TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2018) 48: 794-804
© TÜBİTAK
doi:10.3906/sag-1712-75

The impact of donor and recipient KIR genes and KIR ligands on the occurrence of acute graft-versus-host disease and graft survival after HLA-identical sibling hematopoietic stem cell transplantation

Seyed Ahmad HOSEINIAN[1], Davood JAFARI[1], Mehdi MAHMOODI[2], Kamran ALIMOGHADDAM[3], Mohammadreza OSTADALI[3], Azita TALEBZADEH BONAKDAR[4], Arron Munggela FOMA[4,5], Mir Saeed YEKANINEJAD[6], Ali Akbar AMIRZARGAR[1,4,*]

[1]Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2]Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran
[3]Hematology, Oncology, and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran
[4]Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran
[5]Department of Immunology, School of Medicine, Tehran University of Medical Sciences-International Campus, Tehran, Iran
[6]Department of Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Received: 12.12.2017 • **Accepted/Published Online:** 08.07.2018 • **Final Version:** 16.08.2018

Background/aim: After allogeneic hematopoietic stem cell transplantation (allo-HSCT), donor natural killer (NK) cells trigger alloreactions against potential recipient cells by their killer immunoglobulin-like receptors (KIRs). This study investigated whether KIR/HLA genotypes and KIR haplotypes of donors and recipients exhibit a critical function in the prevalence of acute graft-versus-host disease (aGVHD) and persistence of the graft after HLA-identical sibling allo-HSCT for patients with hematological malignancies.

Materials and methods: We studied KIR and HLA genotypes in 115 related donors and recipients (56 patients with AML and 59 patients with ALL) who had received allo-HSCT from HLA-matched sibling donors. We evaluated 17 KIR genes and some alleles, including their ligands, using the PCR-SSP assay.

Results: KIR gene frequency results between donors and recipients showed that donors had more activating KIR than their recipients. Chi-square comparison of KIR genotype frequencies in donors versus recipients revealed a significant difference ($P < 0.001$). We found a survival association between the donor lacking and the recipient having group B KIR haplotypes, although this was not statistically significant.

Conclusion: This study suggests that we could exploit NK cell alloreactivity as a part of the optimization of donor selection and potential immunotherapeutic regimens to help facilitate good engraftment and reduce the risk of aGVHD incidence after allo-HSCT.

Key words: KIR, HLA, allogeneic hematopoietic stem cell transplantation, graft-versus-host disease

1. Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the definitive choice for treatment of high-risk hematologic malignancies, even in advanced stages (1,2). There is evidence that allogeneic HLA-matched transplantation for patients with lymphoblastic and myeloblastic leukemia is more effective than autogeneic transplantation, because allo-HSCT may induce the graft-versus-leukemia (GVL) effect (3,4). The potential benefit of the GVL effect relies on the elimination of residual malignant cells through immunological antitumor effects, induced by alloreactive T and natural killer (NK) cells, which leads to a lower relapse rate in allo-HSCT patients with hematologic malignancies (3,5,6). Despite all the advantages of allo-HSCT, there are still some complications that limit the success of this important procedure, including the development of graft-versus-host disease (GVHD) (7). Alloreactive cells in the graft are widely considered to mediate both the GVL effect and GVHD (8).

Acute GVHD, a major source of morbidity in HSCT, is mediated by donor allogeneic cytotoxic T cells against host tissues and leads to the recruitment of other effector cells including NK cells (9,10). The pathophysiology of acute GVHD has been described as a three-phase phenomenon: activation of donor-derived T cells, effector phase through

* Correspondence: amirzara@sina.tums.ac.ir

inflammatory immune cells, and host tissue damages mediated by inflammation (11,12).

There are ongoing efforts to identify new immunological targets to minimize severe GVHD and provide the GVL effect and rebuild immunity to infections (6). In allo-HSCT, NK cells have been recognized as a novel factor in the effector system that can overcome natural alloreactive T-cell barriers (6). Alloreactive donor NK cells facilitate engraftment by ablating different cells including residual leukemic cells, APCs, and T cells responsible for triggering GVHD (4,5,13–15).

NK cells are crucial components of the innate immune system and play an important role in early immune protection against virus-infected cells and transformed cells or allogeneic cells, without the need for prior sensitization (16–18). The effector function of NK cells depends on a balance of activating and inhibitory signals that are transmitted via clonally distributed cell receptors, including killer immunoglobulin-like receptors (KIRs) for major histocompatibility complex (MHC) class I molecules on target cells (6,18,19).

In humans, KIR genes are located on chromosome 19q13.4 and to date 15 functional genes have been discovered. This group of tightly clustered genes contains 8 inhibitory KIR (iKIR) genes (KIR2DL1-3, 2DL5A/B, 3DL1-3), 6 activating KIR (aKIR) genes (KIR2DS1-5, 3DS1), and an unusual KIR named KIR2DL4 (encodes receptor with both inhibitory and activating functions). Furthermore, there are 2 pseudogenes (KIR3DP1 and 2DP1) that do not encode any receptor proteins (20,21). A great alternation of KIR gene frequency exists among different peoples and populations (22,23).

To date, a limited number of HLA ligands have been described for some KIR genes (24). Specificity towards HLA class I ligands has been demonstrated for some iKIR receptors. KIR2DL1 interacts with HLA-Cw group 2 and KIR2DL2/2DL3 with HLA-Cw group 1 epitopes distinguished by the amino acid lysine or asparagine at position 80, respectively. Inhibitory receptor KIR3DL1 recognizes the HLA-Bw4 and KIR3DL2 HLA-A3/A11 epitopes (25).

Like T cells, NK cells distinguish self from nonself and play distinct roles during alloreactivity. In vitro studies showed that a wide variety of hematological malignancies are susceptible to alloreactive NK cytotoxicity (26). Functional donor NK cells are thought to play a crucial role in the outcome of allo-HSCT, such as incidence of GVHD, relapse, and graft rejection, based on the 'missing self' and 'missing ligand' hypothesis (3,5,26). Recent data have demonstrated that signaling from the KIR receptors is important in determining the NK cell activation in graft outcome of allo-HSCT, particularly for leukemia patients (9,25). Although our understanding of the role of KIRs in HSCT has recently improved considerably, conflicting data still persist. In order to clarify the impact of KIR and HLA genotypes on the HLA-identical sibling HSCT outcome, patient and donor KIR gene and KIR ligand polymorphisms were genotyped in 115 HLA-identical donor and recipient pairs and correlated with clinical data in simple and multiple models.

