

NKG2D gene polymorphism has a significant impact on transplant outcomes after HLA-fully-matched unrelated bone marrow transplantation for standard risk hematologic malignancies

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Acknowledgments: we are indebted to Drs. Hiroko Oshima, Masanobu Oshima and Atsushi Hirao, Mrs. Shinichi Ohmae and Katsuya Nakata, and Ms. Kaori Matsuura at Kanazawa University, and Drs. Keitaro Matsuo and Takakazu Kawase at the Aichi Cancer Center Research Institute for their technical assistance. We thank all of the JM DP transplant teams who contributed patients and donors to this study.

Funding: this study was supported by grants from the Ministry of Health, Labor and Welfare, and the Ministry of Education, Culture, Sports and Technology, and funds from the Mitani Research and Development Assistance Organization (Kanazawa, Japan) and by the Japan Leukemia Research Fund (Tokyo, Japan).

Manuscript received on March 5, 2009. Revised version arrived on April 13, 2009. Manuscript accepted on April 29, 2009.

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ABSTRACT

Background

NKG2D, an activating and co-stimulatory receptor expressed on natural killer cells and T cells, plays pivotal roles in immunity to microbial infections as well as in cancer immunosurveillance. This study examined the impact of donor and recipient polymorphisms in the *NKG2D* gene on the clinical outcomes of patients undergoing allogeneic T-cell-replete myeloablative bone marrow transplantation using an HLA-matched unrelated donor.

Design and Methods

The *NKG2D* polymorphism was retrospectively analyzed in a total 145 recipients with hematologic malignancies and their unrelated donors. The patients underwent transplantation following myeloablative conditioning; the recipients and donors were matched through the Japan Marrow Donor Program.

Results

In patients with standard-risk disease, the donor *NKG2D-HNK1* haplotype, a haplotype expected to induce greater natural killer cell activity, was associated with significantly improved overall survival (adjusted hazard ratio, 0.44; 95% confidence interval, 0.23 to 0.85; $p=0.01$) as well as transplant related mortality (adjusted hazard ratio, 0.42; 95% confidence interval, 0.21 to 0.86; $p=0.02$), but had no impact on disease relapse or the development of grade II-IV acute graft-versus-host disease or chronic graft-versus-host disease. The *NKG2D* polymorphism did not significantly influence the transplant outcomes in patients with high-risk disease.

Conclusions

These data suggest an association between the donor *HNK1* haplotype and better clinical outcome among recipients, with standard-risk disease, of bone marrow transplants from HLA-matched unrelated donors.

Key words: *NKG2D*, *HNK1*, *LNK1*, unrelated donor; bone marrow transplantation, single nucleotide polymorphism.

Citation: Espinoza JL, Takami A, Onizuka M, Sao H, Akiyama H, Miyamura K, Okamoto S, Inoue M, Kanda Y, Ohtake S, Fukuda T, Morishima Y, Kodera Y, and Nakao S, for the Japan Marrow Donor Program. *NKG2D* gene polymorphism has a significant impact on transplant outcomes after HLA-fully-matched unrelated bone marrow transplantation for standard risk hematologic malignancies. *Haematologica* 2009;94:1427-1434. doi:10.3324/haematol.2009.008318

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Introduction

Hematopoietic stem cell transplantation (SCT) is a potentially curative treatment for a range of hematologic malignancies. Although the use of an HLA-matched unrelated donor is well accepted when an HLA-identical sibling donor is unavailable, the risk of transplantation-related complications may be increased.¹ Despite improvements in clinical and supportive care, transplant-related life-threatening complications, including graft-versus-host disease (GVHD), infections and disease relapse, remain an enormous obstacle to overcome.² Although HLA matching is the major genetic determinant of clinical outcome after allogeneic SCT, recent evidence suggests that non-HLA immune-associated genes are also implicated.³ Previous investigations have revealed that several single nucleotide polymorphisms (SNP) which affect individual immune response to infections and inflammatory reactions are associated with the risk of GVHD and transplant outcomes.⁴⁻¹⁵

