioning regimen (MAC for 369 patients vs RIC for 206 patients) before allo-SCT.

MATERIALS AND METHODS

Study design and data collection

This study was a retrospective analysis of data from a Japanese nationwide multicenter survey. Data for adult ALL patients were provided by the Adult ALL Working Group of the Japanese Society of Hematopoietic Stem Cell Transplantation (JSHCT). Outcomes of 575 adult ALL patients aged ⩾45 years at transplantation who underwent allo-SCT in CR were analyzed according to the type of conditioning regimen (MAC vs RIC) before allo-SCT.

Patient population

This study included ALL patients who received MAC or RIC allo-SCT in CR: and who (1) were aged ⩾45 years at the time of transplantation, (2) underwent transplantation between 2000 and 2009, and (3) received an MAC regimen ($n=369$) as high-dose radiation and chemotherapy usually in combination with CY, or a RIC regimen ($n=206$) defined as the use of fludarabine with low-dose TBI (⩽8 Gy), BU (⩽9 mg/kg) or melphalan (⩽140 mg/m^2).[21]

Transplant procedures

Differences between patients, disease and transplantation-related factors according to conditioning regimens and GVHD prophylaxis are shown in Table 1. As per JSHCT centers' practice for allo-SCT for ALL, patients were eligible to receive an MAC regimen if they were aged <55 years ($n=288$, 78%), and 66 patients (22%) who were aged ⩾55 years without significant comorbidities also received an MAC regimen. In the RIC group, 190 patients (92.2%) received a RIC regimen mainly because of age (⩾50 years), regardless of the presence or absence of significant comorbidities. Sixteen patients (7%) aged <50 years received a RIC regimen possibly as a result of the physician's decision based on significant comorbidities or some clinical reasons.

End points

Primary end points included OS, DFS, relapse (cumulative incidence of relapse) and NRM. Relapse was defined as clinical and hematological leukemia recurrence. NRM was defined as death during continuous CR after transplantation.

Statistical analysis

Characteristics of patients who received MAC and RIC were compared using the χ^2-test for categorical variables and the *t*-test for continuous variables. To compare the prognosis of MAC and that of RIC, univariate survival analyses were conducted for OS, DFS, NRM, cumulative incidence of relapse, engraftment (neutrophil recovery at 100 days), acute GVHD (grades II–IV) and chronic GVHD. Survival curves of OS and DFS for each group were depicted using the Kaplan–Meier method and compared by the log-rank test. In the analysis of NMR, engraftment, cumulative incidence of relapse, acute GVHD and chronic GVHD, probabilities of the incidences were calculated using the cumulative incidence function and compared by Gray's test to accommodate competing risks.[22] To adjust the potential confounders, multivariate analyses were conducted using the Cox proportional hazards model for OS and DFS, and using the Fine-Gray proportional hazards model for cumulative incidence of relapse and NRM.[23] In addition, the interaction terms between treatment (MAC vs RIC) and the above confounders were included in the multivariate model for OS and DFS. If interaction terms were statistically significant (*P*-value <0.05), the adjusted hazard ratios were also calculated on the basis of the multivariate model that included the interaction terms as subgroup analyses. All statistical analyses were conducted using SAS ver 9.2 (SAS Institute Inc., Cary, NC, USA) and R (www.r-project.org, last accessed April 5, 2012).

RESULTS

Patients and clinical characteristics

Table 1 shows clinical and biological characteristics of the 369 MAC and 206 RIC patients who received allo-SCT for ALL. Patients in the RIC group were older (median age, 58 vs 51 years, $P<0.0001$). Seventy-six percent of the RIC patients were aged ⩾55 years, whereas only 22% of the MAC patients were aged ⩾55 years. More RIC patients received related peripheral blood (24% vs 13%, $P<0.002$), and RIC was performed more frequently in the more recent time period (61% vs 52% during 2006–2009, $P=0.035$). There were no significant differences in other prognostic factors such as performance status, WBC at diagnosis, cytogenetics, disease status and HLA matching.

