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

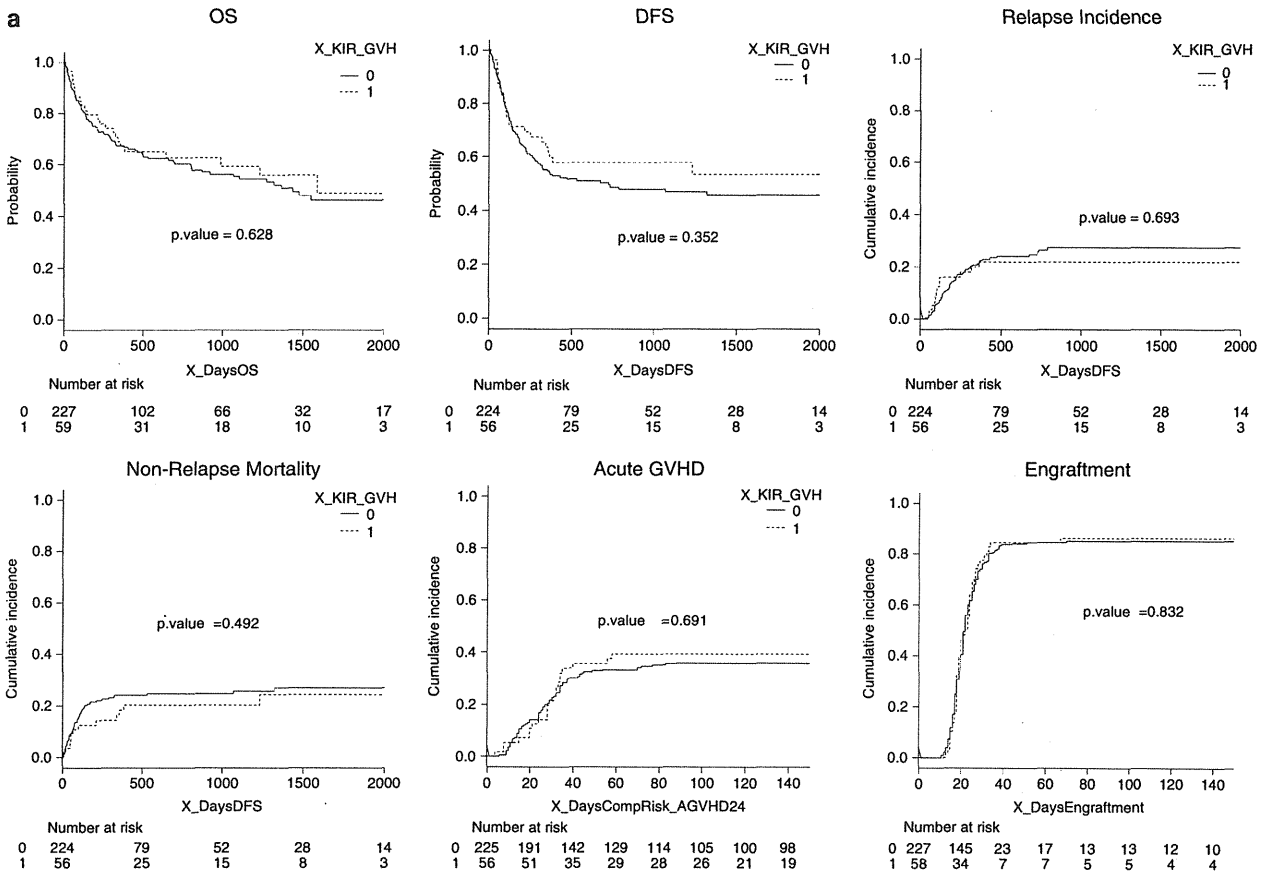
Differences among patients, disease and transplantation-related factors according to conditioning regimens, and GVHD prophylaxis are shown in Tables 1a and b.

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



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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 ($>2.5\times10^7$/kg and $<2.5\times10^7$/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.