2. Materials and methods

2.1. Patient characteristics

An aggregate of 230 donors and recipients of HSCT (115 donor/recipient pairs) was included in this study between March 2014 and February 2016 at the Bone Marrow Transplant Center, Shariati Hospital, Tehran. All 115 patients received HLA identical sibling donor allogeneic bone marrow transplants for acute myelogenous leukemia (AML) (n = 56) or acute lymphoblastic leukemia (ALL) (n = 59). For all BMT recipients who were engrafted, a minimal 90-day follow-up period after transplantation was considered for risk of developing acute GVHD. Criteria for standard grading and staging of acute GVHD were previously reported (27). These 115 transplant pairs were selected in a consecutive manner for patients with AML and ALL who showed aGVHD after HSCT. Patients received hematogenic vegetative cells from bone marrow and peripheral blood. Graft failure was categorized based on a decline in the absolute neutrophil count below 0.2 $\times$ 10^9/L for three consecutive days after HSCT. Clinical characteristics of all patients and donors of BMT are indicated in the Table. Sanitization of $CD34^+$ stem cell grafts was performed with the CliniMACS system (Miltenyi Biotec, Auburn, CA, USA).

Peripheral blood samples from patients and donors were obtained from HSCT pairs and gDNA was harnessed using the phenol-chloroform procedure. All patients provided written informed consent. The ethics and research committee of Tehran University of Medical Sciences approved the study.

2.2. Conditioning regimen and posttransplantation immunosuppression

All patients received intravenous adjusted-dose cyclosporine A and methotrexate (MTX) as a GVHD prophylaxis regimen after HSCT. The conditioning regimen was myeloablative (MA) for HLA-matched sibling transplantations and was determined by chemotherapy, including busulfan and cyclophosphamide with or without antithymocyte globulin (ATG). In 103 cases, oral busulfan and cyclophosphamide were administered as an adjusted conditioning regimen, while for 6 patients, the conditioning regimen was busulfan (i.v. adjusted dose) and cyclophosphamide (adjusted dose). Six patients received intravenous antithymocyte globulin (ATG) plus busulfan (oral adjusted dose) and cyclophosphamide (adjusted dose) (Table). No patient received total body irradiation.

Table. Patient and donor clinical characteristics of donors and recipients of HSCT.

			AML (n = 56)	ALL (n = 59)
Recipient/donor sex no. (%)	M/M		14 (25)	21 (35.6)
	M/F		6 (10.7)	20 (33.9)
	F/M		26 (46.4)	6 (10.15)
	F/F		10 (17.85)	12 (20.3)
Age, mean (range)	Patient		31 (8–63)	21 (7–36)
	Donor		34 (4–61)	27 (8–51)
Graft source no. (%)	BM		0	3 (5.1)
	PBMC		56 (100)	56 (94.9)
Dose of cell infusion (mean)/kg	WBC		14.2	10.4
	MNC × 10^8		8.3	7.9
	CD3 × 10^6		263.3	257
	CD34 × 10^6		4.4	4.6
Grade of aGVHD no. (%)	I		7 (12.5)	4 (6.8)
	II		12 (21.4)	8 (13.6)
	III		14 (25)	10 (16.9)
	IV		0 (0)	2 (3.4)
	Total		33 (58.9)	24 (40.7)
Relapse occurrence			8 (14.3)	8 (13.6)
Conditioning regimen	ATG Bu (30) cyclophosphamide		4 (7.1)	2 (3.4)
	Non-ATG	Bu (30) cyclophosphamide	52 (92.9)	51 (86.4)
		Cyclophosphamide Bu (28)	0 (0)	6 (10.2)
Posttransplant immunosuppressive therapy			MTX-Cyclosporine	MTX-Cyclosporine

ALL, Acute lymphocytic leukemia; AML, acute myelogenous leukemia; F, female; M, male; CMV, cytomegalovirus; MTX, methotrexate; MNC, mononuclear cells.

2.3. HLA and KIR genotyping

All samples were HLA typed (HLA-A, -B, and DRB1) using a low-resolution molecular typing assay. All sibling patients and donors were typed for the existence or lack of 17 KIR genes that included 8 inhibitory (KIR2DL1-3, 2DL5A/B, 3DL1-3), 6 activating (KIR2DS1-5, 3DS1), and KIR2DL4 and 2 pseudogenes (KIR2DP1 and KIR3DP1). In essence, the defined alleles of all KIR genes were considered in the analyses. Typing of HLA and KIR genes was performed by employing the polymerase chain reaction-sequence-specific primer (PCR-SSP) assay. The amplification of KIR genes was performed with an Applied Biosystems thermal cycler (Foster City, CA, USA) using the Kit PCR-SSP (Olerup SSP-KIR Genotyping, Sweden), under conditions described by the manufacturer. The KIR ligands including the HLA-Bw4 and HLA-Cw groups (HLA-C1 and C2) were analyzed in the groups. For typing ligands (HLA-A, -B, -CW), a PCR-SSP kit (Olerup SSP KIR HLA Ligand SSP Typing, Sweden) was used. The amplicons were electrophoresed in a 2% agarose gel and viewed with a UV transilluminator.

2.4. Identification of group A and B haplotypes

To determine haplotypic structures, all samples were considered following the method of Rajalingam et al. (28). Based on the gene content, the KIR genotypes were classified into A and B haplotypes: the A haplotype mostly consisted of iKIR genes (KIR3DL3, 2DL1, 2DL3, 2DL4, 3DL1, and 3DL2) and also one aKIR gene (KIR2DS4). The B haplotype was more variable, comprising the fixed gene content of both stimulating and suppressor KIR genes (KIR2DL5, 2DL2, 2DS1, 2DS2, 2DS3, and 2DS5) (29). The inheritance of distinct paternal and maternal haplotypes (A+A, A+B, or B+B) generated different KIR genotypes, including dominant inhibitory (AA genotype), dominant activating (BB genotype), or balanced (AB genotype). The KIR genes can be separated into centromeric and telomeric

gene regions, with each containing 4 genes. While the centromeric half has KIR2DS2–2DL2–2DL5B–2DS3 genes, the telomeric half mainly consists of KIR3DS1–2DL5A–2DS5–2DS1 genes (20). The associations of KIR haplotypes with transplantation outcome were analyzed by distinguishing between AA or Bx haplotypes, where x stands for either A or B haplotype.