Hematological recovery and GVHD

Engraftment (neutrophil recovery at 100 days) occurred in 92% of the MAC patients and 93% of the RIC patients (Table 2). Acute GVHD grades II–IV occurred in 44% of the MAC patients and 42% of the RIC patients ($P=0.353$). Moreover, chronic GVHD at 3 years occurred in 36% of the MAC patients and 35% of the RIC patients ($P=0.793$). There was no statistically significant difference.

OS and DFS

Despite the older age in the RIC group, OS and DFS at 1 and 3 years were similar to those in the MAC group (Table 2, Figure 1).

OS at 3 years for MAC patients was 51% and that for RIC patients was 53% ($P=0.701$). DFS at 3 years for MAC patients was 47% and that for RIC patients was 39% ($P=0.098$). There was no statistically significant difference.

Relapse

There was no statistically significant difference in relapse at 1 year between the MAC and RIC groups (14% for RIC and 12% for MAC, $P=0.664$). However, a larger percentage of patients relapsed at 3 years in the RIC group than in the MAC group (26% for RIC and 15% for MAC, $P=0.008$).

NRM and cause of death

Conditioning regimen intensity had no impact on NRM at 3 years in the MAC and RIC groups (36% for RIC and 38% for MAC, $P=0.678$). Causes of death are shown in Table 3. Original disease and infection were the most common causes of death, followed by GVHD. Interstitial pneumonitis was more common in the MAC group.

Multivariate analysis for each event

There were no statistically significant differences in OS, DFS, relapse and NRM between the MAC and RIC groups (Table 4). CR2 and HLA mismatching were associated with poor OS (hazard ratio (HR) 1.88, $P=0.002$ for CR2 vs CR1 and 1.67, $P=0.019$ for mismatching vs matching), and female gender was associated with good OS (HR 0.59, $P=0.003$ for females vs males). CR2 was associated with poor DFS (HR 1.95, $P<0.001$ for CR2 vs CR1), and female gender was associated with good DFS (HR 0.65, $P=0.006$ for females vs males). CR2 was associated with higher rate of relapse (HR 2.29, $P=0.007$ for CR2 vs CR1). Interestingly, HLA mismatching was associated with lower rate of relapse (HR 0.27, $P=0.016$ for mismatching vs matching); however, HLA mismatching was associated with higher rate of NRM (HR 2.35, $P=0.001$ for mismatching vs matching). Female gender was associated with lower rate of NRM (HR 0.50, $P=0.001$ for females vs males).

When the interaction terms for each variable and the treatment were evaluated, the interaction between age or HLA status and the treatment was statistically significant. Therefore, subgroup analyses were conducted. As shown in Figure 2, RIC was associated with good OS and DFS in patients who received HLA-mismatch transplantation and were aged 55 years or more compared with MAC by multivariate analysis for each event with interaction (HR 0.35, $P=0.014$ for OS and 0.36, $P=0.013$ for DFS). Conversely, MAC showed good OS and DFS in patients with HLA matching and who were aged <50 years (HR 3.88, $P=0.003$ for OS and 3.51, $P=0.003$ for DFS).