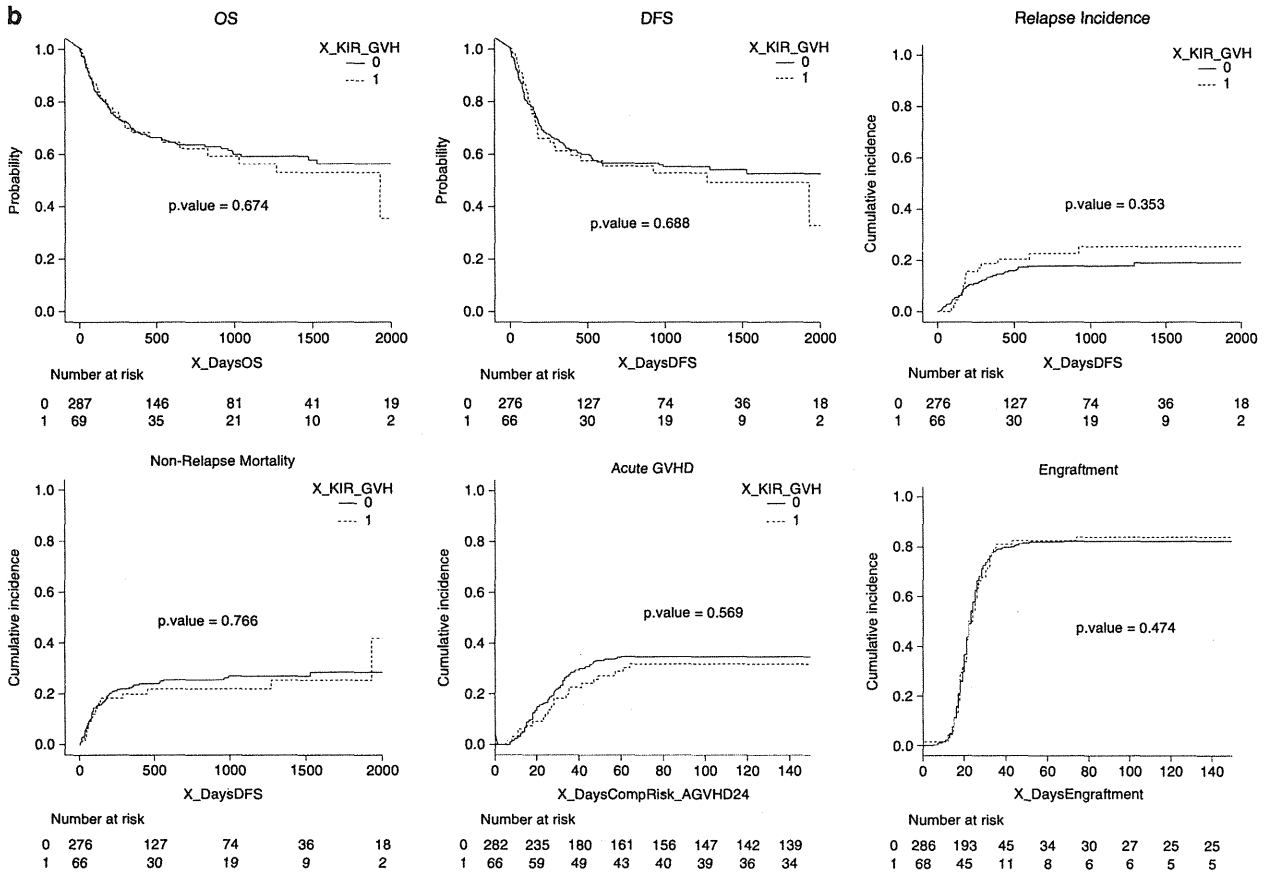
Impact of KIR ligand mismatch in the GVH direction on transplantation outcomes

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)



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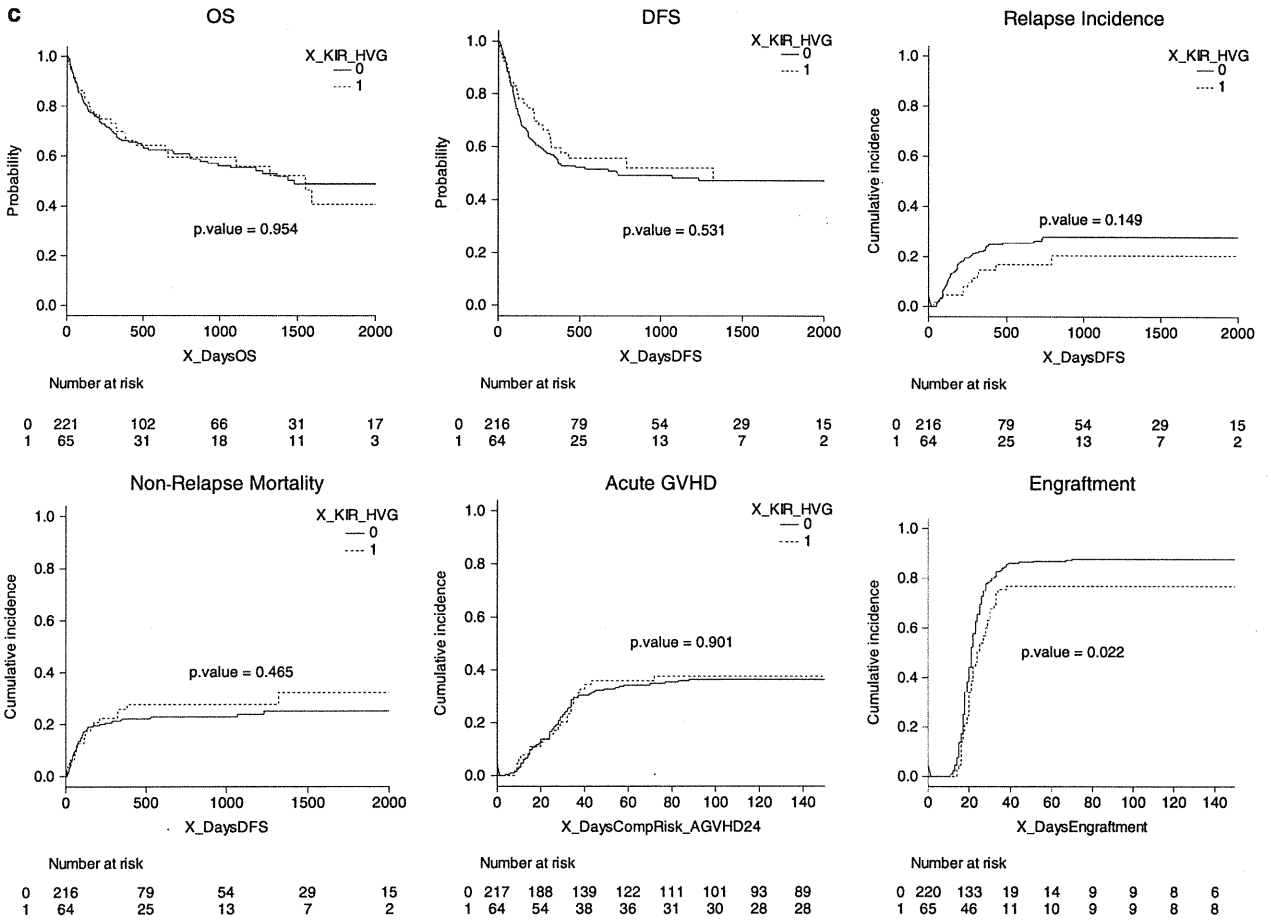