2.5. Statistical analysis

The relationships between genes and aGVHD and rejection were calculated using the chi-square test. The Kaplan–Meier algorithm was used to estimate the probability of survival (overall survival, OS) and defined events, while log-rank statistics provided the difference between the survival curves of the groups. The reverted time between the time of death and HSCT and the last recorded contact criteria was used for defining OS in this study. The association of clinical (transplant characteristics; Table) and genetic variables with each outcome was evaluated using multivariate survival analysis in a Cox regression model. $P < 0.05$ and the 95% confidence interval (95% CI) were the estimated margins for statistical significance in each analysis. All analyses were performed with SPSS 21.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Patient and graft characteristics

Clinical characteristics of all HLA matched recipients and donors of HSCT are provided in the Table. The results of this study showed that 49.5% of patients had aGVHD and the rate of aGVHD in the AML and ALL groups was 58.9% and 40.7%, respectively. The incidence of grades III and IV patients with acute GVHD (highest incidence rate of grades) was 17.4% and 20.8%. In terms of grade, relapse occurrence, and graft source, there was no detectable difference between the groups. The conditioning treatments for ALL and AML patients were almost the same. Considering ATG treatment, we had 4 cases among AML and 2 cases among ALL patients.

3.2. Frequencies of individual donor/recipient KIR genes

One method for determining donor NK alloreactivity involves using gene–gene comparisons for KIR. Donors exhibited more activating KIR than their recipients in a comparative analysis involving individual inhibitory and activating KIR gene frequencies between the two groups. Hence, a significant difference was noticed in the frequency of KIR2DS2 (71.3% in donors and 54.8% of recipients, $P = 0.002$), 2DS3 (53.9% in donors and 39.1% of recipients, $P = 0.005$), 2DS5 (64.3% in donors and 40% of recipients, $P = 0.001$), and 2DL2 (67.8% in donors and 53% of recipients, $P = 0.005$) (Figure 1). The analysis was narrowed to subgroups of patients, for myeloid versus lymphoid malignancies. When KIR gene frequencies in donors versus recipients were compared in patients, it was found that the frequencies of KIR2DS2, 2DS3, 2DS5, and one (KIR2DL2) in mediating inhibitory functions were statistically significant in patients with AML and ALL (data not shown). No statistical difference was observed in the number for the remaining activating and inhibitory KIR genes in donors and recipients.

3.3. KIR genotype mismatch and donor NK alloreactivity forecast

Discrete paternal and maternal haplotype inheritance produces varying KIR genotypes (A+A, A+B, or B+B), an aspect accounted for in the content of each donor and recipient KIR gene. Genotype frequencies of AA among the donors and recipients were 12.2% and 25.2%, while genotype frequencies of Bx between donors and recipients were 87.8% and 74.8%, respectively. Comparison of KIR genotype frequencies in donors versus recipients revealed a significant difference ($P < 0.001$).

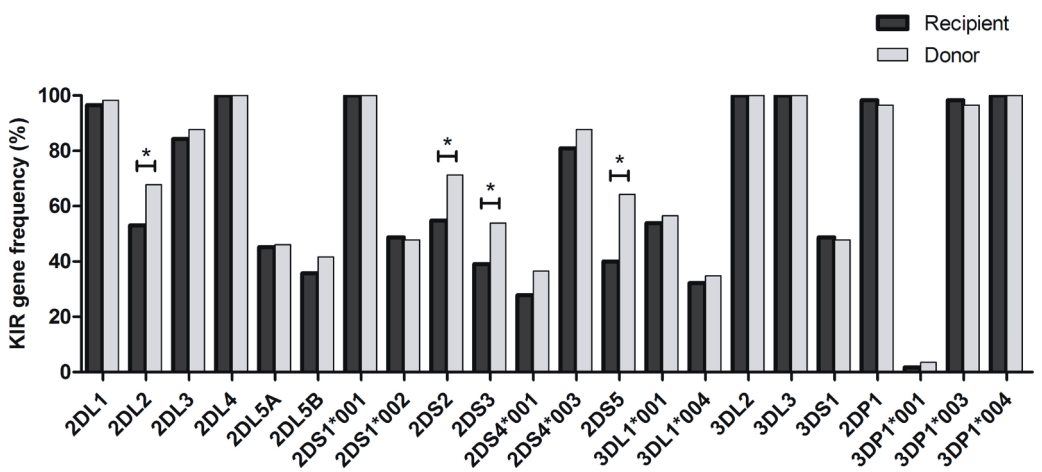

Figure 1. The frequency of KIR genes in donors and recipients of HSCT.

The distribution of each transplant to one of the four groups depending on the donor and the recipient KIR genotype combination was based on the donor and recipient AA or Bx genotype. Based on the four classifications in Figure 2, we explored the hypothesis that differences in outcome correlate with donor–recipient KIR genotype combination. Analysis of the overall survival showed no significant difference in a total comparison of all 115 patients (P = 0.350) by log-rank test (Figure 3). The presence of group B KIR haplotypes in the recipients and their absence in the donors had an association with utmost survival rate. In other words, best survival was achieved by Bx patients receiving an AA graft. By using Cox proportional hazard modeling, the grades of GVHD after transplantation (HR = 5.5, P = 0.043) and ATG conditioning regimen (HR = 7.7, P = 0.019) turned out to be prognostic factors affecting transplant survival. None of the other analyzed potential factors like recipient and donor characteristics, including the transplantation method, had any impact on transplant survival.

Of the 115 patients who underwent transplantation for AML and ALL, the incidence of aGVHD in the four KIR genotype combinations was 42.1% (9/19) for Bx-donor/AA-recipient, 30% (3/10) for AA-donor/AA-recipient, 75% (3/4) for AA donor/Bx-recipient, and 52.4% (43/82) for Bx-donor/Bx recipient. We observed that the combination of Bx recipients given an AA graft was a susceptible factor for occurrence of aGVHD after transplantation for myeloid leukemia, but the study failed to reach a statistically significant level. Figure 4 provides

