Effect of Donor KIR2DL1 Allelic Polymorphism on the Outcome of Pediatric Allogeneic Hematopoietic Stem-Cell Transplantation

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R.B. and W.L. hold the patent for the single nucleotide polymorphism assay used for killer-cell immunoglobulin-like receptors allele typing.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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ABSTRACT

Purpose

Killer-cell immunoglobulin-like receptors (KIRs) that regulate natural-killer cells are highly polymorphic. Some KIR2DL1 alleles encode receptors that have stronger signaling function than others. We tested the hypothesis that the clinical outcomes of allogeneic hematopoietic stem-cell transplantation (HSCT) could be affected by donor KIR2DL1 polymorphism.

Patients and Methods

All 313 pediatric patients received allogeneic HSCT at a single institution. Donor KIR2DL1 functional allele typing was retrospectively performed using single nucleotide polymorphism assay.

Results

Patients who received a donor graft containing the functionally stronger KIR2DL1 allele with arginine at amino acid position 245 (KIR2DL1-R²⁴⁵) had better survival ($P = .0004$) and lower cumulative incidence of disease progression ($P = .001$) than those patients who received a donor graft that contained only the functionally weaker KIR2DL1 allele with cysteine at the same position (KIR2DL1-C²⁴⁵). The effect of KIR2DL1 allelic polymorphism was similar in patients with acute myeloid leukemia or acute lymphoblastic leukemia among all allele groups ($P \geq .71$). Patients who received a KIR2DL1-R²⁴⁵-positive graft with HLA-C receptor-ligand mismatch had the best survival ($P = .00003$) and lowest risk of leukemia progression ($P = .0005$) compared with those who received a KIR2DL1-C²⁴⁵ homozygous graft.

Conclusion

Donor KIR2DL1 allelic polymorphism affects recipient outcomes after allogeneic HSCT. These findings have substantial implications for prognostication and donor selection.

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INTRODUCTION

Allogeneic hematopoietic stem-cell transplantation (HSCT) is a curative treatment for many malignancies or genetic diseases. Donor selection is a critical element for successful transplantation. In addition to the consideration of donor's health status and HLA matching, routine typing of killer cell immunoglobulin like receptors (KIRs) has recently been proposed.¹⁻³

KIRs are present on the surface of natural killer (NK) cells and a subset of cytotoxic T cells.⁴ The receptors recognize class I HLA molecules on targets cells to control the effector cell activity. Analogous to T cells, NK cells may facilitate engraftment, combat infection, and control cancer recurrence in HSCT; however, NK cells may reduce rather than increase the risk of graft-versus-host disease (GvHD).^{5,6}

Recent clinical data have confirmed that both inhibitory and activating KIRs are important determinants in the outcome of HSCT in both malignant and nonmalignant disorders.⁷⁻⁹ The KIR genes are encoded on chromosome 19q13 and are highly polymorphic.¹⁰⁻¹² Fifteen members (plus two pseudogenes) of the KIR family have been identified in humans thus far. There is substantial variability among people in KIR haplotype content, gene expression, and allele function.

KIR2DL1 is one of the KIR family members known to be important in HSCT and is present in approximately 90% to 95% of healthy individuals.¹³⁻¹⁵ The KIR2DL1 receptor inhibits NK cell functions as well as educates NK cells for "missing self" recognition of its ligand group 2 HLA-C^{Lys80} (C2).^{16,17} Based on this biologic principle, some HSCT studies have shown that the presence of

donor KIR2DL1 and the absence of recipient C2 ligand (ie, KIR2DL1 receptor-ligand mismatch) were associated with favorable outcomes.^{2,18-23} Similar findings were observed for the corresponding receptors KIR2DL2 and KIR2DL3 that recognize group 1 HLA-C^{Asn80} (C1). However, there were conflicting reports from different transplantation centers, in part because prior NK alloreactivity models did not take into account the functional heterogeneity in KIR alleles. Until recently, all KIR alleles of the same family have been considered functionally identical in HSCT analyses.⁹ Although significant variability in terms of function as well as surface expression among different alleles of the same KIR gene has been reported,²⁴⁻²⁷ the effect of KIR allelic polymorphism in HSCT remains unknown. We recently reported²⁸ that KIR2DL1 alleles with arginine in position 245 of the transmembrane domain (KIR2DL1-R²⁴⁵) are stronger than those with cysteine in the same position (KIR2DL1-C²⁴⁵). Specifically, KIR2DL1-R²⁴⁵ can recruit more Src-homology-2 domain-containing protein tyrosine phosphatase 2 and beta-arrestin 2, shows higher inhibition of lipid raft polarization at the immune synapse, and has less downregulation of cell-surface expression on interaction with its ligand. Based on this knowledge of the molecular determinants, we developed a high-throughput method to type different KIR2DL1 functional allelic groups.²⁹ In this study, we investigated the influence of donor KIR2DL1-R²⁴⁵ and KIR2DL1-C²⁴⁵ on the outcome of allogeneic HSCT. Furthermore, we examined the effect of receptor-ligand mismatch in the context of KIR2DL1 allelic polymorphism.