Table 1. Patient characteristics

Patient characteristics	*MAC*	*RIC*	P
No. of patients	369	206	
Median age, years (range)	51 (45–70)	58 (45–70)	<0.0001
Sex			
Female (%)	189 (51)	106 (52)	0.957
PS before transplantation			
0–1 (%)	285 (96)	172 (95)	0.461
2–4 (%)	12 (4)	10 (5)	
Missing	72	24	
Lineage			
T cell (%)	19 (5)	10 (5)	0.837
B cell (%)	305 (86)	163 (84)	
Others (%)	33 (9)	21 (11)	
Missing	12	12	
WBS at diagnosis, × 10^9 L			
<25 (%)	208(59)	121(63)	0.381
25–100 (%)	102(29)	46(24)	
>100 (%)	40(12)	26(13)	
Missing	19	13	
Conditioning TBI			
No (%)	27 (7)	92 (46)	<0.0001
Yes (%)	336 (93)	107 (54)	
Missing	6	7	
Median dose (range)	12 (3–13.5)	4 (2–8)	
Cytogenesis			
None	105 (28)	46 (22)	0.205
t(9;22)(Ph) (%)	188 (51)	125 (61)	
t(4;11) (%)	10 (3)	5 (2)	
Others (%)	52 (14)	21 (10)	
Missing	14	9	
Disease status before transplantation			
CR1 (%)	310 (85)	160 (80)	0.134
CR2 (%)	55 (15)	40 (20)	
Missing	4	6	
HLA matching			
6/6 (%)	246 (74)	121 (65)	0.051
5/6, 4/6 (%)	45 (14)	39 (21)	
Others (%)	40 (12)	27 (14)	
Missing	38	19	
Graft type of donor			
Related BMT (%)	62 (17)	19 (9)	0.002
Related PBSCT (%)	47 (13)	49 (24)	
Unrelated BMT (%)	172 (48)	90 (44)	
Unrelated CBSCT (%)	80 (22)	47 (23)	
Missing	8	1	
Donor/recipient sex match			
Female/female (%)	80 (23)	47 (24)	0.989
Male/female (%)	102 (29)	55 (28)	
Female/male (%)	55 (16)	31 (16)	
Male/male (%)	113 (32)	63 (32)	
Missing	19	10	
Year of transplantation			
2000–2005 (%)	177 (48)	80 (39)	0.035
2006–2009 (%)	192 (52)	126 (61)	
Age at transplantation, years			
<50 (%)	137 (37)	16 (8)	<0.0001
50–54 (%)	151 (41)	34 (16)	
55 >(%)	81 (22)	156 (76)	
Missing	2	11	
GVHD prophylaxis of CyA			
No (%)	133 (41)	106 (54)	0.004
Yes (%)	191 (59)	90 (46)	
Missing	45	10	
GVHD prophylaxis of FK			
No (%)	164 (49)	83 (42)	0.153
Yes (%)	174 (51)	114 (58)	
Missing	31	9	
GVHD prophylaxis of MTX			
No (%)	36 (10)	47 (23)	<0.0001
Yes (%)	321 (90)	156 (77)	
Missing	12	3	
Acute GVHD grade			
0–I (%)	194 (54)	118 (59)	0.483
II–IV (%)	148 (41)	75 (37)	
Not evaluable (%)	19 (5)	8 (4)	
Missing	8	5	
Chronic GVHD grade			
Extensive	84 (24)	42 (20)	0.253
Limited	33 (9)	30 (15)	
Not evaluable	64 (18)	34 (17)	
No	173 (49)	97 (48)	
Missing	15	3	

Abbreviations: MAC = myeloablative conditioning; RIC = reduced-intensity conditioning.

Table 2. Univariate analysis for outcomes after transplantation

	MAC probability (95% CI)	*RIC probability (95% CI)*	*P-value*
Engraftment (neutrophil recovery at 100 days)	92 (88–94)	93 (88–96)	0.063
Acute GVHD at 100 days (grades II–IV)	44 (38–49)	42 (34–49)	0.353
Chronic GVHD at 3 years	36 (30–42)	35 (27–42)	0.793
OS			
1 year	65 (60–70)	67 (60–73)	0.606
3 year	51 (45–56)	53 (45–60)	0.701
Disease-free survival			
1 year	59 (53–63)	60 (53–66)	0.734
3 year	47 (42–53)	39 (31–47)	0.098
Relapse			
1 year	12 (9–16)	14 (9–19)	0.664
3 year	15 (11–19)	26 (19–33)	0.008
Non-relapse mortality			
1 year	30 (25–34)	26 (21–33)	0.268
3 year	38 (33–44)	36 (28–43)	0.678

Abbreviations: CI = confidence interval; MAC = myeloablative conditioning; RIC = reduced-intensity conditioning.