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and also with poor DFS (HR 2.41, $P=0.002$ and HR 1.67, $P=0.011$, respectively). Also, age >40 years was associated with higher NRM and lower engraftment rate (HR 6.96, $P<0.001$ and HR 0.55, $P<0.001$, respectively). For AML patients, age >40 years 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

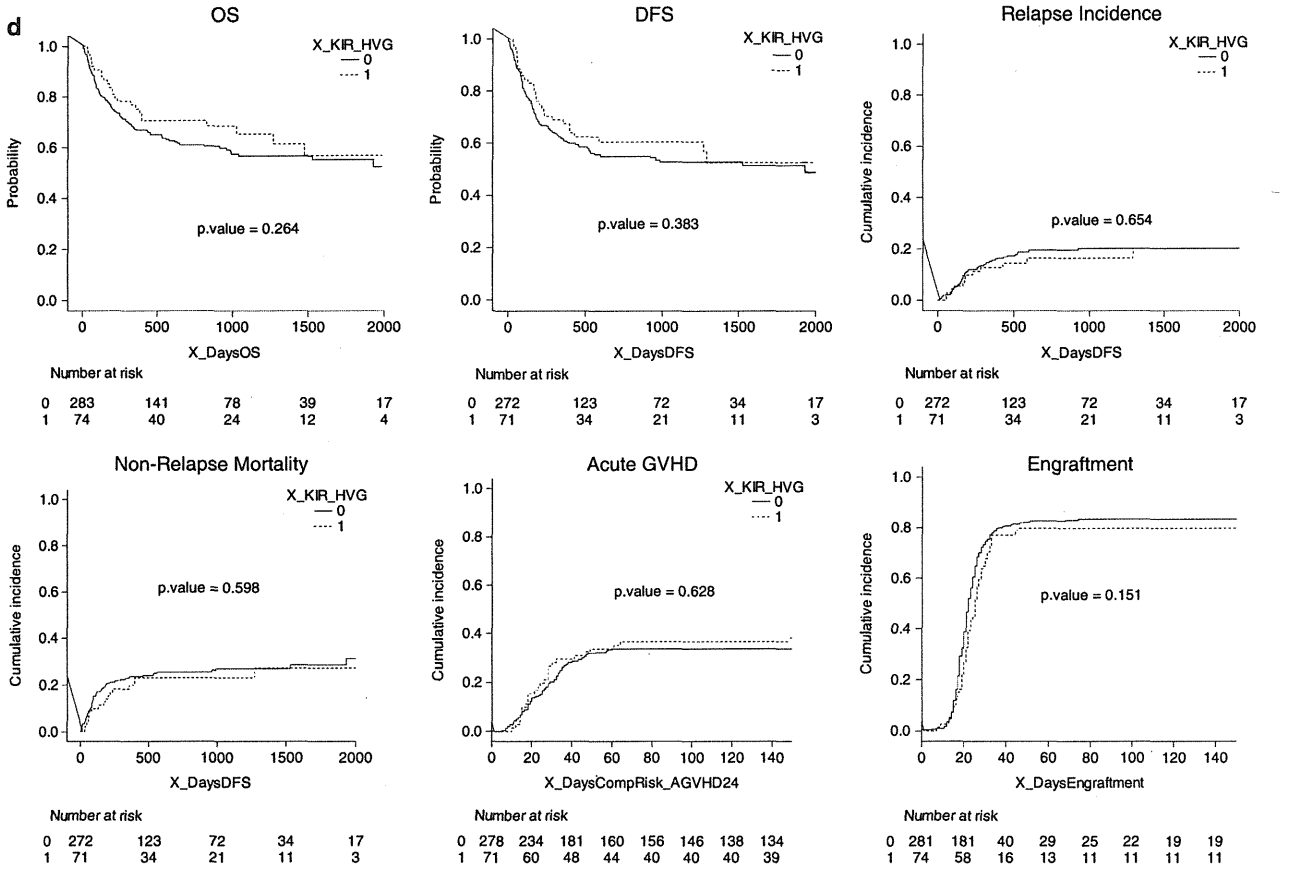

Figure 1. Kaplan–Meier curves forOS, DFS, CIR, NRM, acute GVHD and engraftment in (**a**) ALL and (**b**) AML patients transplanted from KIR-compatible and incompatible donors in the GVH direction and in (**c**) ALL and (**d**) AML patients transplanted from KIR-compatible and incompatible donors in 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 $>2.5 \times 10^7$/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.