PATIENTS AND METHODS

Patients and Donors

KIR2DL1 functional allelotyping was performed using DNA samples from 313 donors for patients who underwent allogeneic HSCT at St Jude Children's Research Hospital over the 10-year period from January 1, 2000, to January 1, 2010. Of the 313 patients, their median age at the time of transplantation was 9.9 years (range, 1 month to 18.6 years). The primary diagnoses included 231 hematologic malignancies (lymphoid, 116 patients; myeloid, 115 patients; total, 74%), 25 solid tumors (embryonal tumors, 19 patients; bone sarcomas, six patients; total, 8%), and 57 nonmalignant diseases (marrow failure, 10 patients; genetic diseases, 47 patients; total, 18%). No patients were excluded based on primary disease type to allow examination of generalizability of our findings. Of the 256 patients with cancer, 143 patients (56%) were in remission and 113 (44%) had persistent disease at the time of transplantation. About half (175 patients; 56%) received a conditioning regimen including total-body irradiation. The majority (76.7%) was myeloablative, defined as total-body irradiation ≥ 5 Gy in single dose or ≥ 8 Gy fractionated; busulfan more than 9 mg/kg; or melphelan more than 150 mg/m². The grafts were obtained from matched-sibling donors (27%), matched-unrelated donors (31%), or haploidentical donors (41%). General supportive care for transplantation recipients at our institution has been described recently.³⁰ This study was approved by the institutional review board.

Laboratory Assay for KIR and HLA Genotyping

The single-nucleotide polymorphism (SNP) assay for KIR2DL1 functional allele typing was performed on an HT7900 Sequence Detection System (Applied Biosystems, South San Francisco, CA) as described previously.²⁹ The probes were designed based on a single-nucleotide difference at amino acid position 245 in the mature protein. The sequence of the probes was 6FAM-CATCGCTGGTGCTC-MGBNFQ and VIC-CATTGCTGGTGCTCC-MGBNFQ. A universal primer set was designed that could specifically amplify all the alleles of KIR2DL1: forward primer 5'-CTCTTCATCCTCTCCTC TTCTTTC-3' and reverse primer 5'-GAAAGTCCTGCCTCTGTGGC-3'. Each reaction mix (total volume, 25 μ L) contained 250 nmol/L probes, 100 ng donor's genomic DNA, 5 pM forward and reverse primer, and 1 $\times$ TaqMan

genotyping master mix. The allelic discrimination assay software SDS2.3 (Applied Biosystems) was used to set up the background in the preread program. Then, absolute quantification program SDS2.3 was run to amplify the samples. The polymerase chain reaction steps were stage 1: 2 minutes at 50°C; stage 2: 10 minutes at 95°C; stage 3: 95°C for 15 seconds and 60°C for 1 minute. Stage 3 was repeated for 45 cycles. Finally, the allelic discrimination assay program was used for postreading. HLA typing for HLA-A, -B, -C, and -DRB1 was performed by DNA methods.^{30,31} KIR genotyping for haplotype A and B assignment was performed using polymerase chain reactions with specific primers as described previously.³²

End Points and Statistical Analysis

The χ^2 or Fisher's exact test was used to compare baseline variables among various KIR2DL1 allelic groups. Overall survival, progression-free survival (PFS) and grade II to IV GVHD probabilities were estimated using the Kaplan-Meier method. Overall survival was defined as the time from transplantation until death from any cause, censoring those alive at last follow-up. PFS was defined as the time from transplantation until the first event (relapse, progression, or death from any cause), censoring patients alive and progression free at last follow-up. PFS, rather than disease-free survival, was estimated, because 44% of the patients had active disease at the time of HSCT. The cumulative incidences of events were estimated using the method of Kelb-fleisch and Prentice. In the estimation of cumulative incidence of disease progression, deaths unrelated to disease were considered competing events. Univariate analyses of survival probabilities were performed using log-rank tests. Cumulative incidences were compared using Gray's tests. The parameters associated with outcomes in univariate analyses at a nominal level of 0.15 were included in multivariate analyses using Cox proportional hazards regression models. The assumption of proportional hazard was confirmed in all analyses.