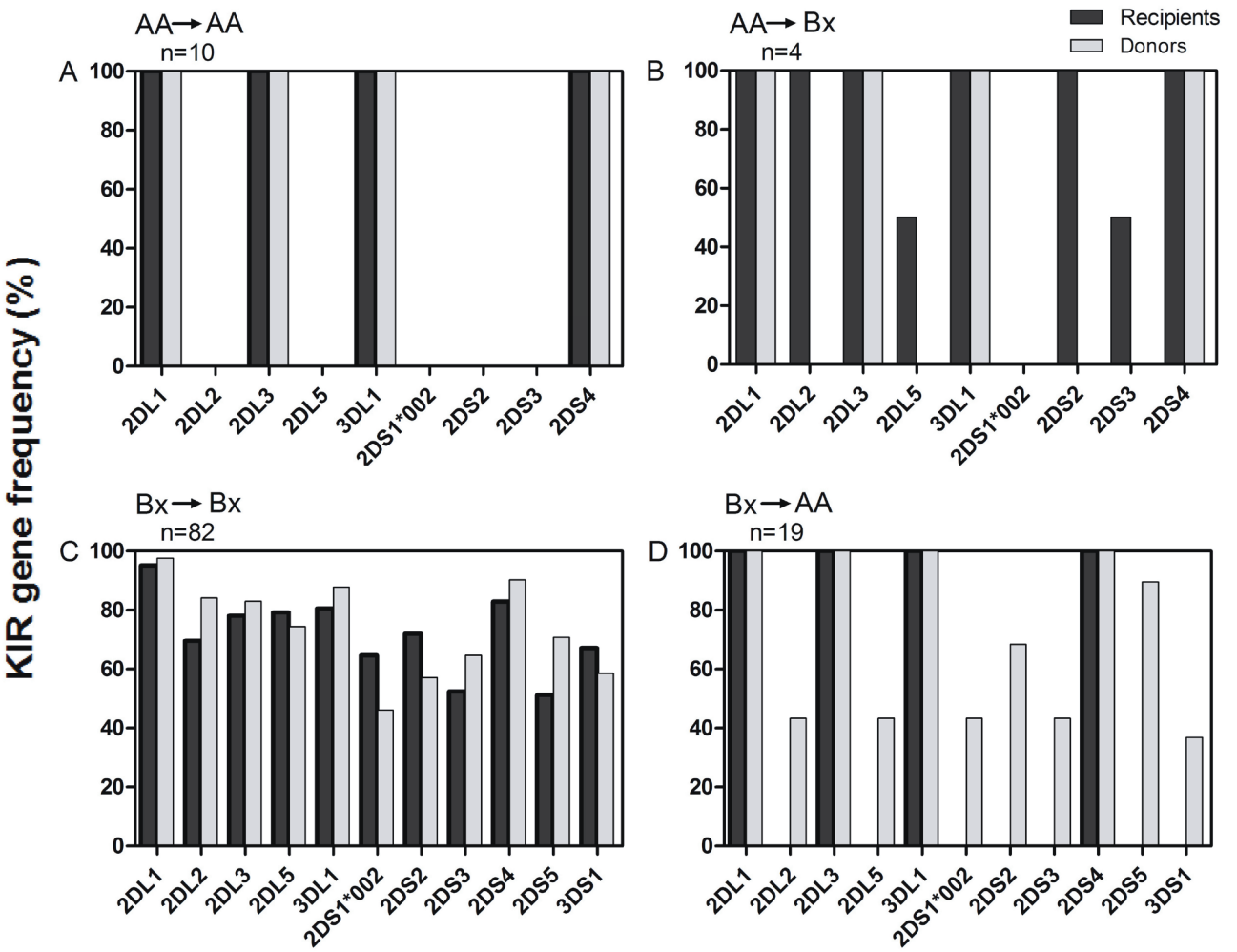

Figure 2. Frequency distribution of KIR genes of patients with hematologic malignancies based on the donor and recipient KIR genotypes. A) Donor-AA/recipient-AA: donors and patients have identical KIR gene content and KIR gene frequencies. B) Donor-AA/recipient-Bx: recipients have more activating KIR than their donors. C) Donor-Bx/recipient-Bx: donors and recipients have almost the same KIR gene frequencies. D) Donor-Bx/recipient-AA: donors have more activating KIR than their recipients. KIR2DL4, -3DL2, and -3DL3 were not typed for in this panel as these genes are present in all individuals.

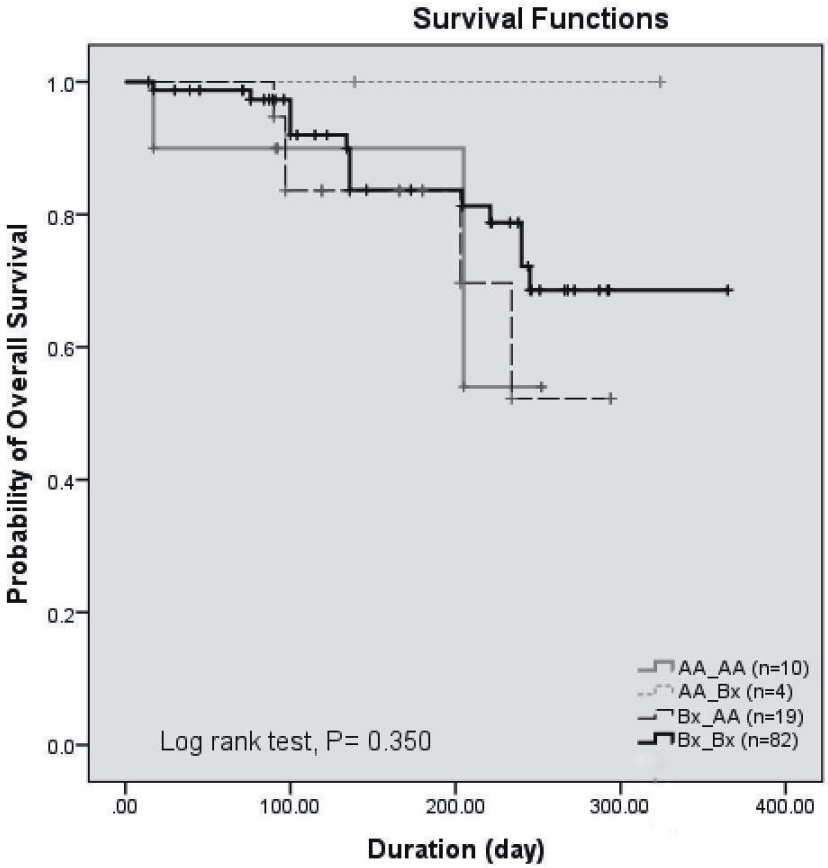

Figure 3. Kaplan–Meier graft survival curve. The figure summarizes the comparison of graft survival for KIR genotypes. Kaplan–Meier analysis of overall survival for patients with the donor-recipient KIR genotype combinations including donor-AA/recipient-AA, donor-AA/recipient-Bx, donor-Bx/recipient-AA, and donor-Bx/recipient-Bx.

a comparison of hazard modeling of the different KIR genotype combinations of the 115 patients who underwent transplantation for leukemia.

