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Title	Effects of KIR ligand incompatibility on clinical outcomes of umbilical cord blood transplantation without ATG for acute leukemia in complete remission
Author(s)	Tanaka, J.; Morishima, Y.; Takahashi, Y. et al.
Citation	Blood cancer journal, 3, e164 https://doi.org/10.1038/bcj.2013.62
Issue Date	2013-11
Doc URL	https://hdl.handle.net/2115/54115
Rights(URL)	https://creativecommons.org/licenses/by/3.0/
Type	journal article
File Information	Blood Cancer J_3_e164.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

OPEN

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

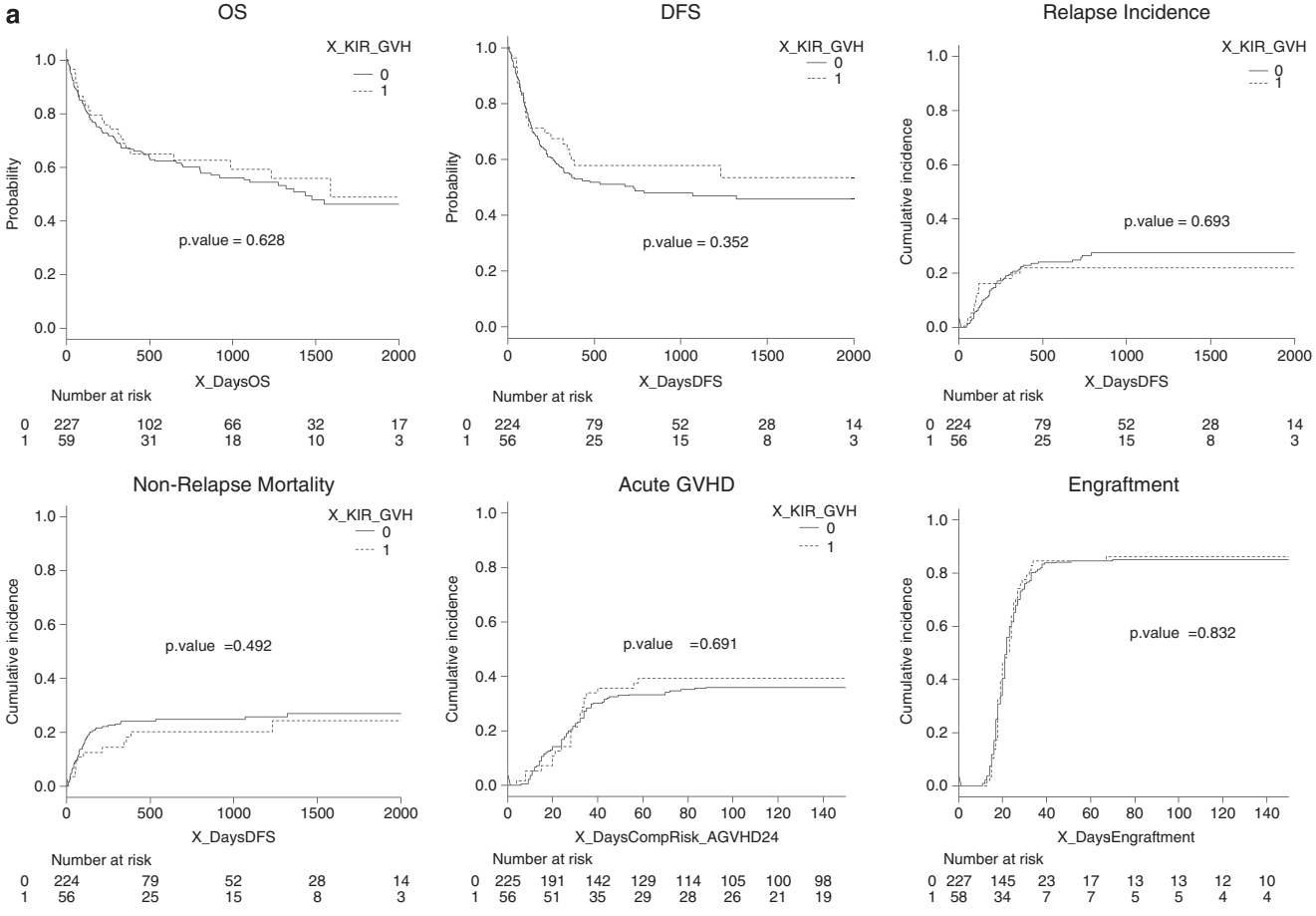


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transplantation (40 years or more, 16–39 years and <15 years), performance status before transplantation (2–4 and 0–1), year of transplantation (2006–2009 and 2001–2005), sex (female and male), disease status (CR2 and CR1), conditioning regimens (RIC 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)