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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)

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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.

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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

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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

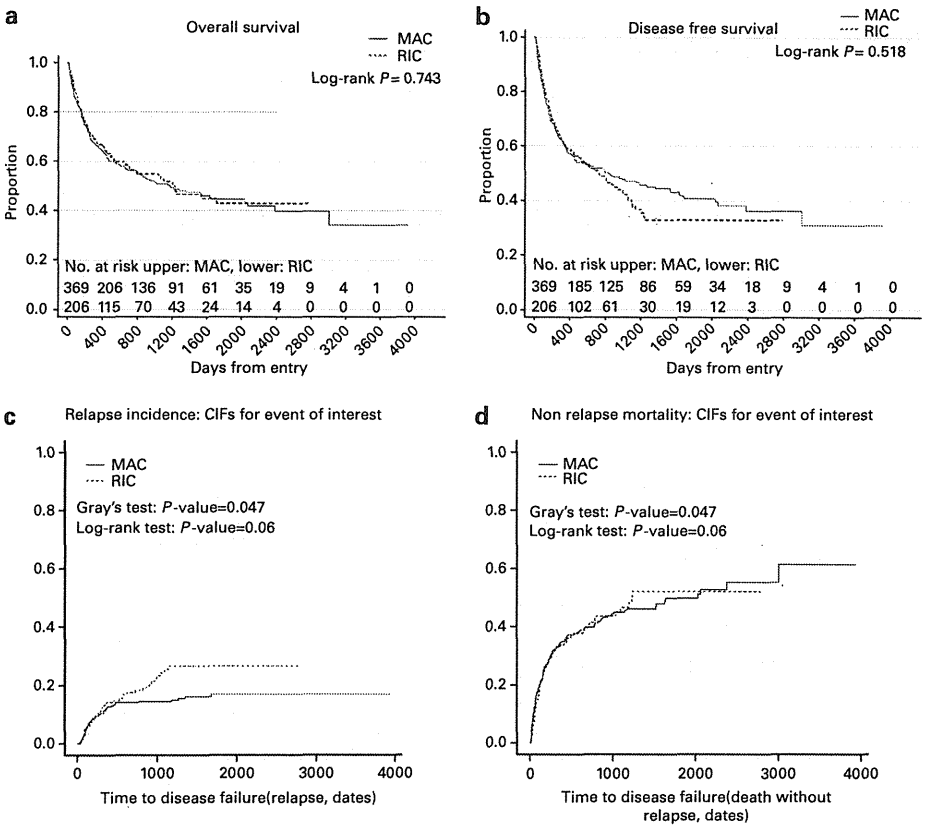

Figure 1. Kaplan–Meier curves for OS (**a**), disease-free survival (**b**), cumulative incidence of relapse (**c**) and non-relapse mortality (**d**).

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.

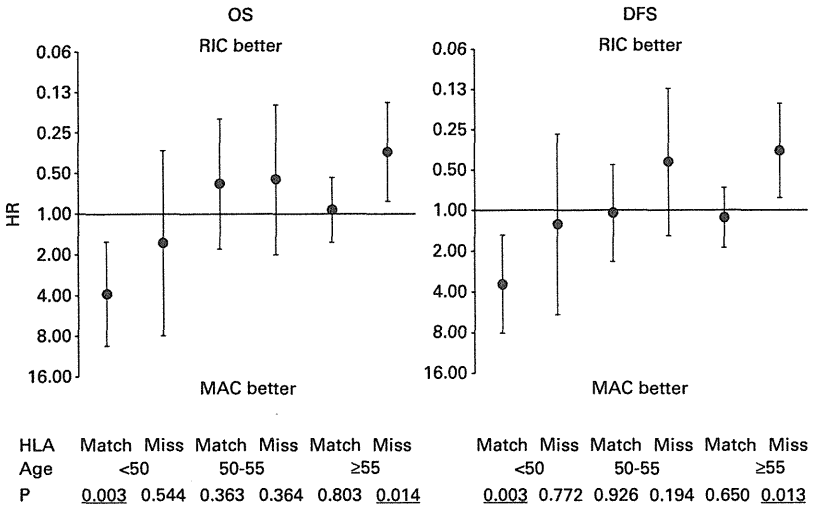

Figure 2. Adjusted hazard ratios for OS and DFS of RIC patients compared with MAC patients in subgroups of HLA matching and age. RIC was associated with good OS and DFS in patients who received HLA-mismatch transplantation and were aged ⩾55 years compared with MAC by multivariate analysis for each event 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.

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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.