3.4. Combinatorial analysis and its effect on posttransplantation outcome

Combinatorial analysis is a combination of recipient HLA class I genotype with donor-recipient KIR genotypes. Due to the HLA-C type's significance in clinical associations, and also its consideration as major ligands for KIR, including beneficial NK-cell alloreactions after haploidentical transplantation, HLA-Cw was assessed in 115 donor/recipient pairs. Frequencies of C1, C2, and C1C2 in the donor/recipient pairs were compared, as shown in Figure 5. However, the analyses of HLA-Cw frequencies failed to show a statistically significant association.

The ligand incompatibility model was used to predict NK alloreactive donors. All transplants were divided into two groups, based on whether the recipients and their HLA-identical donors had C2. The reason for this division was the difference between the C1 and C2 ligands and their cognate inhibitory KIR, which was such that C2 produced more robust interactions than C1. Recipients lacking C2 were assigned as C1 homozygotes (C1C1), as such recipients having C2 included both C2C2 homozygotes and C2Cx heterozygotes (where x stands for either C1 or C2).

The combination of HLA-C with either the donor or the recipient KIR genotypes when considered showed that there was no significant relationship between genotype ligands and aGVHD, although mean survival of recipient-AA/C1C1 was lower compared with recipient-AA/C2Cx. Thus, the combination showed increased aGVHD when the recipient was C1C1. The highest survival rate was observed for the combination of AA donor, AA recipient, and C2Cx (Figure 6).

Recipient HLA-B epitopes and the association with donor-recipient KIR genes was analyzed, since the Bw4 epitope is a robust ligand for KIR3DL1. Patients with the four donor-recipient KIR genotype combinations were divided according to HLA-B type. No differences were observed in the occurrence of aGVHD irrespective

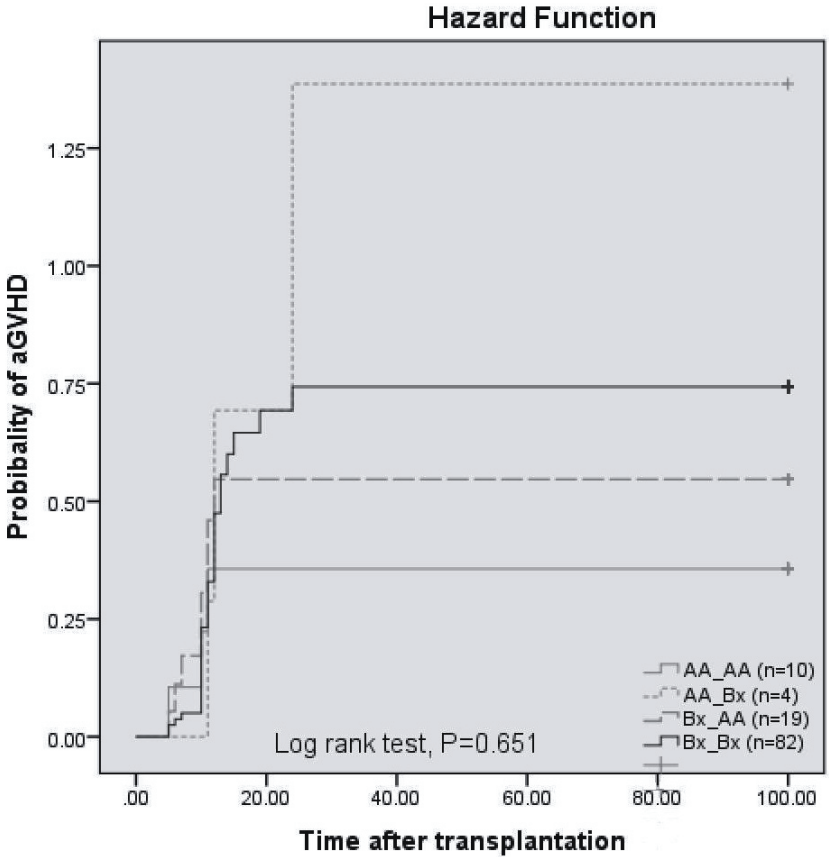

Figure 4. Kaplan–Meier analysis of acute graft-versus-host disease (aGVHD) in AML and ALL patients (n = 115) with different KIR genotype combinations in hazard modeling approach. Comparison of aGVHD between donor-AA/recipient-AA patients, donor-AA/recipient-Bx, donor-Bx recipient-AA, and donor-Bx/recipient-Bx.

of whether Bw4 was present or absent in different KIR genotype combinations.

4. Discussion

NK cells are thought to contribute to enhanced GVL effect and improved survival after allo-HSCT in a variety of conditions. The effect is associated with NK cell alloreactivity in the context of transplantation and depends on donor–recipient incompatibility regarding NK cell receptors and their ligands (3,30,31). Variation in gene number and sequence polymorphism influence interindividual variability in the KIR gene family (22). The difference in the genes and alleles may raise the question of whether the genetic content influences susceptibility and resistance in the occurrence of aGVHD. The present study was undertaken to investigate whether KIR and HLA genotypes play a crucial role in the occurrence of aGVHD and graft survival after HLA-identical sibling allo-HSCT.

A variety of immune-related diseases have been associated with specific KIR genotypes (22). We hypothesized that the number of activating KIR genes may have an improvement effect on survival after allo-HSCT. The comparison of KIR gene frequencies between donors and recipients showed that donors have more activating KIR than their recipients, as reflected in the significant differences observed in the frequency of KIR2DS2, 2DS3, and 2DS5. Genotypes with a greater number of aKIR genes and a lower number of iKIR genes have been shown to be associated with autoimmune diseases (22). In the case of allo-HSCT, a study revealed that the presence of activating KIR in the donor graft promoted immune sensibility; meanwhile, the presence of repressive KIR in the recipient promoted immune tolerance (32). Additionally, the existence of more favorable activating KIRs as opposed to their absence was the focal point of qualifying potential donors based on the KIR repertoire (8). Despite the establishment that outcome was impacted by KIRs in a majority of cases, contradictory results prevailed. The enhancement or depreciation of survival ensued from donor/recipient incompatibilities and existence of activating KIRs (30).