NKG2D is an activating and co-stimulatory receptor belonging to the C-type lectin-like family of transmembrane proteins and is expressed as a homodimer on natural killer (NK) cells, CD8⁺ $\alpha\beta$ ⁺ T cells, $\gamma\delta$ ⁺ T cells and activated macrophages.¹⁶⁻¹⁸ The ligands for NKG2D, such as MHC class I-chain related proteins (MICA and MICB), UL16 binding proteins are usually absent or expressed at very low levels in normal cells but are up-regulated by cellular stress including heat shock and microbial infections and are frequently expressed in epithelial tumor cells.¹⁹ Ligand engagement of NKG2D triggers cell-mediated cytotoxicity and co-stimulates cytokine production through a DAP10-phosphoinositol 3-kinase dependent pathway and plays an important role in the elimination of tumors and infected cells.^{16-18,20}

Recently, SNP were identified between *LNK1* and *HNK1* haplotypes of the *NKG2D* gene.²¹ In Japanese individuals, the *HNK1* haplotype is associated with greater activity of NK cells in the peripheral blood^{21,22} and a lower prevalence of cancers originating from epithelial cells.^{21,23,24} The present study investigates the impact of donor and recipient polymorphisms in the *NKG2D* gene on the clinical outcomes of patients undergoing allogeneic myeloablative bone marrow transplantation using an HLA allele-matched unrelated donor.

Design and Methods

Patients

NKG2D genotyping was performed on a total 145 recipients with hematologic malignancies and their unrelated donors who were part of the Japan Marrow Donor Program (JMDP). The recipients underwent transplantation, following myeloablative conditioning, with T-cell-replete marrow from an HLA-A, -B, -C, -DRB1 allele-matched donor between November 1995 and March 2000. HLA genotypes of the HLA-A, -B, -C, and -DRB1 alleles of the patients and donors were determined by the Luminex microbead method described previously. (Luminex 100 System; Luminex, Austin, TX, USA).^{25,26} No

patient had a history of prior transplantation. The final clinical survey of these patients was completed by November 1, 2007. Diagnoses were acute myeloid leukemia (n=49; 34%), acute lymphoblastic leukemia (n=37; 26%), chronic myeloid leukemia (n=41; 28%), myelodysplastic syndrome (n=11; 8%) and malignant lymphoma (n=7; 5%), (Table 1). The recipients were defined as having standard risk disease if they had acute myeloid or lymphoblastic leukemia in first complete remission, malignant lymphoma in complete remission, chronic myeloid leukemia in any chronic phase or myelodysplastic syndrome. All other patients were designated as having high-risk disease. Myeloid malignancies included acute myeloid leukemia, chronic myeloid leukemia and myelodysplastic syndrome, whereas lymphoid malignancies included acute lymphoblastic leukemia and malignant lymphomas. Cyclosporine or tacrolimus-based regimens were used in all patients for GVHD prophylaxis whereas anti-T-cell therapy, such as anti-thymocyte globulin and *ex vivo* T-cell depletion, was not. All patients and donors gave their written informed consent to molecular studies, according to the declaration of Helsinki, at the time of transplantation. The project was approved by the Institutional Review Board of Kanazawa University Graduate School of Medicine and the JMDP.

NKG2D genotyping

NKG2D was genotyped using the TaqMan-Allelic discrimination method²⁷ with a 9700-HT real time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA, USA) and results were analyzed using allelic discrimination software (Applied Biosystems). The genotyping assay was conducted in 96-well PCR plates. The amplification reaction contained template DNA, TaqMan universal master mix and a specific probe (product No. C_9345347_10; Applied Biosystems) for rs1049174, a single locus featuring a G-C substitution to distinguish between the *HNK1* (G) and *LNK1* (C) haplotypes of the *NKG2D* gene.^{21,23,24}