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once sufficient numbers of mice in other colonies are observed to 1 year and are subjected to similar analyses. If disease progression is unique to mice in our facility, it might suggest that environmental or infectious agents can play a role in the progression to the more severe phenotype in a subset of mice in the context of 12/15-lipoxygenase deficiency. It is important to stress, however, that the defects we reported in the majority of Alox15 mice that are asymptomatic do not in our opinion appear to be unique to the Wistar colony, and these mice remain a valuable tool for defining the role of 12/15-lipoxygenase in hematopoiesis.

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To the editor:

Dasatinib enhances the expansion of CD56$^+$CD3$^-$ NK cells from cord blood

Dasatinib can inhibit T-cell activation through inhibition of the Scr family of tyrosine kinases such as p56 (Lck).[1] It has been reported that some chronic myeloid leukemia (CML) patients who were treated with dasatinib developed chronic large-granular lymphocytosis (LGL) with natural killer (NK) or NK T-cell lineage, and that these patients achieved optimal molecular response.[2] In addition, Mustjoki et al reported clonal expansion of NK T cells during dasatinib therapy.[3] Kreutzman et al reported that mono/oligoclonal T and NK cells were present in CML patients at diagnosis and expanded during dasatinib therapy and that LGL expansion is linked to cytomegalovirus infection.[4,5] Therefore, dasatinib may have a favorable effect on NK-cell proliferation. In this study, we analyzed the effects of dasatinib on the expansion of NK cells from cord blood and transcriptional factors during expansion.

Umbilical cord blood cells (1×10^6/mL; Hokkaido Cord Blood Bank) were cultured with IL-15 (10 ng/mL; PeproTech), IL-2 (5 ng/mL; R&D Systems), and anti-CD3 mAb (OKT3, 10 ng/mL; Janssen Pharmaceutical); with or without dasatinib (10nM; a kind gift from Bristol-Myers Squibb) in culture medium stem cell growth medium (CeeGenix) with 5% human AB serum in 24-well plates, as we reported previously.[6] After a 7-day culture of umbilical cord blood cells (1×10^6/mL), the absolute number of CD56$^+$CD3$^-$ NK cells had significantly increased in the culture with dasatinib compared with the culture with cytokines only (before culture $5.3 \pm 1.4 \times 10^4$ in 10^6 cord blood cells, after culture with IL-2 + IL-15 $26.0 \pm 17.8 \times 10^4$, and after culture with IL-2 + IL-15 and dasatinib $66.6 \pm 29.1 \times 10^4$; $P < .05$, means ± SDs, n = 6; Figure 1A). In addition, the proportion of CD56$^+$CD3$^-$ cells, CD56$^+$NKG2D$^+$ cells, and CD56$^+$granzyme$^+$ cells significantly increased after culture with dasatinib (Figure 1B).

We analyzed the transcriptional factors Eomesodermin (Eomes) and T-bet using an Applied Biosystems 7300 Real-Time PCR System and GAPDH as an endogenous control. Before stimulation, cord blood of CD56$^+$ cells showed increased expression of Eomes and T-bet compared with the expression in unfractionated whole cord blood cells and CD3$^+$ cells. After 24 hours, Eomes expression was significantly increased in cord blood cells cultured with dasatinib compared with cells cultured with cytokines only (5.96 ± 3.95 vs 0.81 ± 0.62, $P < .05$; Figure 1C).

At present, there are only a few transcription factors that are known to play an essential role in NK-cell development, especially in humans. T-box proteins, T-bet, and Eomes are involved in NK-cell development.[7-9] T-bet and Eomes are both later required for the differentiation in DX5$^+$(CD49b) CD11b$^+$ NK cells. In addition, Eomes is highly expressed in fully differentiated NK cells. In this study, we showed NK-cell expansion after culture with dasatinb and increased expression of Eomes after 24 hours. Therefore, dasatinib has some role in NK-cell expansion from cord blood under the condition of IL-2 and IL-15 stimulation through increased expression of transcription factors such as Eomes. This observation may have potentially important implication for the treatment of other diseases with dasatinib.[10]