DISCUSSION

The role of allo-SCT in adult ALL is still controversial; however, allo-SCT is a potentially curative treatment for patients with ALL. However, the majority of older adult ALL patients are not candidates for MAC regimens. Although significant reduction of

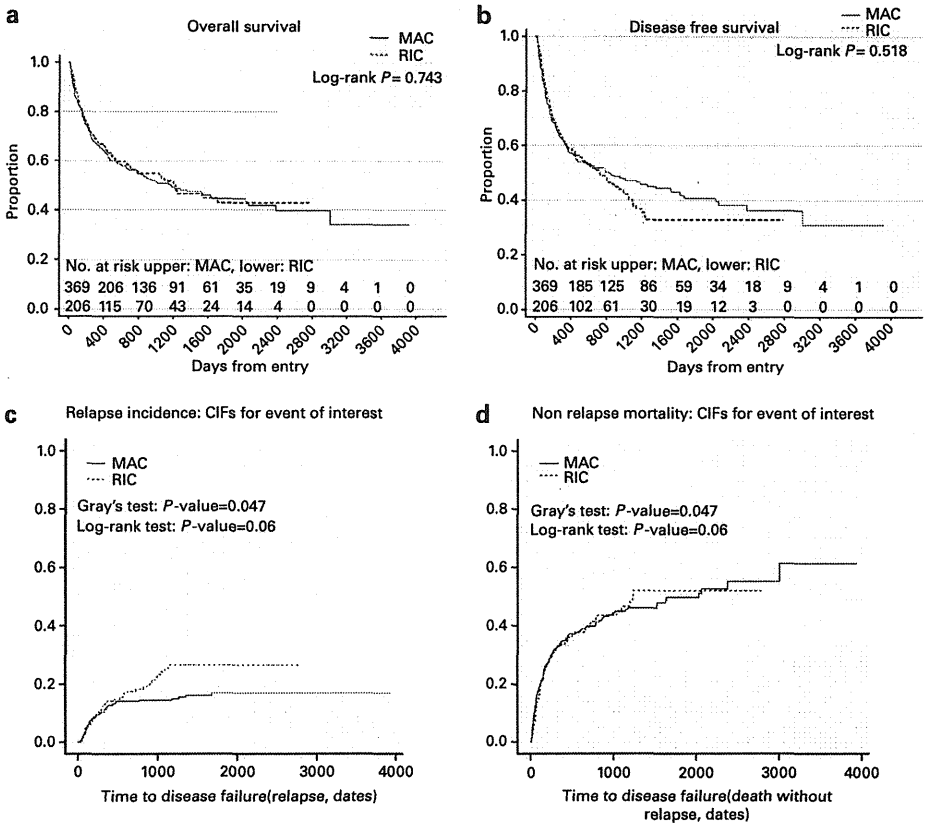

Figure 1. Kaplan–Meier curves for OS (**a**), disease-free survival (**b**), cumulative incidence of relapse (**c**) and non-relapse mortality (**d**).

Table 3. Cause of death

	MAC, n *(%)*	*RIC*, n *(%)*
Original disease	38 (22)	21 (23)
Acute GVHD	9 (5)	9 (10)
Chronic GVHD	6 (4)	2 (2)
Graft rejection	3 (2)	3 (3)
Infection	38 (22)	22 (24)
Hemorrhage	6 (4)	5 (5)
Interstitial pneumonitis	21 (12)	4 (4)
Organ failure	26 (15)	11 (12)
Others	25 (15)	15 (16)

Abbreviations: MAC = myeloablative conditioning; RIC = reduced-intensity conditioning.