Data management and statistical analysis

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year and annually after transplantation. Pre-transplant cytomegalovirus serostatus was routinely tested only in patients but not in their donors. Engraftment was confirmed by an absolute neutrophil count of more than $0.5 \times 10^9/L$ for at least 3 consecutive days. Acute and chronic GVHD were diagnosed and graded using established criteria.^{28,29} Overall survival was defined as the number of days from transplantation to death from any cause. Disease relapse was defined as the number of days from transplantation to disease relapse. Transplant-related mortality was defined as death without relapse. Any patients who were alive at the last-follow-up date were censored. When collecting data, only the main cause of death was recorded if two or more causes were combined. Data on etiological agents of infections, post-mortem changes and supportive care (including prophylaxis of infections and therapy of GVHD, which were given on an institutional basis), were not available for this

cohort of patients. The analysis was performed using Excel 2007 (Microsoft Corp, Redmond, WA, USA), OriginPro version 8.0J (Lightstone Inc, Tokyo, Japan), and R (The R Foundation for Statistical Computing, Perugia, Italy).³⁰ The probability of overall survival was calculated using the Kaplan-Meier method and compared using the log-rank test. The probabilities of transplant-related mortality, disease relapse, acute GVHD, chronic GVHD, and each cause of death were compared using the Grey test³¹

and analyzed using cumulative incidence analysis,³⁰ considering relapse, death without disease relapse, death without acute GVHD, death without chronic GVHD, and death without each cause as respective competing risks. The analysis was stratified for patients with standard-risk disease and high-risk disease to take into account the already recognized prognostic differences. The variables considered were recipient age at time of transplantation, sex, recipient cytomegalovirus serosta-

Table 1. Characteristics of the donors and recipients.

Variable	Standard-risk disease (n=93, 64%) Donor NKG2D haplotype				p	High-risk disease (n=52, 36%) Donor NKG2D haplotype				p
	HNK1 negative n=55, 59%		HNK1 negative n=38, 41%			HNK1 positive n=28, 54%		HNK1 negative n=24, 46%		
	N.	Ratio	N.	Ratio		N.	Ratio	N.	Ratio	
Age, years										
Recipient					0.08					0.39
Median	31		23			23		22		
Range	1-50		1-50			7-46		2-48		
Donor					0.54					
Median	33		28			34		29		0.02
Range	22-49		21-50			21-47		21-50		
Recipient NKG2D haplotype					0.17					0.48
HNK1 positive	33	60%	28	74%		19	68%	14	58%	
HNK1 negative	22	40%	10	26%		9	32%	10	42%	
Sex, male					0.37					0.77
Recipient	30	55%	23	61%		19	68%	15	63%	
Donor	42	76%	23	61%		19	68%	13	54%	
Recipient/donor sex					0.23					0.86
Sex matched	31	56%	20	53%		18	64%	16	67%	
Male/female	6	11%	9	24%		5	18%	5	21%	
Female/male	18	33%	9	24%		5	18%	3	13%	
Disease					0.86					0.99
Acute myeloid leukemia	14	25%	9	24%		14	50%	12	50%	
Acute lymphoblastic leukemia	10	18%	8	21%		10	36%	9	38%	
Myelodysplastic syndrome	6	11%	5	13%		0	0%	0	0%	
Malignant lymphoma	2	4%	3	8%		1	4%	1	4%	
Chronic myeloid leukemia	23	42%	13	34%		3	11%	2	8%	
ABO matching					0.37					0.18
Matched	35	64%	19	50%		14	50%	17	71%	
Major mismatch	11	20%	10	26%		6	21%	5	21%	
Minor mismatch	9	16%	9	24%		8	29%	2	8%	
Bi-directional	0	0%	1	3%		0	0%	1	4%	
Conditioning regimen					0.93					0.51
With total body irradiation	43	78%	30	79%		26	93%	21	88%	
Without total body irradiation	12	22%	8	21%		2	7%	3	13%	
Pretransplant CMV serostatus					0.30					0.99
CMV-negative recipient	14	25%	5	13%		6	27%	5	21%	
Missing data	4	7%	2	5%		5	18%	4	17%	
GVHD prophylaxis					0.58					0.11
With cyclosporine	51	93%	34	89%		27	96%	20	83%	
With tacrolimus	4	7%	4	11%		1	4%	4	17%	
TNC, ×10 ⁶ /kg					0.40					0.04
Median	5.4		5.8			5.8		8.2		
Range	2.3-14.6		2.3-57.6			2.9-20.0		2.4-42.8		
Engraftment	53	96%	38	100%	0.23	28	100%	23	96%	0.28

CMV: cytomegalovirus; TNC: total nucleated cell count harvested.