We used the Memphis receptor-ligand mismatch model to examine the statistical interactions between KIR2DL1 allele polymorphism and its HLA-C ligand effects. HLA-C ligands are dimorphic, as the C2 group is the ligand for KIR2DL1 and the C1 group is the ligand for KIR2DL2/3. If a patient had HLA C1/C1 and the donor was KIR2DL1-positive, or if a patient had HLA C2/C2 and the donor was KIR2DL2/3-positive, then they were classified as receptor-ligand mismatched. In contrast, the donors for patients who were C1/C2 were defined as HLA-C receptor-ligand matched. All the reported *P* values are two-sided and are considered significant if less than .05. Statistical analyses were performed with SAS software version 9.2 and R version 2.14.0.

RESULTS

Patient and Donor Characteristics

The patient and donor characteristics, stratified by donor KIR2DL1 functional allelic groups, are summarized in Table 1. Among the 313 donors, 215 were homozygous for KIR2DL1-R²⁴⁵ (RR; RR, 68.7%), 22 were homozygous for KIR2DL1-C²⁴⁵ (CC; CC, 7%), and 76 were heterozygous for KIR2DL1-R²⁴⁵/C²⁴⁵ (RC; RC, 24.3%). As expected, KIR2DL1-R²⁴⁵ was associated with centromeric A (Cen A) –containing haplotypes and KIR2DL1-C²⁴⁵ with Cen-B haplotypes (*P* < .00001); otherwise, there was no statistically significant difference among the three allelic groups in any demographic or transplantation variables (all *P* > .23).

Overall Survival

Compared with the patients who received a CC donor graft, the risk of death after HSCT was significantly lower in those who received an RR graft (hazard ratio [HR], 0.4; 95% CI, 0.25 to 0.64; *P* = .0001) or an RC graft (HR, 0.42; 95% CI, 0.25 to 0.71; *P* = .0013; Table 2). Thus, the Kaplan-Meier estimates of survival probability in the patients who received an RR or RC graft were significantly higher than the estimates

Table 1. Patient and Donor Characteristics Stratified by Donor KIR2DL1 Functional Allelic Groups

Characteristic	Donor KIR2DL1 Alleles								P
	Total		RR		RC		CC		
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	
No. of patients	313		215	68.7	76	24.3	22	7.0	
Donor KIR haplotype									< .001
Cen A/A	104	33.4	89	41.8	15	19.7	0	0	
Cen A/B	117	37.6	80	37.6	30	39.5	7	31.8	
Cen B/B	90	28.9	44	20.6	31	40.8	15	68.2	
Age, years									.68
< 10	158	50.5	112	52.1	35	46.1	11	50	
> 10	155	49.5	103	47.9	41	53.9	11	50	
Sex									.45
Female	113	36.1	73	34	32	42.1	8	36.4	
Male	200	63.9	142	66	44	57.9	14	63.6	
Race									.29
White	198	63.3	140	65.1	41	53.9	17	77.3	
Black	57	18.2	38	17.7	17	22.4	2	9.1	
Other	58	18.5	37	17.2	18	23.7	3	13.6	
Primary disease									.44
Hematologic malignancies	231	73.8	157	73	54	71	20	90.9	
Solid tumors	25	8	18	8.4	6	7.9	1	4.5	
Nonmalignant diseases	57	18.2	40	18.6	16	21.1	1	4.5	
Acute leukemia									.89
ALL	98	54.4	67	54.5	21	56.8	10	50	
AML	82	45.6	56	45.5	16	43.2	10	50	
Malignant disease status									.73
CR1	77	30.1	54	30.9	16	26.7	7	33.3	
CR2	52	20.3	33	18.9	16	26.7	3	14.3	
CR3/CR4	14	5.5	9	5.1	5	8.3	0	0	
Refractory/progressive	113	44.1	79	45.1	23	38.3	11	52.4	
Conditioning type									.40
TBI containing	175	55.9	116	54	44	57.9	15	68.2	
Non-TBI	138	44.1	99	46	32	42.1	7	31.8	
Conditioning intensity									.93
Myeloablative	240	76.7	164	76.3	58	76.3	18	81.8	
Nonmyeloablative	73	23.3	51	23.7	18	23.7	4	18.2	
Donor type									.56
Sibling	86	27.5	57	26.5	22	29	7	31.8	
Unrelated	98	31.3	73	34	21	27.6	4	18.2	
Haploidentical	129	41.2	85	39.5	33	43.4	11	50	
T cell depleted									.9
Yes	154	49.2	105	48.8	37	48.7	12	54.5	
No	159	50.8	110	51.2	39	51.3	10	45.5	
Donor CMV status									.23
Positive	165	52.7	108	50.2	44	57.9	13	59.1	
Negative	137	43.8	96	44.7	32	42.1	9	40.9	
Unknown	11	3.5	11	5.1	0	0	0	0	