the intensity of the preparative regimen may have a negative impact on long-term leukemic control,[24,25] RIC is a reasonable preparative option for older ALL patients in order to reduce regimen-related toxicities. There is little information on RIC allo-SCT for ALL patients.[14–19,26] It was reported in 2008 by Mohty *et al.*[14] that 2-year OS, leukemia-free survival and NRM were 52, 18 and 18%, respectively, after RIC allo-SCT for 97 adult ALL patients. RIC allo-SCT with cord blood and RIC allo-SCT with PBSCs were both feasible for adult ALL patients.[15,16] Moreover, RIC allo-SCT was suggested to be a potential therapeutic approach for adult high-risk ALL patients in remission based on the results of a prospective phase 2 study.[17] Marks *et al.*[18] found that conditioning intensity did not affect TRM or relapse risk by multivariate analysis of a comparison of 93 Ph chromosome-negative ALL patients >16 years of age after RIC with 1482 patients who received MAC. Mohty *et al.*[19] found by multivariate analysis that NRM was decreased in RIC recipients, whereas it was associated with higher relapse rate in 576 ALL patients (RIC for 127 and MAC for 499 patients) aged ⩾45 years. For Ph chromosome-positive ALL patients in first remission, RIC allo-SCT with post-grafting imatinib resulted in favorable long-term survival.[26] Lee *et al.*[27] reported that the BuFlu regimen (BU plus fludarabine) is not a suitable replacement for the BuCy regimen (BU plus CY) in young adults who are eligible for MAC therapy for allo-SCT.

In this study, outcomes for 575 adult ALL patients aged ⩾45 years at the first transplantation who underwent allo-SCT in CR were analyzed according to the type of conditioning regimen (MAC for 369 vs RIC for 206). The survival rate of RIC patients was similar to that of MAC patients, despite an older median age of RIC patients. Relapse rate at 3 years was higher in the RIC group; however, OS, DFS and NRM were similar in the two groups. We divided patients into two age groups, one group with age of <55 years and one group with age of ⩾55 years. There were no significant differences in OS and DFS between the MAC and RIC patients in the two age groups (data not shown). We found that HLA mismatching was associated with lower rate of relapse, and it seems that allo-SCT for ALL induces a GVL effect. However, HLA mismatching was associated with higher rate of NRM. RIC was associated with good OS and DFS in patients who underwent HLA-mismatch transplantation and were aged ⩾55 years compared with MAC by multivariate analysis for each event with interaction. Conversely, MAC resulted in good OS and DFS in patients with HLA matching and who were aged <50 years. Therefore, patients with HLA-mismatch transplantation and who are aged ⩾55 years would be candidates for RIC rather than MAC. Female gender was associated with good OS and DFS, but donor/recipient sex mismatch did not affect survival. The reason for this is not clear, but lower rate of NRM in female patients may be

Table 4. Multivariate analysis for each event

Variables	*Reference*	*HR*	*95% CI*		*P-value*
OS					
RIC	Full intensity	0.86	0.56	1.33	0.507
Female	Male	0.59	0.42	0.83	0.003
CR2	CR1	1.88	1.26	2.80	0.002
HLA mismatching	Complete matching (6/6)	1.67	1.09	2.57	0.019
Disease-free survival					
RIC	Full intensity	0.99	0.66	1.48	0.969
Female	Male	0.65	0.48	0.89	0.006
CR2	CR1	1.95	1.35	2.82	<0.001
HLA Mismatching	Complete matching (6/6)	1.29	0.85	1.95	0.229
Relapse incidence					
RIC	Full intensity	1.58	0.83	2.99	0.160
Female	Male	0.97	0.58	1.61	0.900
CR2	CR1	2.29	1.25	4.19	0.007
HLA mismatching	Complete matching (6/6)	0.27	0.09	0.78	0.016
Non-relapse mortality					
RIC	Full intensity	0.74	0.42	1.32	0.310
Female	Male	0.50	0.33	0.74	0.001
CR2	CR1	1.39	0.85	2.28	0.190
HLA mismatching	Complete matching (6/6)	2.35	1.41	3.93	0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; RIC = reduced-intensity conditioning.