tus before transplantation, disease characteristics (disease type and disease lineage), donor characteristics (age, sex, sex compatibility, and ABO compatibility), transplant characteristics (total body irradiation-containing regimen, tacrolimus versus cyclosporine, and total nucleated cell count harvested per recipient weight). The median was used as the cut-off point for continuous variables. The χ^2 test and Mann-Whitney test were used to compare results of two groups. The Hardy-Weinberg equilibrium for the *NKG2D* gene polymorphism was tested using the Haploview program.³² Multivariate Cox models were used to evaluate the hazard ratio associated with the *NKG2D* polymorphism. Co-variables found to be statistically significant in univariate analyses ($p \leq 0.10$) were included in the models. For both the univariate and multivariate analyses, p values were two-sided and outcomes were considered to be statistically significant with $p \leq 0.05$.

Results

Frequencies of *NKG2D* haplotype

The *NKG2D* gene polymorphism was analyzed in 145 pairs of unrelated donors-recipients of bone marrow following myeloablative conditioning (Table 1). The haplotype frequencies of *LNK1/LNK1*, *HNK1/LNK1* and *HNK1/HNK1* were 43%, 42% and 15%, respectively in donors and 35%, 45% and 20%, respectively in recipients. These frequencies were similar to those reported in previous studies in Japanese populations^{21,24} and were in accordance with the Hardy-Weinberg equilibrium ($p=0.80$).

Transplant outcomes according to *NKG2D* haplotype

With a median follow-up of 115 months among survivors (range, 74 to 140 months), 30 recipients (21%) had relapsed or progressed and 62 (47%) had died. Three patients (2%) died before engraftment. The analysis of the influence of the *NKG2D* genotype on clinical out-

comes after transplantation was stratified according to whether the recipients had standard-risk disease or high-risk disease to account for the already recognized prognostic difference. The overall survival at 5 years in patients with standard-risk disease was 63% while that of patients with high-risk disease was 44% ($p=0.06$). The 5-year cumulative incidences of transplant-related mortality were 32% and 27%, respectively ($p=0.33$) and those of disease relapse were 10% and 31%, respectively ($p=0.0006$).

The transplant outcomes according to *NKG2D* genotype are summarized in Table 2. Patients with standard-risk disease receiving transplants from donors with the *HNK1* haplotype had a significantly better 5-year overall survival (73% vs. 49%, $p=0.01$; Figure 1A) and lower transplant-related mortality rate (22% vs. 45%, $p=0.02$; Figure 1B) than those receiving transplants from donors without the *HNK1* haplotype. No difference was noted in disease relapse in relation to the donors' polymorphism (9% vs. 11%, $p=0.81$; Figure 1C) or in the development of grades II to IV acute GVHD (28% vs. 41%, $p=0.25$) or chronic GVHD (37% vs. 41%, $p=0.83$). When patients with acute myeloid leukemia or myelodysplastic syndrome were separately analyzed, there was still no difference in disease relapse in relation to *NKG2D* polymorphisms (*data not shown*). In patients with high-risk disease, the donor *HNK1* haplotype had no significant effects on transplant outcomes (Table 2).

Multivariate analysis

Any factors found to be significant in univariate analyses were included in the multivariate analysis. When patients with standard-risk disease were analyzed, the *HNK1* haplotype in donors remained statistically significant in multivariate analyses for both overall survival and transplant-related mortality (Table 3). The presence of the *HNK1* haplotype in the donor resulted in better overall survival (hazard ratio, 0.44; 95% confidence interval, 0.23 to 0.85; $p=0.01$) and transplant-related mortality (hazard ratio, 0.42; 95% confidence interval, 0.21 to 0.86; $p=0.02$).

Table 2. Univariate analysis of the association of *NKG2D* polymorphisms with clinical outcomes after transplantation.