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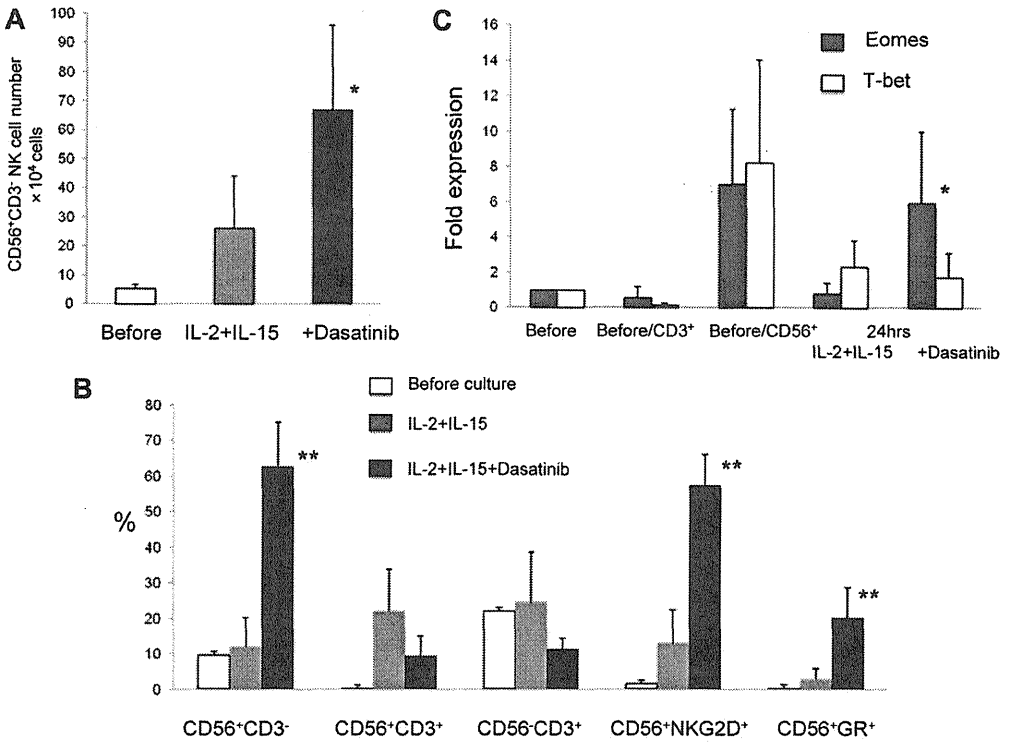

Figure 1. Expansion of CD56$^+$CD3$^-$ NK cells with dasatinib from cord blood cells. (A) The absolute number of CD56$^+$CD3$^-$ NK cells had significantly increased in the culture with dasatinib compared with those in the culture with cytokines only. (B) The proportion of CD56$^+$CD3$^-$, CD56$^+$NKG2D$^+$, and CD56$^+$granzyme$^+$ cells significantly increased after culture with dasatinib compared with culture without dasatinib. (C) After 24 hours, Eomes expression was significantly increased in cord blood cells cultured with dasatinib compared with that in cells cultured with cytokines only. T-bet expression was increased after 24 hours culture compared with that before culture, but there was no significant difference between expression level in culture with dasatinib and that without dasatinib (bars indicate means ± SDs, n = 6; $^*P < .05$ and $^{**}P < .01$).

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ORIGINAL ARTICLE

Positive impact of chronic graft-versus-host disease on the outcome of patients with *de novo* myelodysplastic syndrome after allogeneic hematopoietic cell transplantation: a single-center analysis of 115 patients

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Abstract

To evaluate the impact of graft-versus-host disease (GVHD) and prognostic factors for patients with myelodysplastic syndrome (MDS) after allogeneic hematopoietic cell transplantation (allo-HCT), we retrospectively reviewed 115 patients with MDS or acute myeloid leukemia with multilineage dysplasia (AML-MLD) after allo-HCT at our center. Eighty one patients received reduced-intensity conditioning (RIC) regimens, whereas 34 received myeloablative conditioning regimens. Although the RIC group was significantly older and included more patients with poor cytogenetic risk, no difference in 4-yr overall survival (OS) was seen between the two groups. In a multivariate analysis, covariates associated with a worse OS were the French-American-British stage of refractory anemia excess blasts in transformation/AML-MLD at peak, poor cytogenetic risk, bone marrow blasts of 20% or higher at HCT and the absence of chronic GVHD (cGVHD). By using semi-landmark analyses, we found that the presence of cGVHD significantly improved OS in high-risk patients or the RIC group. However, there was no difference in OS between those with and without cGVHD among low-risk MDS patients. These findings suggest that the graft-versus-leukemia effect may be more beneficial in high-risk patients who do not receive intensive preparative regimens.

Key words myelodysplastic syndrome; allogeneic hematopoietic cell transplantation; graft-versus-host disease; graft-versus-leukemia effect

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Allogeneic hematopoietic cell transplantation (allo-HCT) has been assumed to be the only treatment modality with curative potential for patients with myelodysplastic syndrome (MDS). However, about 90% of MDS cases occur in elderly patients above the age of 60 yrs (1) and a substantial proportion of them are more likely to have a worse performance status and an increased comorbidity. As a result, myeloablative conditioning (MAC) regimens are less commonly used for patients with MDS because of an increased risk of non-relapse mortality (NRM). However, some studies have reported that the dose intensity of the conditioning regimen plays an important role in controlling the disease after allo-HCT for MDS or acute myeloid leukemia (AML) (2, 3). Reduced-intensity conditioning regimens (RIC) have been developed to decrease the risk of NRM with less-intensive conditioning for elderly or less-fit patients while preserving a graft-versus-leukemia (GVL) effect by an alloimmune reaction as an antitumor effect (4, 5). The European Group for Blood and Marrow Transplantation reported that, among patients with MDS who underwent allo-HCT from a sibling donor, the RIC group was associated with a lower incidence of NRM and a higher risk of relapse in comparison with the

MAC group, whereas overall survival (OS) was similar in both groups (6).

Although an alloimmune reaction by donor T-cells is important for disease control after allo-HCT, especially in the RIC setting, the significance of this effect has not been well documented in patients with MDS. Therefore, we retrospectively reviewed the medical records of 115 patients with *de novo* MDS or AML with multilineage dysplasia (AML-MLD) who underwent their first allo-HCT at our center, and evaluated the impact of graft-versus-host disease (GVHD) and prognostic factors for the outcome in patients with MDS after allo-HCT.