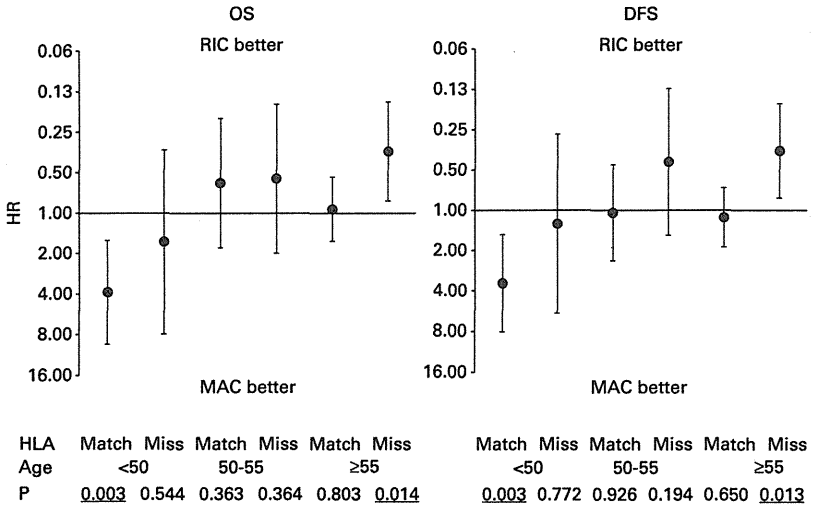

Figure 2. Adjusted hazard ratios for OS and DFS of RIC patients compared with MAC patients in subgroups of HLA matching and age. RIC was associated with good OS and DFS in patients who received HLA-mismatch transplantation and were aged ⩾55 years compared with MAC by multivariate analysis for each event with interaction (HR and 95% CI: 0.35 and 0.15–0.81, $P = 0.014$ for OS and 0.36 and 0.16–0.81, $P = 0.013$ for DFS).

associated with good survival. This study has some limitations that would influence data interpretation because the patient populations were different. More of the RIC patients received PBSCs and more received a transplantation after 2006. The reason for selecting RIC is not always apparent. Therefore, our retrospective study had these serious limitations and there is a need for prospective randomized trials. However, the results of this study suggest that RIC allo-SCT is feasible and is a potential option for ALL patients aged ⩾45 years in CR who are not eligible for MAC allo-SCT for some reason. Moreover, RIC may be a useful preparative regimen for patients aged ⩾55 years, especially those with HLA-mismatch donors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan and a Japanese Grant-in-Aid for Scientific Research.

AUTHOR CONTRIBUTIONS

JT and NM designed the study and prepared the manuscript; NN and KO performed the statistical analysis; SN, KO, ST, TE, HN, Ke M, Ko M, HS, YM, KK and RS participated in interpretation of data and approval of the final manuscript.

REFERENCES

1 Rowe JM, Buck G, Burnett AK, Chopra R, Wiernik PH, Richards SM *et al.* Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. *Blood* 2005; **106**: 3760–3767.

2 Hunault M, Harousseau JL, Delain M, Truchan-Graczyk M, Cahn JY, Witz F *et al.* Better outcome of adult acute lymphoblastic leukemia after early genoidentical allogeneic bone marrow transplantation (BMT) than after late high-dose therapy and autologous BMT: a GOELAMS trial. *Blood* 2004; **104**: 3028–3037.

3 Thomas X, Boiron JM, Huguet F, Dombret H, Bradstock K, Vey N *et al.* Outcome of treatment in adults with acute lymphoblastic leukemia: analysis of the LALA-94 trial. *J Clin Oncol* 2004; **22**: 4075–4086.

4 Goldstone AH, Richards SM, Lazarus HM, Tallman MS, Buck G, Fielding AK *et al.* In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). *Blood* 2008; **111**: 1827–1833.

5 Cornelissen JJ, van der Holt B, Verhoef GE, van't Veer MB, van Oers MH, Schouten HC *et al.* Myeloablative allogeneic versus autologous stem cell transplantation in adult patients with acute lymphoblastic leukemia in first remission: a prospective sibling donor versus no-donor comparison. *Blood* 2009; **113**: 1375–1382.