	N.	5-year OS	p	5-year TRM	p	5-year relapse	p	Grade II-IV acute GVHD	p	Chronic GVHD	p
Standard-risk disease											
Donor <i>NKG2D</i> haplotype			0.01		0.02		0.81		0.25		0.83
<i>HNK1</i> -positive	55	73%		22%		9%		28%		37%	
<i>HNK1</i> -negative	38	49%		45%		11%		41%		41%	
Recipient <i>NKG2D</i> haplotype			0.39		0.31		0.93		0.48		0.98
<i>HNK1</i> -positive	61	62%		33%		10%		37%		39%	
<i>HNK1</i> -negative	32	66%		28%		9%		25%		38%	
High-risk disease											
Donor <i>NKG2D</i> haplotype			0.91		0.77		0.93		0.08		0.47
<i>HNK1</i> -positive	28	43%		26%		33%		54%		44%	
<i>HNK1</i> -negative	24	46%		29%		29%		30%		35%	
Recipient <i>NKG2D</i> haplotype			0.41		0.43		0.10		0.40		0.68
<i>HNK1</i> -positive	33	42%		23%		39%		39%		37%	
<i>HNK1</i> -negative	19	47%		35%		18%		50%		47%	

OS: overall survival; TRM: transplant-related mortality.

The donor and recipient *HNK1* haplotype did not significantly influence the transplant outcomes in patients with high-risk disease.

Main causes of death

The main causes of death according to the *HNK1* haplotype of the donors and recipients are illustrated in Figure 2A for patients with standard-risk disease, and in Figure 2B for those with high-risk disease. In patients with standard-risk disease receiving transplants from *HNK1*-negative donors, the most frequent cause of death was acute GVHD, followed by interstitial pneumonia. Transplants from *HNK1*-positive donors resulted in a statistically significantly reduced incidence of death attributed to acute GVHD (Figure 3A; $p=0.006$) as well as a trend toward a lower incidence of death attributed to interstitial pneumonia (Figure 3B; $p=0.09$). Other causes of death did not differ according to the *HNK1* haplotype.

Discussion

The current study showed an association between the *NKG2D-HNK1* haplotype in unrelated donors of HLA-matched myeloablative bone marrow transplants (haplotype frequency, 61%) and a significantly reduced transplant-related mortality and better overall survival for their recipients with standard-risk disease. The polymorphism of the donor *NKG2D* gene did not influence disease relapse or the development of grades II to IV acute GVHD or chronic GVHD in the patients. One possible explanation for the absence of the beneficial effects of the *HNK1* haplotype in patients with high-risk disease may be that the number of cases in the study was insufficient for a meaningful assessment of the effect. Alternatively, disease progression may precede the emergence of the potential advantageous effects of the *HNK1* donor haplotype that could protect the recipient from severe transplant-related complications. There was a larger difference in disease relapse between patients with

standard-risk disease and those with high-risk disease: 10% and 31% at 3 years after transplantation, respectively.

NKG2D plays important roles in immunity to microbial infections and is especially prominent in controlling viral and bacterial infections.¹⁶ Therefore, the reduced transplant-related mortality in patients with standard-risk disease receiving grafts from donors with the *HNK1* haplotype in this study might be a consequence of increased resistance to infections in the recipients. However, the hypothesis is too speculative because of the unavailability of data on causes of infections in this cohort. Further studies will be needed to clarify whether the *HNK1* haplotype in donors can effectively protect patients against infections.

Several studies have shown that NK cell activity has an important role in the outcomes of patients undergoing allogeneic transplantation.^{33,34} Alloreactive NK cells reduced the risk of relapse of acute myeloid leukemia without increasing the incidence of GVHD, resulting in a marked improvement of event-free survival in a series of haploidentical transplant recipients.^{35,36} In HLA-identical sibling transplants, the absence of HLA-C and HLA-B ligand for donor-inhibitory killer immunoglobulin-like receptors (KIR) provided benefits in terms of survival and relapse of patients with acute myeloid leukemia and myelodysplastic syndrome in recipients of T-cell-depleted SCT.³⁷ On the other hand, the JMDP found that KIR ligand mismatch was unfavorably correlated with relapse of leukemia and survival in patients undergoing T-cell-replete unrelated bone marrow transplants.³⁸ All patients in the present study received grafts from an HLA-A, -B, and -C allele-matched donor, implying KIR ligand match between each patient and donor. It is an open question whether the *NKG2D* polymorphism could affect the outcomes of patients undergoing transplantation with KIR-mismatched grafts.

In this study, major and minor ABO incompatibilities between the donor and recipient tended to be associated with poorer transplant outcomes, regardless of the risk

Table 3. Multivariate analysis of the association of *NKG2D* polymorphisms with clinical outcomes after transplantation.