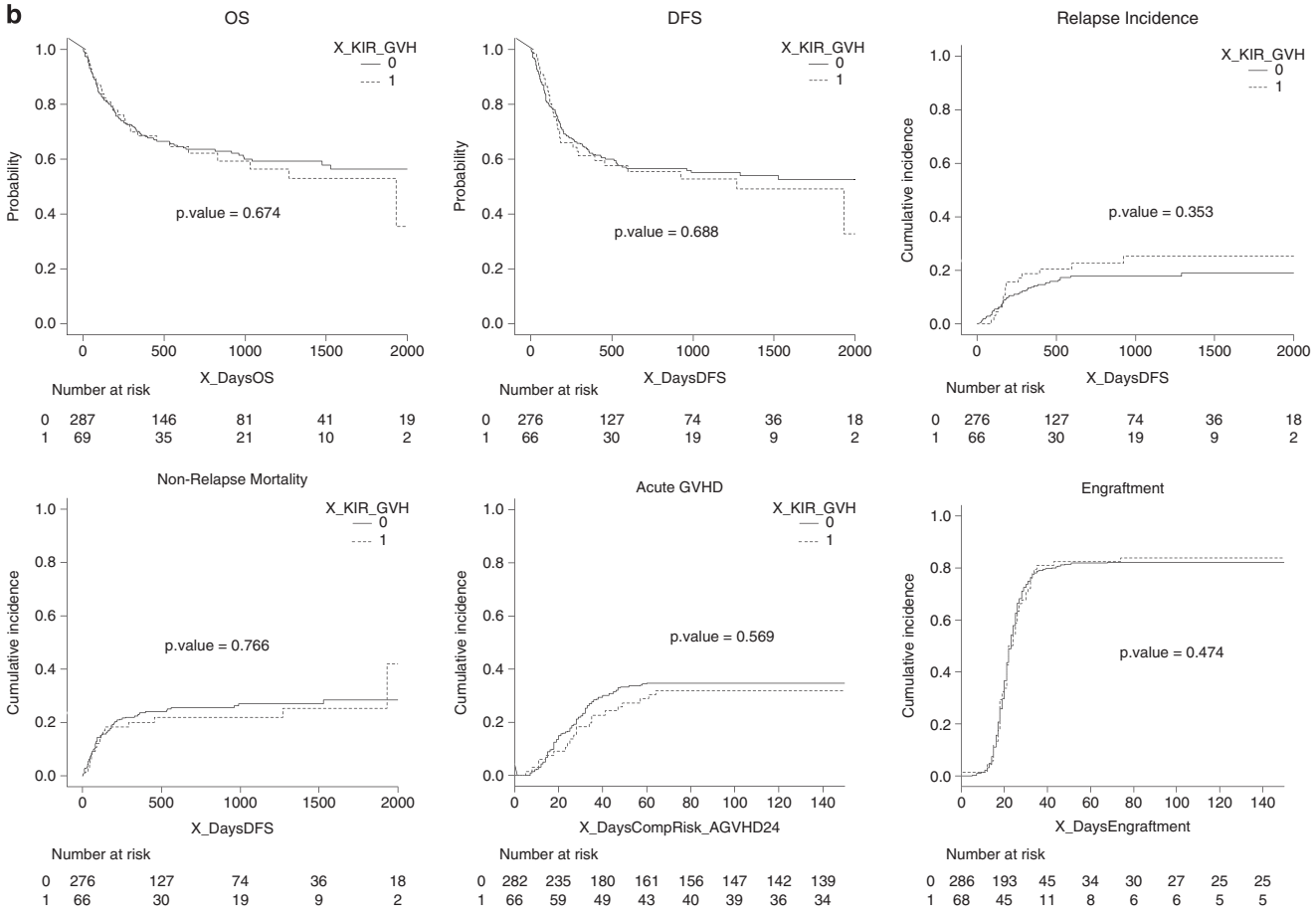


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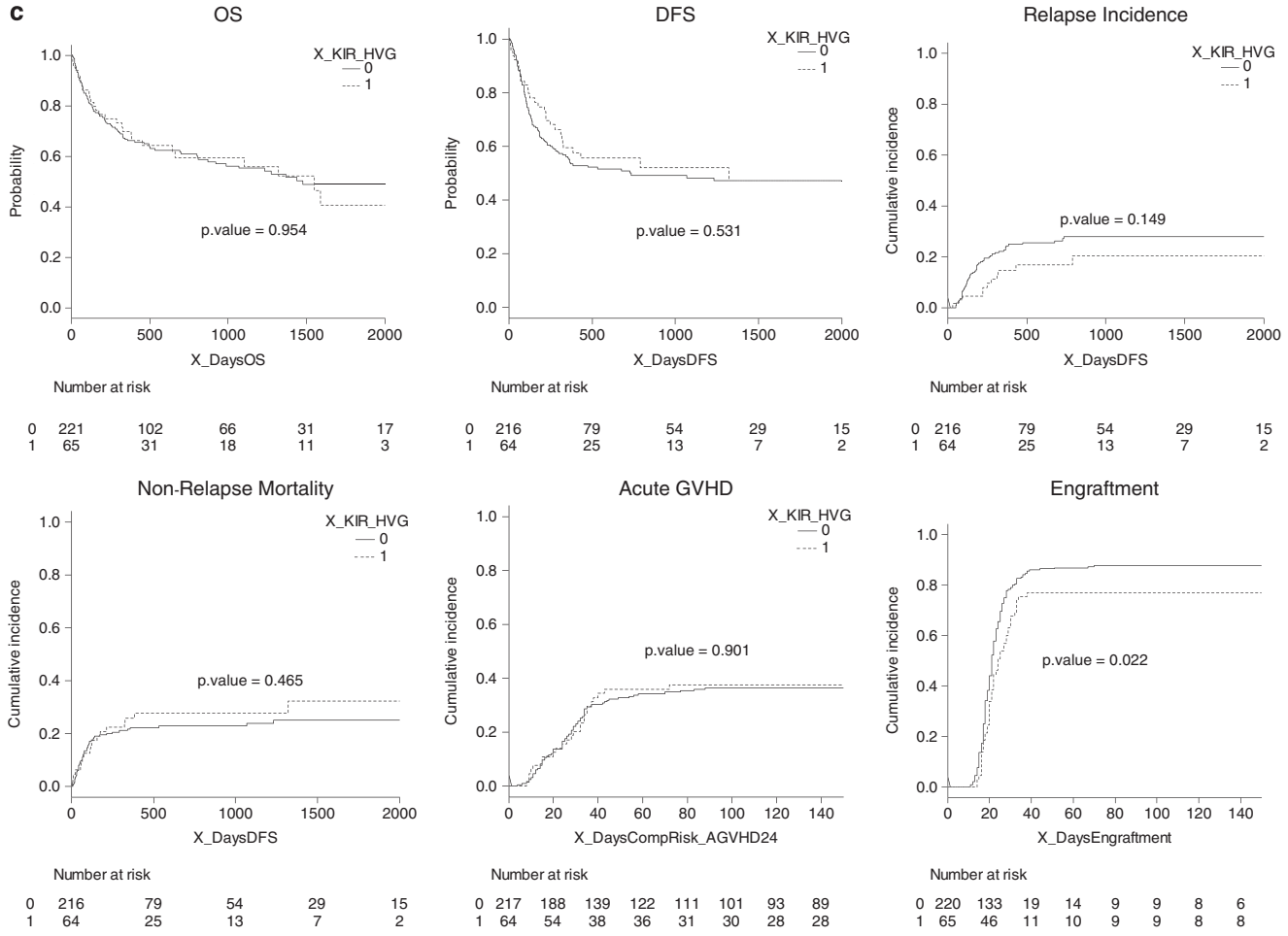


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and also with poor DFS (HR 2.41, $P=0.002$ and HR 1.67, $P=0.011$, respectively). Also, age >40 years was associated with higher NRM and lower engraftment rate (HR 6.96, $P<0.001$ and HR 0.55, $P<0.001$, respectively). For AML patients, age >40 years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