When the frequencies of KIR genes of all 115 patients were compared based on the four KIR genotype sequences, analysis of the overall survival revealed no significant

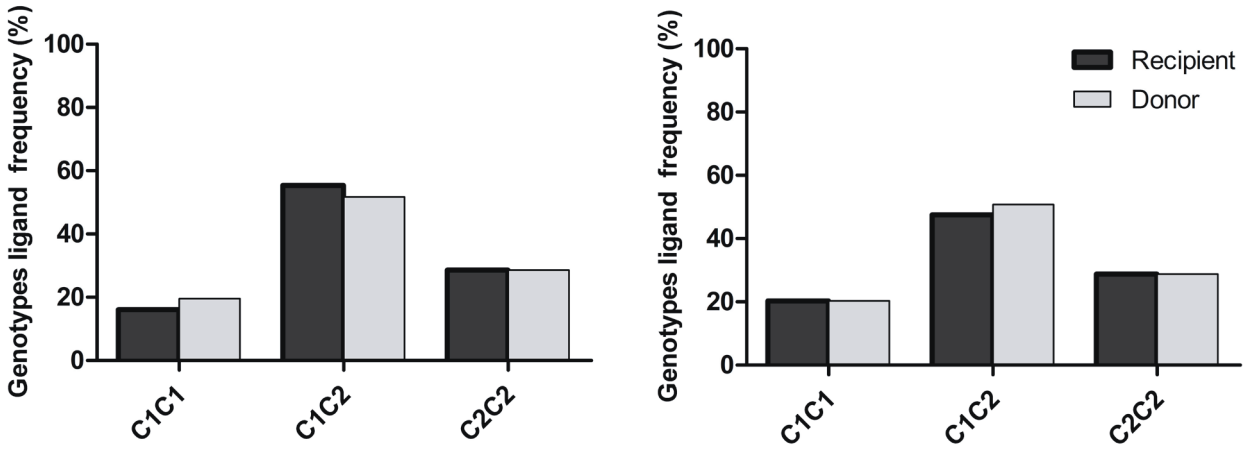

Figure 5. Genotype ligand frequencies between AML and ALL disease donor and recipients.

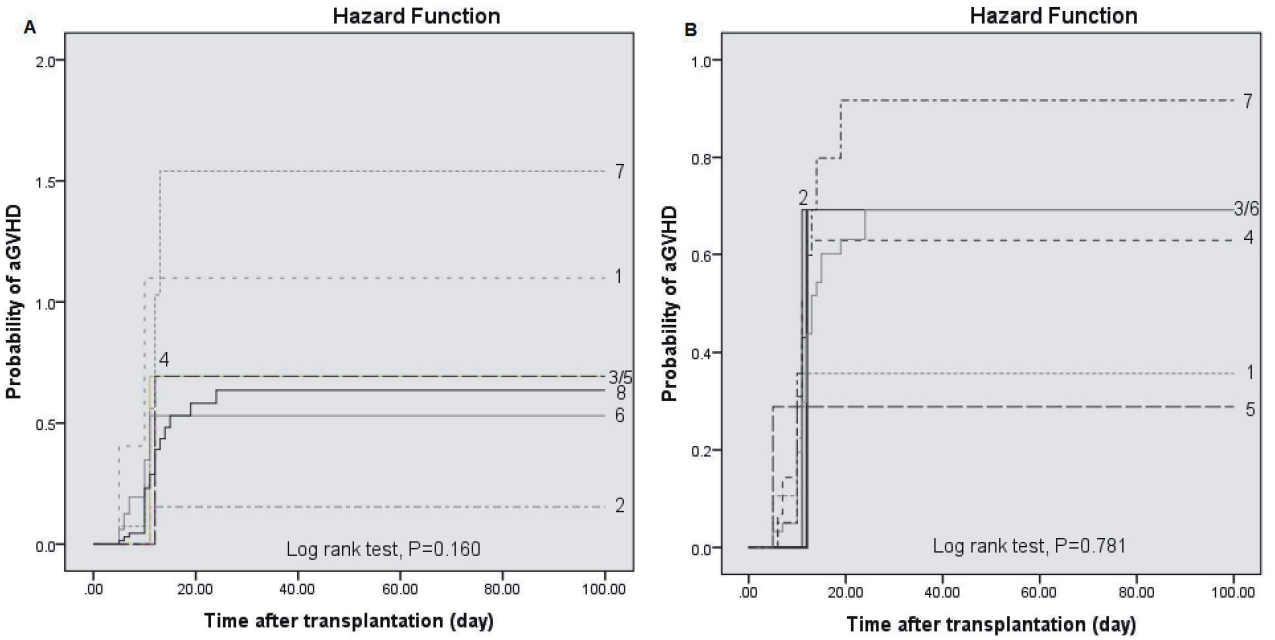

Figure 6. Influence of HLA-C and HLA-B genotype on acute graft-versus-host disease. A) Kaplan–Meier analysis of aGVHD in patients who have been grouped according to donor-recipient KIR and recipient HLA-C genotype combination: 1) donor-AA/recipient-AA: recipient C1; 2) donor-AA/recipient-AA: recipient C2; 3) donor-AA/recipient-Bx: recipient C1; 4) donor-AA/recipient-AA: recipient C2; 5) donor-Bx/recipient-AA: recipient C1; 6) donor-Bx/recipient-AA: recipient C2; 7) donor-Bx/recipient-Bx: recipient C1; 8) donor-Bx/recipient-Bx: recipient C2. B) Kaplan–Meier analysis of aGVHD in patients who have been grouped according to donor-recipient KIR and recipient HLA-B genotype combination: 1) donor-AA/recipient-AA: recipient BW4+; 2) donor-AA/recipient-Bx: recipient BW4+; 3) donor-AA/recipient-Bx: recipient BW4-; 4) donor-Bx/recipient-AA: recipient BW4+; 5) donor-Bx/recipient-AA: recipient BW4-; 6) donor-Bx/recipient-Bx: recipient BW4+; 7) donor-Bx/recipient-Bx: recipient BW4-.

difference, despite the observed inclination favoring best survival in Bx patients receiving an AA graft. The results showed that the combination of KIR haplotypes in the donor and recipient could have an influence on the outcome of HCT. However, a much bigger sample size is necessary to get a significant result. Differences in outcome that correlated with donor–recipient KIR genotype combination were assessed based on four classifications. Analysis of OS showed no significant association when all 115 patients were compared, although Karina et al. observed this effect in myeloid leukemia patients (32).

Giebel et al. reported that activating KIR receptor incompatibilities enhanced graft-versus-host disease and affected survival after allogeneic hematopoietic stem cell

transplantation. They established that a negative patient and a positive donor resulted in a higher risk of acute (KIR2DS1) and chronic (KIR2DS3) GVHD including relapse (KIR2DS5) (30).

We showed that KIR2DS3 and KIR2DS5 have statistically different frequencies between donor and recipient, but we found no significance for survival analysis based on GVHD and relapse (data not shown).