Variable	Overall survival			Transplant-related mortality			Relapse			Grades II-IV acute GVHD			Chronic GVHD		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Standard-risk disease															
<i>HNK1</i> -positive donor	0.44	0.23-0.85	0.01	0.42	0.21-0.86	0.02	0.71	0.19-2.67	0.61	0.83	0.39-1.75	0.63	0.83	0.39-1.75	0.62
<i>HNK1</i> -positive recipient	1.22	0.60-2.50	0.58	1.32	0.61-2.87	0.48	1.11	0.28-4.48	0.88	1.54	0.66-3.57	0.32	1.06	0.49-2.31	0.88
Donor age, >31 years	-	-	-	-	-	-	-	-	-	2.17	0.95-4.96	0.07	-	-	-
Major ABO incompatibility	-	-	-	-	-	-	-	-	-	3.12	1.49-6.56	0.003	0.50	0.17-1.45	0.20
Minor ABO incompatibility	2.42	1.17-5.03	0.02	-	-	-	-	-	-	-	-	-	0.29	0.07-1.24	0.10
High-risk disease															
<i>HNK1</i> -positive donor	0.68	0.30-1.51	0.34	0.62	0.20-1.91	0.40	1.25	0.41-3.80	0.69	1.87	0.69-5.07	0.22	1.55	0.60-4.01	0.37
<i>HNK1</i> -positive recipient	1.41	0.65-3.07	0.39	0.76	0.25-2.29	0.63	2.35	0.66-8.44	0.19	0.47	0.18-1.22	0.12	0.92	0.35-2.38	0.86
Age, >26 years	1.95	0.93-4.09	0.08	6.30	1.86-21.32	0.003	-	-	-	-	-	-	-	-	-
Donor age, >31 years	-	-	-	-	-	-	0.53	0.17-1.65	0.27	-	-	-	-	-	-
Minor ABO incompatibility	2.94	1.19-7.25	0.02	-	-	-	-	-	-	5.10	2.08-12.52	0.004	-	-	-

category of the disease. These findings are compatible with those of a previous study by the JMDF,³⁹ although the impact of ABO incompatibilities on SCT outcomes is controversial.

This study also identified age as a significant predictive factor for transplant-related mortality in the patients with

standard-risk disease. This is consistent with the results of a previous study⁴⁰ showing that age over 35 years increased the risk of transplant-related mortality after allogeneic myeloablative SCT in high-risk patients.

A possible limitation of this study is the fact that no direct evidence is yet available regarding the ability of *NKG2D* polymorphisms to protect against microbial infections. The association observed between the *NKG2D* haplotype and transplant outcome might be due to another genetic polymorphism in linkage disequilibrium responsible for a better transplant outcome. One candidate gene is *NKG2F* (*KLRC4*), which is located in the NK complex region adjacent to the *NKG2D* gene, because an intrinsic SNP (rs2617171) in the gene has been reported to be in complete linkage with the *NKG2D* genotype.²⁴ Alternatively, polymorphisms may not be directly associated with controlling infection, but rather may be associated with other factors, such as sensitivity to treatment against GVHD or protection against organ toxicities related to transplants, which also influence the transplant outcome. These hypotheses have yet to be verified give the insufficient evidence.

Polymorphisms in genes encoding for nucleotide-binding oligomerization domain 2 (*NOD2*)/caspase recruitment domain 15 (*CARD15*),⁹ heme oxygenase-1 (*HO-1*) promoter,⁶ the Toll-like receptor 4,⁴ CC chemokine ligand (*CCL*) 5 promoter,³² transforming growth factor (*TGF*) β 1,¹¹ interleukin (*IL*) 12, tumor necrosis factor (*TNF*) α ,¹⁵ *IL-23*,⁵ mannose-binding lectin (*MBL*),¹⁰ *Fc* γ receptor IIa (*Fc γ RIIa*), myeloperoxidase (*MPO*), *Fc γ RIIIb*, *IL-1Ra*, *IL-10*,¹² *Fc* receptor-like 3 (*FCRL3*), peptidylarginine deimi-