6 Tomblyn MB, Arora M, Baker KS, Blazar BR, Brunstein CG, Burns LJ *et al.* Myeloablative hematopoietic cell transplantation for acute lymphoblastic leukemia: analysis of graft sources and long-term outcome. *J Clin Oncol* 2009; **27**: 3634–3641.

7 Marks DI, Pérez WS, He W, Zhang MJ, Bishop MR, Bolwell BJ *et al.* Unrelated donor transplants in adults with Philadelphia-negative acute lymphoblastic leukemia in first complete remission. *Blood* 2008; **112**: 426–434.

8 Fielding AK, Richards SM, Chopra R, Lazarus HM, Litzow MR, Buck G *et al.* Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. *Blood* 2007; **109**: 944–950.

9 Bishop MR, Logan BR, Gandham S, Bolwell BJ, Cahn JY, Lazarus HM *et al.* Long-term outcomes of adults with acute lymphoblastic leukemia after autologous or unrelated donor bone marrow transplantation: a comparative analysis by the National Marrow Donor Program and Center for International Blood and Marrow Transplant Research. *Bone Marrow Transplant* 2008; **41**: 635–642.

10 Marks DI, Forman SJ, Blume KG, Pérez WS, Weisdorf DJ, Keating A *et al.* A comparison of cyclophosphamide and total body irradiation with etoposide and total body irradiation as conditioning regimens for patients undergoing sibling allografting for acute lymphoblastic leukemia in first or second complete remission. *Biol Blood Marrow Transplant* 2006; **12**: 438–453.

11 Marks DI, Aversa F, Lazarus HM. Alternative donor transplants for adult acute lymphoblastic leukaemia: a comparison of the three major options. *Bone Marrow Transplant* 2006; **38**: 467–475.

12 Toubai T, Tanaka J, Mori A, Hashino S, Kobayashi S, Ota S *et al.* Efficacy of etoposide, cyclophosphamide, and total body irradiation in allogeneic bone marrow transplantation for adult patients with hematological malignancies. *Clin Transplant* 2004; **18**: 552–557.

13 Shigematsu A, Kondo T, Yamamoto S, Sugita J, Onozawa M, Kahata K *et al.* Excellent outcome of allogeneic hematopoietic stem cell transplantation using a conditioning regimen with medium-dose VP-16, cyclophosphamide and total-body irradiation for adult patients with acute lymphoblastic leukemia. *Biol Blood Marrow Transplant* 2008; **14**: 568–575.

14 Mohty M, Labopin M, Tabrizzi R, Theorin N, Fauser AA, Rambaldi A *et al.* Reduced intensity conditioning allogeneic stem cell transplantation for adult patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. *Haematologica* 2008; **93**: 303–306.

15 Bachanova V, Verneris MR, DeFor T, Brunstein CG, Weisdorf DJ. Prolonged survival in adults with acute lymphoblastic leukemia after reduced-intensity conditioning with cord blood or sibling donor transplantation. *Blood* 2009; **113**: 2902–2905.

16 Stein AS, Palmer JM, O'Donnell MR, Kogut NM, Spielberger RT, Slovak ML *et al.* Reduced-intensity conditioning followed by peripheral blood stem cell transplantation for adult patients with high-risk acute lymphoblastic leukemia. *Biol Blood Marrow Transplant* 2009; **15**: 1407–1414.

17 Cho BS, Lee S, Kim YJ, Chung NG, Eom KS, Kim HJ *et al.* Reduced-intensity conditioning allogeneic stem cell transplantation is a potential therapeutic approach for adults with high-risk acute lymphoblastic leukemia in remission: results of a prospective phase 2 study. *Leukemia* 2009; **23**: 1763–1770.

18 Marks DI, Wang T, Pérez WS, Antin JH, Copelan E, Gale RP *et al.* The outcome of full-intensity and reduced-intensity conditioning matched sibling or unrelated donor transplantation in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia in first and second complete remission. *Blood* 2010; **116**: 366–374.