Patients and methods

Patients

This study included patients with *de novo* MDS or AML-MLD who underwent their first allo-HCT at our center between January 2000 and December 2009. The study protocol was reviewed and approved by the institutional ethics committee. Therapy-related MDS and cord blood transplant recipients were excluded. Therapy-related MDS was defined as disease arising in patients who were treated with irradiation, chemotherapy, or both for hematologic malignancies or other cancers. Disease stages were categorized according to the French-American-British (FAB) classification (7). AML-MLD was defined as AML with more than 30% bone marrow (BM) myeloblasts and morphological features of myelodysplasia, or a prior history of MDS. Patients with MDS were classified into two diagnostic groups (Low/Intermediate-1 and Intermediate-2/High) at diagnosis and at peak according to the International Prognostic Scoring System (IPSS) (8). Cytogenetic risk groups were determined according to IPSS using the cytogenetic information at diagnosis. Matching between the donor and recipient was determined according to donor–recipient HLA-A, HLA-B, and HLA-DR compatibility.

Myeloablative conditioning regimens included cyclophosphamide (Cy, 60 mg/kg for 2 d) plus busulfan (Bu, orally 4 mg/kg for 4 d or i.v. 3.2 mg/kg for 4 d) (Bu/Cy) or total body irradiation (TBI, 12 Gy) (TBI/Cy). RIC regimens included Bu (orally 4 mg/kg for 2 d or i.v. 3.2 mg/kg for 2 d) plus fludarabine (Flu, 30 mg/m^2 for 6 d) (Flu/Bu) or cladribine (2-CdA, 0.11 mg/kg for 6 d) (2-CdA/Bu). In a subset of patients who received RIC, low-dose TBI (2 or 4 Gy) and/or low-dose antithymocyte globulin (ATG) (total dose 5–10 mg/kg Fresenius or 2.5–5 mg/kg Thymoglobulin) were added. GVHD prophylaxis included either cyclosporine or tacrolimus alone or a combination of either of the calcineurin inhibitors and methotrexate. The decision regarding the intensity of the conditioning regimen and GVHD prophylaxis for each patient was made at the discretion of the attending physicians based on a review of the patient's age, disease status, comorbidities, performance status and HLA compatibility.

Neutrophil and platelet engraftment dates were defined as the first of three consecutive days with an absolute neutrophil count of 0.5 × 10^9/L or higher and an untransfused platelet count of 2.0 × 10^9/L or higher. Acute and chronic GVHD (cGVHD) were diagnosed and graded according to standard criteria (9). Response and relapse of the disease were defined according to standard hematologic criteria.

Statistical analysis

We used the Chi-square analysis and Fisher's exact test to compare categorical covariates and the Mann–Whitney *U* test to compare continuous covariates. OS was estimated by the Kaplan–Meier method, and differences between groups were evaluated by the log-rank test. Relapse and NRM were considered as competing risk events for each other. The probabilities of relapse and NRM were estimated by the cumulative incidence functions, and differences between groups were evaluated by the Gray test (10, 11). OS and the incidences of relapse and NRM were estimated as probabilities at 4 yrs from allo-HCT. To evaluate the effect of cGVHD on OS, we performed semi-landmark analyses (12). For patients with cGVHD, OS was estimated as the probability from the onset of cGVHD by the Kaplan–Meier method. A landmark comparison group consisted of survivors without cGVHD at day 138 (landmark day), which was the median time of the onset of cGVHD with OS for this group estimated as the probability from the landmark day. The Cox proportional hazards regression model was used for univariate and multivariate analyses, and a hazard ratio was calculated in conjunction with a 95% confidence interval (CI). For the assumption of proportional hazards over time, acute GVHD (aGVHD) and cGVHD were treated as time-dependent covariates (13). For multivariate analyses, we decided to include covariates with a *P*-value of <0.1 in univariate analyses. In addition, we included conditioning regimens and GVHD in these models to evaluate their effects on the outcome. The statistical analysis was performed with R-Project (version 2.2.1; http://www.r-project.org/).

Results

Patient characteristics

The characteristics of a total of 115 patients are summarized in Table 1. The median age was 55 yrs (range: 19–68) and the median follow-up of surviving patients was 40 months (range: 4–130). Eighty one patients (70%) received RIC regimens, whereas 34 (30%) received MAC regimens. According to the FAB stage at peak, the proportions of patients with refractory anemia (RA)/refractory anemia with ringed sideroblasts (RARS), refractory anemia