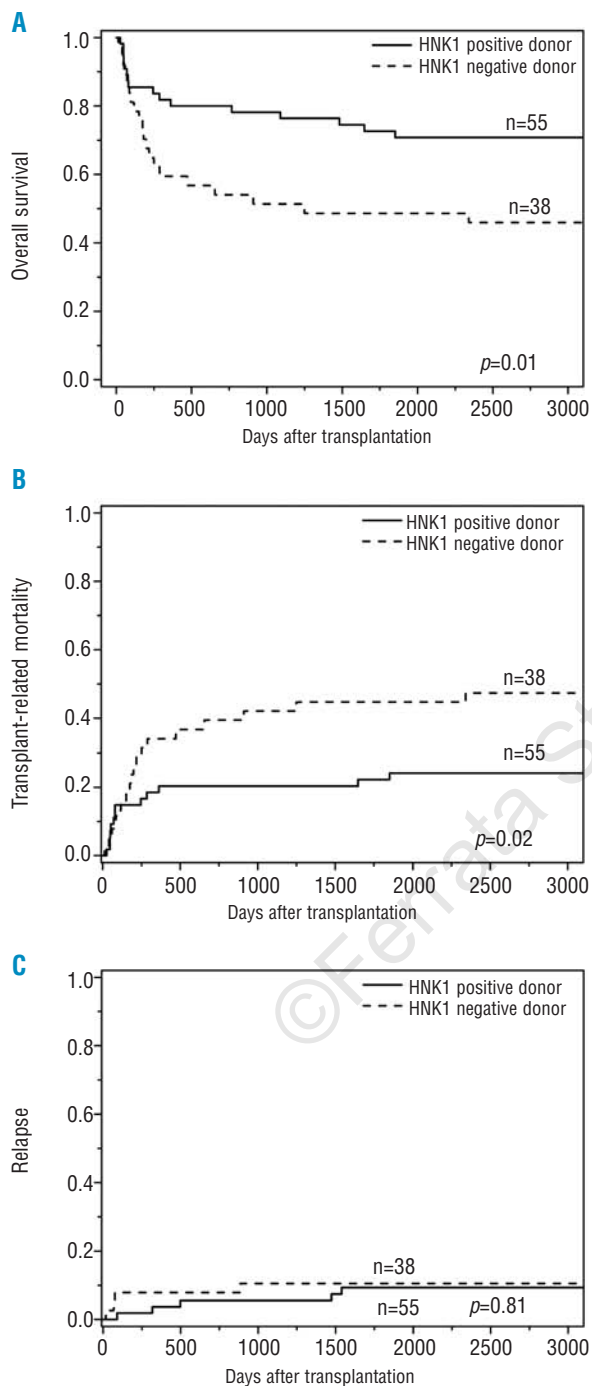


Figure 1. Kaplan-Meier analysis of (A) overall survival, (B) cumulative incidence of transplant-related mortality and (C) disease relapse after transplantation according to the donor *NKG2D* polymorphism in patients with standard-risk disease. Patients with donors with the *HNK1* haplotype had better overall survival and lower transplant-related mortality. Donor haplotype had no significant impact on disease relapse.

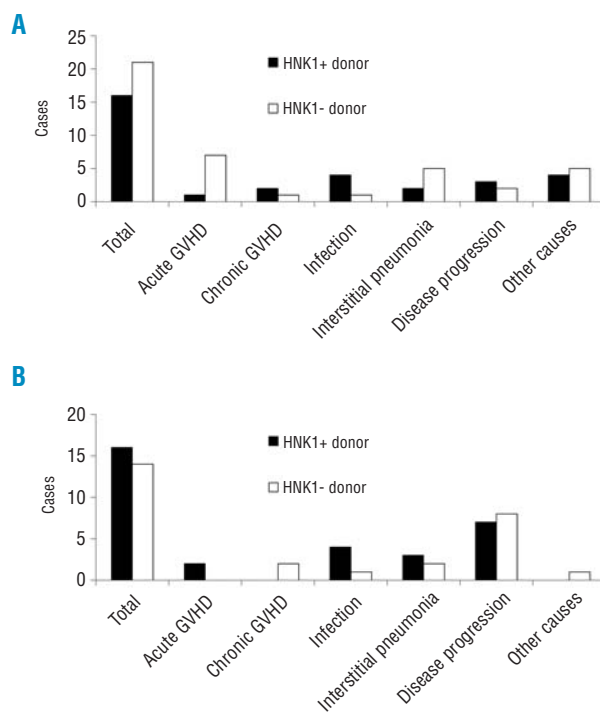


Figure 2. Main causes of death after transplantation according to the *NKG2D* polymorphism in patients with (A) standard-risk disease (B) high-risk disease.