19 Mohty M, Labopin M, Volin L, Gratwohl A, Socié G, Esteve J *et al.* Reduced-intensity versus conventional myeloablative conditioning allogeneic stem cell transplantation for patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. *Blood* 2010; **116**: 4439–4443.

20 Kim SW, Matsuo K, Fukuda T, Hara M, Matsue K, Taniguchi S *et al.* Reduced-intensity unrelated donor bone marrow transplantation for hematologic malignancies. *Int J Hematol* 2008; **88**: 324–330.

21 Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M *et al.* Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant* 2009; **15**: 367–369.

22 Pepe MS, Mori M. Kaplan-Meier, marginal or conditional probability curves in summarizing competing risks failure time data? *Stat Med* 1993; **12**: 737–751.

23 Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; **94**: 496–509.

24 Martino R, Iacobelli S, Brand R, Jansen T, van Biezen A, Finke J *et al.* Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. *Blood* 2006; **108**: 836–846.

25 Shimoni A, Hardan I, Shem-Tov N, Yeshurun M, Yerushalmi R, Avigdor A *et al.* Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. *Leukemia* 2006; **20**: 322–328.

26 Ram R, Storb R, Sandmaier BM, Maloney DG, Woolfrey A, Flowers ME *et al.* Non-myeloablative conditioning with allogeneic hematopoietic cell transplantation for the treatment of high-risk acute lymphoblastic leukemia. *Haematologica* 2011; **96**: 1113–1120.

27 Lee JH, Joo YD, Kim H, Ryoo HM, Kim MK, Lee GW *et al.* Randomized trial of myeloablative conditioning regimens: busulfan plus cyclophosphamide versus busulfan plus fludarabine. *J Clin Oncol* 2013*20* **31**: 701–709.

Bone Marrow Transplantation (2013) 48, 1513–1518

www.nature.com/bmt

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.

Bone Marrow Transplantation (2013) **48,** 1513–1518; doi:10.1038/bmt.2013.140; published online 23 September 2013

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.

[1]Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan; [2]Department of Hematology, Fujita Health University Hospital, Toyoake, Japan; [3]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan; [4]Division of Molecular Medicine, Aichi Cancer Center Research Institute, Nagoya, Japan; [5]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [6]Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan; [7]Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [8]Department of Hematology, Osaka City University Graduate School of Medicine, Osaka, Japan; [9]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [10]Third Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Japan; [11]Division of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan; [12]Division of Hematology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; [13]Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; [14]Department of Cell Processing and Transfusion, Research Hospital, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [15]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan; [16]Department of HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [17]Division of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan and [18]Department of Hematology, Tokyo Women's Medical University, Tokyo, Japan. Correspondence: Dr H Kanamori, Department of Hematology, Kanagawa Cancer Center, 1-1-2 Nakao, Asahi-ku, Yokohama 241-8515, Japan.
E-mail: kanamori@kcch.jp
Received 9 June 2013; revised 18 July 2013; accepted 29 July 2013; published online 23 September 2013

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.

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

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.

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

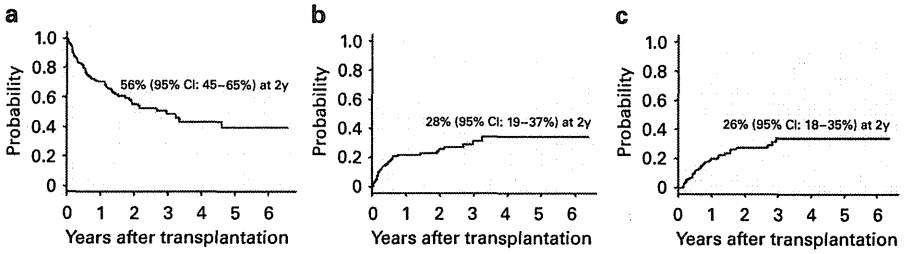

Figure 1. (a) OS, (b) cumulative incidence of relapse and (c) cumulative incidence of non-relapse mortality.

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