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CC, KIR2DL1-C²⁴⁵ homozygous; Cen, centromeric; CMV, cytomegalovirus; CR, complete remission; KIR, killer-cell immunoglobulin-like receptors; RC, KIR2DL1-R²⁴⁵/C²⁴⁵ heterozygous; RR, KIR2DL1-R²⁴⁵ homozygous; TBI, total-body irradiation.

for those with a CC graft ($P_{RR,RC/CC} = .0004$; Fig 1A). There was no statistical difference in survival in the patients who received an RR graft and those who received an RC graft ($P_{RR/RC} = .81$; Fig 1A). Among the 256 patients with malignant disorders, the patients who received a CC graft had the worst survival rates ($P_{RR,RC/CC} = .001$; Fig 1B). When the analysis was further limited to the 231 patients with hematologic malignancies, the survival probability was still significantly higher in patients who received an RR or RC graft than in those who received a CC graft ($P_{RR,RC/CC} = .0007$; Fig 1C). When compared

with the patients with acute myeloid leukemia (AML), patients with acute lymphoblastic leukemia (ALL) had similar survival probabilities among all three donor KIR2DL1 allelic groups (all $P \geq .71$; Fig 1D). The effects of donor KIR2DL1 alleles were comparable in the following subsets: sibling, unrelated, and haploidentical HSCT (Appendix Fig A1A; online-only); T-cell depleted and replete grafts (Appendix Fig A1B); and myeloablative HSCT (Appendix Fig A1C). The difference in nonmyeloablative HSCT was not significant statistically ($P_{RR,RC/CC} = .42$).

Table 2. Univariate Analysis of Mortality, PFS, and Acute GvHD

Variable	Mortality			PFS			Grade II-IV Acute GvHD		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Donor KIR2DL1			.0004			.0013			.62
CC	1			1			1		
RC	0.42	0.25 to 0.71	.0013	0.48	0.28 to 0.82	.0075	1.12	0.46 to 2.75	.8
RR	0.4	0.25 to 0.64	.0001	0.42	0.26 to 0.68	.0003	0.89	0.38 to 2.06	.78
Donor KIR haplotype			.92			.34			.16
Cen A/A	1			1			1		
Cen A/B	1.03	0.72 to 1.47	.87	1.11	0.76 to 1.61	.59	1.28	0.75 to 2.2	.36
Cen B/B	1.08	0.74 to 1.56	.69	1.33	0.9 to 1.95	.15	1.68	0.98 to 2.88	.057
Age, years									
< 10	1			1			1		
> 10	1.33	0.99 to 1.8	.06	1.17	0.85 to 1.59	.33	1.5	0.97 to 2.31	.067
Sex									
Female	1			1			1		
Male	0.95	0.7 to 1.29	.74	1.04	0.76 to 1.43	.79	1	0.64 to 1.56	.99
Race			.054			.11			.048
Black	1			1			1		
Other	0.66	0.38 to 1.14	.14	0.61	0.34 to 1.08	.092	1.18	0.51 to 2.74	.69
White	1.14	0.77 to 1.69	.51	1.01	0.67 to 1.52	.98	1.99	1.02 to 3.87	.044
Primary diseases			.0001						.0064
Nonmalignant	1						1		
Hematologic malignancy	2.32	1.4 to 3.84	.0011	1			1.4	0.75 to 2.61	.29
Solid tumors	4.26	2.26 to 8.01	.0001	1.67	1.07 to 2.6	.024	3.27	1.46 to 7.31	.0039
Hematologic malignancies									
ALL	1			1			1		
AML	1.04	0.71 to 1.52	.85	1.07	0.73 to 1.56	.72	1.17	0.66 to 2.06	.59
Malignant diseases status			.7			.079			.39
CR1	1			1			1		
CR2	1.31	0.82 to 2.11	.26	1.38	0.87 to 2.21	.17	0.64	0.3 to 1.41	.27
CR3/CR4	1.09	0.51 to 2.35	.82	1.32	0.64 to 2.74	.45	0.55	0.13 to 2.34	.42
Refractory/progressive	1.2	0.82 to 1.76	.35	1.67	1.13 to 2.46	.01	1.1	0.66 to 1.83	.7
Conditioning type									
Non-TBI	1			1			1		
TBI based	1.06	0.78 to 1.43	.7	0.78	0.57 to 1.07	.13	0.89	0.58 to 1.37	.59
Conditioning intensity									
Nonmyeloablative	1			1			1		
Myeloablative	0.61	0.44 to 0.85	.0034	0.57	0.41 to 0.8	.001	0.52	0.33 to 0.82	.0052
Donor type			.19			.26			.61
Haploidentical	1			1			1		
Sibling	0.71	0.49 to 1.03	.069	0.75	0.51 to 1.1	.14	0.9	0.53 to 1.55	.71
Unrelated	0.9	0.64 to 1.27	.56	0.79	0.55 to 1.14	.21	1.19	0.72 to 1.95	.5
T cell depleted									
No	1			1			1		
Yes	1.38	1.02 to 1.86	.034	1.29	0.95 to 1.76	.1	1.18	0.77 to 1.81	.45
Donor CMV status			.19			.57			.23
Negative	1			1			1		
Positive	1.26	0.93 to 1.71	.13	1.17	0.85 to 1.6	.33	1.45	0.92 to 2.28	.11
Unknown	0.72	0.29 to 1.78	.48	0.88	0.32 to 2.42	.81	1.71	0.6 to 4.85	.31