In the present study, using Cox proportional hazard modeling, we showed that grade of GVHD after the time of transplantation and ATG conditioning regimen were prognostic factors affecting transplant survival. Multivariate analysis using a Cox proportional hazard model from the findings of Yabe and Clausen suggested that ATG preadministration was a critical factor in grade III–IV aGVHD under consideration of the HLA-C KIR-L status (33,34).

Donor compatibility based on the combination of HLA and KIR data was extended to the HLA-B and HLA-C epitope groups. In this research, genotype ligands and aGVHD had no significant relationship, despite the observed higher recipient-AA/C2Cx mean survival as compared to the lower recipient-AA/C1C1. A significant relation was reported between increased risk of aGVHD and absent donor HLA-C2 ligand for iKIR2DL1, including AA KIR haplotypes in patients and donors in HLA-C1Cx (25). Another report established serious side reactions of KIR ligand mismatch consequences on T cell-replete unrelated HSCT in the graft-versus-host direction. Increased incidence of aGVHD was related to patient cognate C1 ligand and donor KIR2DS2 gene (33). Additionally, there is evidence of the association of donor KIR2DS1-2 positivity with a better overall outcome after HSCT in C1-negative patients with myeloid malignancies group (35).

Additionally, Gagne et al. reported that the transplantation of KIR3DL1^{+}/3DS1^{+} donor NK cells into HLA-Bw4^{-} patients without KIR3DL1/HLA-Bw4 interactions resulted in harmful outcomes (36).

In the context of unrelated cord blood transplantation, the absence of a C-ligand (C1 or C2) of inhibitory KIR in a patient with hematologic malignancies is associated with lower probability of relapse, which is almost certainly due to the GVL effect caused by NK cells of umbilical cord blood that lack a ligand for the inhibitory KIR 2DL1/2DL2/2DL3 (37).

An increase in prevalence of aGVHD in non-T-cell-depleted graft HLA-C1C1 patients has also been shown (32). Low signal intensity during NK cell development produces low activation potential in HLA-C1C1 and HLA-Bw4^{-} patients. Hence, this suggests a possible connection between higher T-cell allogenic reaction and NK cells, even though the exact process is still unknown. Alternating opinions by other researchers have revealed either a condensed aGVHD (III–IV) in HLA-C1C1 CML patients or no influence of KIR ligands on aGVHD in AML patients (25).

Mismatch of KIR and KIR ligands has been shown to induce engraftment and a reduction in the outcome of aGVHD occurrence by alloreactive donor NK cells, which interrupts the contact between recipient APCs and pathogenic T cells, thus suppressing aGVHD prevalence (5). A severe increase in the T/B cell immune partitions, possibly caused by inhibition of NK cell activity, has also been proposed (22).

To summarize, intentional mismatch of KIR and KIR ligands between donors and patients by clinicians in order to utilize NK cell characteristics has led to a reduced GVHD occurrence and higher patient survival rate after HSCT. Even though NK cell activation might intensify with an increase in diverse activating KIR receptor signals, it appears that activating KIR in the donor graft induces higher aGVHD outcomes, which are conflicting and incomprehensible. A probable explanation could be that donor-derived NK cells expressing activated KIRs induce graft-to-host alloreactivity, thereby inhibiting restoration of the immune system function. Our study results suggest that NK alloreactivity could be considered as a rational strategy in donor recipient allocation in order to improve the outcome of HSCT, by potentiating the GVL effect and reducing the aGVHD, although elucidation of the underlying pathogenic mechanisms requires further investigation.

Acknowledgment

This study (as an MSc thesis) was financially supported by Tehran University of Medical Sciences, Research Affairs, Grant Number 13302, Tehran, Iran.

References

1. Shlomchik WD. Graft-versus-host disease. Nat Rev Immunol 2007; 7: 340-352.
2. Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. Blood 2008; 112: 4371-4383.
3. Bishara A, De Santis D, Witt C, Brautbar C, Christiansen F, Or R, Nagler A, Slavin S. The beneficial role of inhibitory KIR genes of HLA class I NK epitopes in haploidentically mismatched stem cell allografts may be masked by residual donor-alloreactive T cells causing GVHD. Tissue Antigens 2004; 63: 204-211.

4. Willemze R, Ruggeri A, Purtill D, Rodrigues CA, Gluckman E, Rocha V. Is there an impact of killer cell immunoglobulin-like receptors and KIR-ligand incompatibilities on outcomes after unrelated cord blood stem cell transplantation? Best Pract Res Clin Haematol 2010; 23: 283-290.

5. Merindol N, Charrier E, Duval M, Soudeyns H. Complementary and contrasting roles of NK cells and T cells in pediatric umbilical cord blood transplantation. J Leukoc Biol 2011; 90: 49-60.

6. Ruggeri L, Aversa F, Martelli MF, Velardi A. Allogeneic hematopoietic transplantation and natural killer cell recognition of missing self. Immunol Rev 2006; 214: 202-218.

7. Gagne K, Brizard G, Gueglio B, Milpied N, Herry P, Bonneville F, Chéneau ML, Schleinitz N, Cesbron A, Folléa G. Relevance of KIR gene polymorphisms in bone marrow transplantation outcome. Hum Immunol 2002; 63: 271-280.

8. Schellekens J, Rozemuller EH, Petersen EJ, van den Tweel JG, Verdonck LF, Tilanus MG. Patients benefit from the addition of KIR repertoire data to the donor selection procedure for unrelated haematopoietic stem cell transplantation. Mol Immunol 2008; 45: 981-989.

9. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol 2012; 12: 443-458.

10. Matte CC, Liu J, Cormier J, Anderson BE, Athanasiadis I, Jain D, McNiff J, Shlomchik WD. Donor APCs are required for maximal GVHD but not for GVL. Nat Med 2004; 10: 987-992.

11. Jacobsohn DA, Vogelsang GB. Acute graft versus host disease. Orphanet J Rare Dis 2007; 2: 35.

12. Couriel D, Saliba R, Hicks K, Ippoliti C, de Lima M, Hosing C, Khouri I, Andersson B, Gajewski J, Donato M. Tumor necrosis factor-α blockade for the treatment of acute GVHD. Blood 2004; 104: 649-654.

13. Bignon JD, Gagne K. KIR matching in hematopoietic stem cell transplantation. Curr Opin Immunol 2005; 17: 553-559.

14. Bouchlaka MN, Redelman D, Murphy WJ. Immunotherapy following hematopoietic stem cell transplantation: potential for synergistic effects. Immunotherapy 2010; 2: 399-418.