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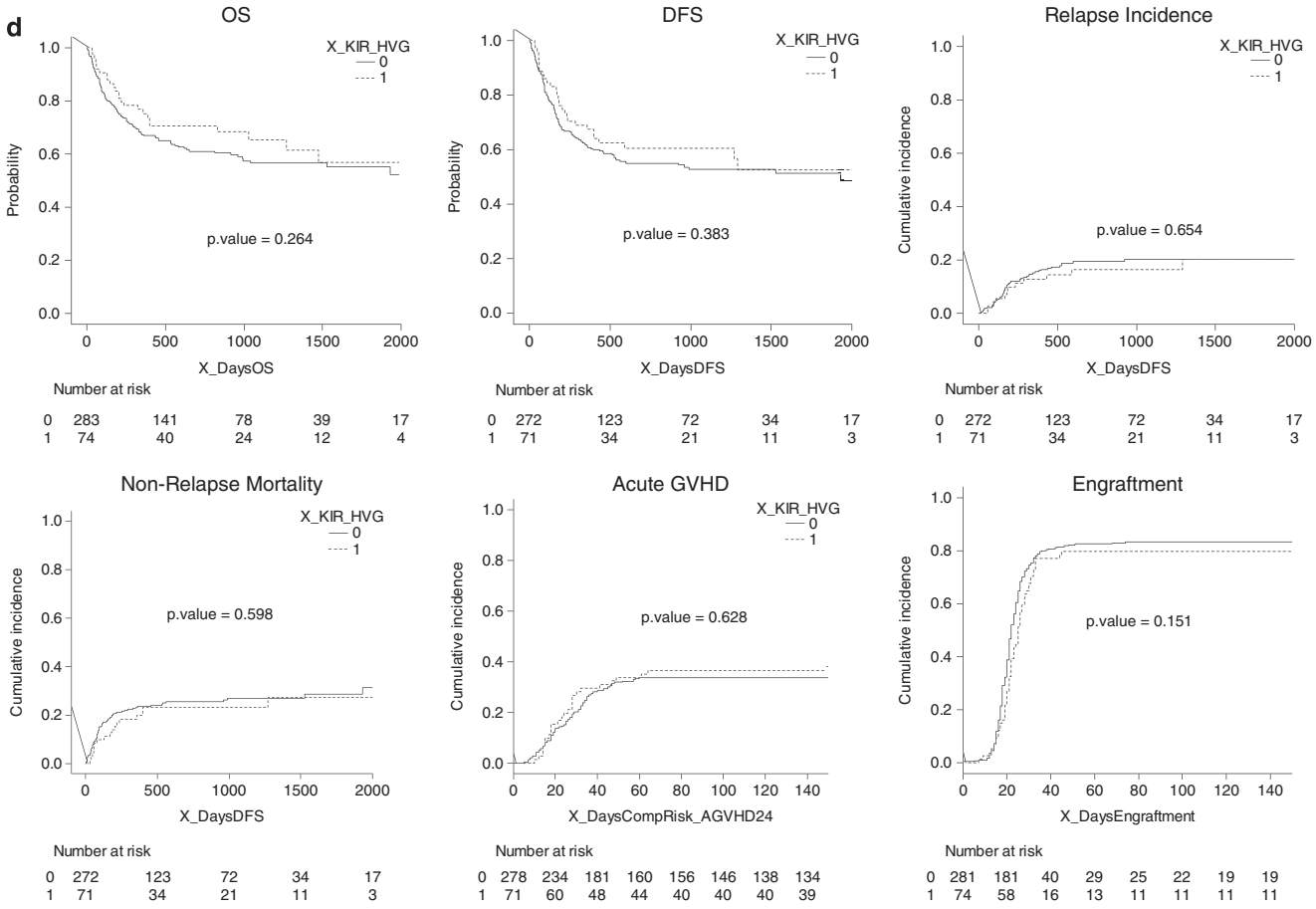


Figure 1. Kaplan–Meier curves forOS, DFS, CIR, NRM, acute GVHD and engraftment in (**a**) ALL and (**b**) AML patients transplanted from KIR-compatible and incompatible donors in the GVH direction and in (**c**) ALL and (**d**) AML patients transplanted from KIR-compatible and incompatible donors in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

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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.

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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 $>2.5\times10^7$/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

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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 $>2.5\times10^7$/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 $>2.5\times10^7$/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.

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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 $>2.5\times10^7$/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.

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