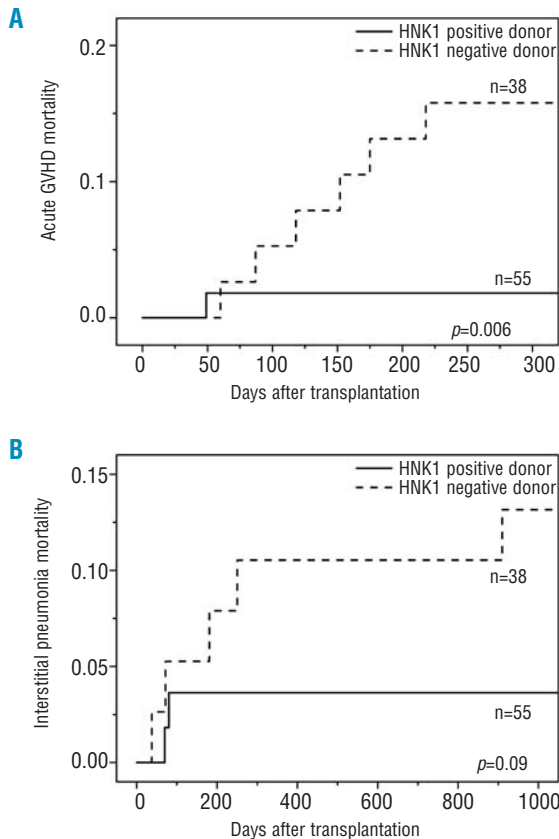


Figure 3. Cumulative incidence of deaths due to (A) acute GVHD and (B) interstitial pneumonia after transplantation in patients with standard-risk disease. The *HNK1* haplotype in donors was associated with a significantly lower incidence of deaths due to acute GVHD ($p=0.006$) as well as a trend toward a lower incidence of deaths due to interstitial pneumonia ($p=0.09$).

ciated with overall survival in the present study. This may prompt the determination of the donor *NKG2D* polymorphism prior to SCT in order to choose the best donor, expected to minimize transplant-related mortality after SCT, when multiple donors for a patient are available. Otherwise, prior information on the donor *NKG2D* polymorphism may be helpful in selecting risk-specific appropriate precautions following transplantation.

In conclusion, the present data suggest that the *NKG2D* polymorphism, in addition to HLA disparity between recipients and donors, affects prognosis after a bone marrow transplant from an unrelated donor. However, care should be made in drawing conclusions because the number of patients in the present study was small. The finding of a gene polymorphism may not be equivalent to differences in gene expression, which may be influenced by multiple factors because the *NKG2D* receptor is found on many tissues and cells.⁴¹ Experimental evidence is required to substantiate the effect of the *NKG2D* polymorphism on immune function. We next plan to conduct a prospective study to confirm these results and to extend this investigation to other transplantation settings, such as related donor SCT, reduced-intensity SCT, HLA-mismatched SCT and SCT for patients with non-hematologic malignancies.

nase citullinating enzymes 4 (*PADI4*)¹³ and methylenetetrahydrofolate reductase (*MTHFR*)¹⁴ have been shown to influence the outcome after allogeneic SCT. Most of them are associated with the development of GVHD. Only the *NOD2/CARD15* and *HO-1* promoter polymorphisms have a significant impact on overall survival after SCT. Furthermore, the impact of the *HO-1* promoter polymorphisms depends on donor cells but not on recipient cells, as observed with the *NKG2D* polymorphism which, in the donor, was shown to be significantly asso-

Authorship and Disclosures

JLE and AT designed and performed the research, and contributed to the same aspects of the work; AT, JLE and SN wrote the paper; AT, YKa, and SOh performed the statistical analyses; MO, HS, HA, KM, SOk, MI, TE, YM, and YKo contributed to data collection.

The authors reported no potential conflicts of interest.

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