15. Abdolvahabi R, Sarrafnejad A, Nafar M, Jafari D, Razaghi E, Lessan-Pezashki M, Yekaninejad M, Sharif-Paghaleh E, Amirzargar A. Association between TLR2, TLR4, and CD14 gene polymorphisms and acute rejection in kidney transplant. Exp Clin Transplant 2017; 16: 31-37.

16. Farag SS, Fehniger TA, Ruggeri L, Velardi A, Caligiuri MA. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood 2002; 100: 1935-1947.

17. Hiby SE, Ashrafian-Bonab M, Farrell L, Single RM, Balloux F, Carrington M, Moffett A. Distribution of killer cell immunoglobulin-like receptors (KIR) and their HLA-C ligands in two Iranian populations. Immunogenetics 2010; 62: 65-73.

18. Giebel S, Nowak I, Wojnar J, Markiewicz M, Dziaczkowska J, Wylezol I, Krawczyk-Kulis M, Bloch R, Kusnierczyk P, Holowiecki J. Impact of activating killer immunoglobulin-like receptor genotype on outcome of unrelated donor–hematopoietic cell transplantation. Transplant Proc 2006; 38: 287-291.

19. Velardi A. Role of KIRs and KIR ligands in hematopoietic transplantation. Curr Opin Immunol 2008; 20: 581-587.

20. Solgi G, Ghafari H, Ashouri E, Alimoghdam K, Rajalingam R, Amirzargar A. Comparison of KIR gene content profiles revealed a difference between northern and southern Persians in the distribution of KIR2DS5 and its linked loci. Hum Immunol 2011; 72: 1079-1083.

21. Vairo F, Portela P, Salim PH, Jobim M, Netto C, Dorneles A, Mittlestadt S, Jobim LF, Schwartz IVD. KIR genes and HLA class I ligands in Gaucher disease. Gene 2013; 516: 53-57.

22. Ashouri E, Dabbaghmanesh MH,Omrani GR. Presence of more activating KIR genes is associated with Hashimoto's thyroiditis. Endocrine 2014; 46: 519-525.

23. Jafari D, Nafar M, Yekaninejad MS, Abdolvahabi R, Pezeshki ML, Razaghi E, Amirzargar AA. Investigation of killer immunoglobulin-like receptor (KIR) and HLA genotypes to predict the occurrence of acute allograft rejection after kidney transplantation. Iran J Allergy Asthma Immunol 2017; 16: 245.

24. Dastmalchi R, Farazmand A, Noshad S, Mozafari M, Mahmoudi M, Esteghamati A, Amirzargar A. Polymorphism of killer cell immunoglobulin-like receptors (KIR) and their HLA ligands in Graves' disease. Mol Biol Rep 2014; 41: 5367-5374.

25. Ludajic K, Balavarca Y, Bickeböller H, Rosenmayr A, Fae I, Fischer G, Kouba M, Pohlreich D, Kalhs P, Greinix H. KIR genes and KIR ligands affect occurrence of acute GVHD after unrelated, 12/12 HLA matched, hematopoietic stem cell transplantation. Bone Marrow Transplant 2009; 44: 97-103.

26. Ruggeri L, Mancusi A, Burchielli E, Capanni M, Carotti A, Aloisi T, Aversa F, Martelli MF, Velardi A. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. Blood Cells Mol Dis 2008; 40: 84-90.

27. Glucksberg H, Storb R, Fefer A, Buckner C, Neiman P, Clift R, Lerner K, Thomas E. Clinical manifestations of graft-versus-host disease in human recipients of marrow from hl-a-matched sibling donor. Transplantation 1974; 18: 295-304.

28. Rajalingam R, Du Z, Meenagh A, Luo L, Kavitha VJ, Pavithra-Arulvani R, Vidhyalakshmi A, Sharma SK, Balazs I, Reed EF. Distinct diversity of KIR genes in three southern Indian populations: comparison with world populations revealed a link between KIR gene content and pre-historic human migrations. Immunogenetics 2008; 60: 207-217.

29. Hsu KC, Chida S, Geraghty DE, Dupont B. The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism. Immunol Rev 2002; 190: 40-52.

30. Giebel S, Nowak I, Dziaczkowska J, Czerw T, Wojnar J, Krawczyk-Kulis M, Holowiecki J, Holowiecka-Goral A, Markiewicz M, Kopera M. Activating killer immunoglobulin-like receptor incompatibilities enhance graft-versus-host disease and affect survival after allogeneic hematopoietic stem cell transplantation. Eur J Haematol 2009; 83: 343-356.

31. Shimoni A, Labopin M, Lorentino F, van Lint M, Koc Y, Gülbas Z, Tischer J, Bruno B, Blaise D, Pioltelli P. KIR-ligand mismatching is associated with inferior survival in patients with acute leukemia following T-cell replete haplo-identical transplantation with post-transplant cyclophosphamide. A study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT). Blood 2017; 130: 3302.

32. McQueen KL, Dorighi KM, Guethlein LA, Wong R, Sanjanwala B, Parham P. Donor–recipient combinations of group A and B KIR haplotypes and HLA class I ligand affect the outcome of HLA-matched, sibling donor hematopoietic cell transplantation. Hum Immunol 2007; 68: 309-323.

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

34. Clausen J, Böhm A, Straßl I, Stiefel O, Buxhofer-Ausch V, Machherndl-Spandl S, König J, Schmidt S, Steitzer H, Danzer M. HLA-C KIR-ligands determine the impact of anti-thymocyte globulin (ATG) on graft versus host and graft versus leukemia effects following hematopoietic stem cell transplantation. Biomedicines 2017; 5: 13.

35. Neuchel C, Fürst D, Niederwieser D, Bunjes D, Tsamadou C, Wulf G, Pfreundschuh M, Wagner E, Stuhler G, Einsele H. Impact of donor activating KIR genes on HSCT outcome in C1-ligand negative myeloid disease patients transplanted with unrelated donors—a retrospective study. PLoS One 2017; 12: e0169512.

36. Gagne K, Busson M, Bignon JD, Balère-Appert ML, Loiseau P, Dormoy A, Dubois V, Perrier P, Jollet I, Bois M. Donor KIR3DL1/3DS1 gene and recipient Bw4 KIR ligand as prognostic markers for outcome in unrelated hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2009; 15: 1366-1375.

37. Martínez-Losada C, Martín C, Gonzalez R, Manzanares B, García-Torres E, Herrera C. Patients lacking a KIR-ligand of HLA group c1 or c2 have a better outcome after umbilical cord blood transplantation. Front Immunol 2017; 8: 810.