Table 1 Patient characteristics

No. of patients	All $N = 115$	MAC $N = 34$	RIC $N = 81$
Period of HCT (%)			
2000–2004	71 (62)	18 (53)	53 (65)
2005–2009	44 (38)	16 (47)	28 (35)
Age at HCT, median (range)	55 (19–68)	46 (23–57)	57 (19–68)
Age at HCT, yrs			
≧50 yrs (%)	84 (73)	10 (29)	74 (91)
Patient sex, male (%)	82 (71)	24 (71)	58 (72)
FAB stage at diagnosis (%)			
RA/RARS	45 (39)	13 (38)	32 (40)
RAEB/CMMoL	44 (38)	12 (36)	32 (40)
RAEB-T/AML-MLD	26 (23)	9 (26)	17 (20)
IPSS at diagnosis (%)			
Low/Intermediate-1	37 (32)	13 (38)	24 (30)
Intermediate-2/High	64 (56)	16 (47)	48 (59)
Unknown	14 (12)	5 (15)	9 (11)
FAB stage at peak (%)			
RA/RARS	22 (19)	6 (18)	16 (20)
RAEB/CMMoL	38 (33)	10 (29)	28 (34)
RAEB-T/AML-MLD	55 (48)	18 (53)	37 (46)
IPSS at peak (%)			
Low/Intermediate-1	24 (21)	6 (18)	18 (22)
Intermediate-2/High	77 (67)	23 (68)	54 (67)
Unknown	14 (12)	5 (14)	9 (11)
Cytogenetic risk group (%)			
Good/Intermediate	75 (65)	27 (79)	48 (59)
Poor	40 (35)	7 (21)	33 (41)
BM blasts at HCT, median (range)	5 (0–78)	3 (0–46)	4 (0–78)
≦4%	60 (52)	18 (53)	42 (52)
5–19%	38 (33)	10 (29)	28 (35)
≧20%	10 (9)	3 (9)	7 (8)
Unknown	7 (6)	3 (9)	4 (5)
Disease duration, months, median (range)	9 (1–200)	8 (2–200)	10 (1–172)
Karnofsky score at HCT (%)			
90–100	96 (83)	29 (85)	67 (83)
Transfusion dependence (%)	89 (77)	27 (79)	62 (77)
Prior chemotherapy (%)	68 (59)	22 (65)	46 (57)
Donor (%)			
Related	55 (48)	12 (35)	43 (53)
Unrelated	60 (52)	22 (65)	38 (47)
HLA matching (%)			
HLA match (6/6)	101 (88)	31 (91)	70 (86)
HLA mismatch (5/6)	14 (12)	3 (9)	11 (14)
Source of stem cells (%)			
Peripheral blood	52 (45)	11 (32)	41 (51)
BM	63 (55)	23 (68)	40 (49)
Sex mismatch (%)			
Female donor/Male recipient	36 (31)	13 (38)	23 (28)
Other combination	79 (69)	21 (62)	58 (72)
Follow-up duration for survivors, months, median (range)	40 (4–130)	40 (4–130)	47 (4–125)

(continued)

Table 1. (contiuned)

No. of patients	All $N = 115$	MAC $N = 34$	RIC $N = 81$
Conditioning regimen			
MAC (%)			
CY/TBI		15 (44)	
Bu/CY		19 (56)	
Reduced intensity conditioning			
Flu/Bu-based			65 (80)
2-CdA/Bu-based			16 (20)
TBI-containing			23 (28)
ATG-containing			26 (32)
GVHD prophylaxis (%)			
CSP			26 (32)
CSP+MTX		24 (71)	37 (46)
TAC			2 (2)
TAC+MTX		10 (29)	16 (20)

MAC, myeloablative conditioning; RIC, reduced intensity conditioning; HCT, allogeneic hematopoietic cell transplantation; FAB, French-American-British; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; CMMoL, chronic myelomonocytic leukemia; RAEB-T, refractory anemia with excess blasts in transformation; AML-MLD, acute myeloid leukemia with multilineage dysplasia; BM, bone marrow; mons, months; CY, cyclophosphamide; TBI, total body irradiation; Bu, busulfan; ATG, antithymocyte globulin; Flu, fludarabine; 2-CdA, cladribine; CSP, cyclosporine; MTX, methotrexate; TAC, tacrolimus; GVHD, graft-versus-host disease; IPSS, International Prognostic Scoring System; MDS, myelodysplastic syndrome.

with excess blasts (RAEB)/chronic myelomonocytic leukemia (CMMoL), and refractory anemia excess blasts in transformation (RAEB-T)/AML-MLD were 19%, 33%, and 48%, respectively. According to the cytogenetic risk at diagnosis, the proportions of patients with good/intermediate and poor risk were 65% and 35%, respectively. According to the IPSS risk at peak, the proportions of patients with Low/Intermediate-1 and Intermediate-2/High were 21% and 67%, respectively, and 12% of the patients did not have evaluable data. BM blast counts at allo-HCT were 4% or less in 52%, 5–19% in 33%, 20% or higher in 9%, and not evaluable in 6%. The RIC group was significantly older than the MAC group (median, 57 vs. 46 yrs, $P < 0.001$) and included more patients with poor cytogenetic risk (41% vs. 21%, $P = 0.03$).

Conditioning regimen and GVHD prophylaxis

The conditioning regimen and GVHD prophylaxis are shown in Table 1. The MAC group included either Bu/CY or TBI/CY, followed by a combination of methotrexate and tacrolimus or cyclosporine. The RIC group included Flu/Bu or 2-CdA/Bu, followed by either cyclosporine or tacrolimus alone or a combination of either of the calcineurin inhibitors and methotrexate.