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CC, KIR2DL1-C²⁴⁵ homozygous; Cen, centromeric; CMV, cytomegalovirus; GvHD, graft-versus-host disease; HR, hazard ratio; KIR, killer-cell immunoglobulin-like receptors; PFS, progression-free survival; RC, KIR2DL1-R²⁴⁵/C²⁴⁵ heterozygous; RR, KIR2DL1-R²⁴⁵ homozygous; TBI, total-body irradiation.

Other than donor KIR2DL1 polymorphism, we found that primary disease ($P = .0001$), T-cell depletion ($P = .034$), and conditioning intensity ($P = .0034$) were also statistically significant in association with patient survival in univariate analysis (Table 2). After adjusting for all these non-KIR2DL1 factors, we found that survival was still significantly higher in patients receiving an RR (HR, 0.4; 95% CI, 0.25 to 0.64; $P = .0001$) or RC graft (HR, 0.44; 95% CI, 0.26 to 0.74; $P = .0024$) than in those who received a CC graft (Table 3).

Progression-Free Survival

In univariate analysis, the PFS in the 256 patients with cancer was significantly higher in those with an RR (HR, 0.42; 95% CI, 0.26 to 0.68; $P = .0003$) or RC donor (HR, 0.48; 95% CI, 0.28 to 0.82; $P = .0075$) than in those with a CC donor (Table 2). There was no statistical difference in the cumulative incidence of progression between the patients who received an RR graft or an RC graft (Fig 2A). When the analysis was limited to the 231 patients with hematologic

Fig 1. Survival of patients stratified by donor KIR2DL1 functional allelic groups. Shown are the overall survival of (A) the entire cohort of 313 transplantation recipients, (B) the 256 patients with malignant disease, (C) the 231 patients with a hematologic malignancy, and (D) the patients with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) displayed separately after receiving a KIR2DL1-R²⁴⁵ homozygous (RR), KIR2DL1-R²⁴⁵/C²⁴⁵ heterozygous (RC), or KIR2DL1-C²⁴⁵ homozygous (CC) graft. $P_{RR/RC}$, P value between RR and RC group; $P_{RR, RC/CC}$, P value comparing RR and RC groups with CC group; ALL:RR, patients with ALL who received an RR donor graft; ALL:RC, patients with ALL who received an RC donor graft; ALL:CC, patients with ALL who received a CC donor graft; AML:RR, patients with AML who received an RR donor graft; AML:RC, patients with AML who received an RC donor graft; AML:CC, patients with AML who received a CC donor graft; $P_{\times RR, RC/CC}$, P value when comparing RR and RC groups with CC group for patients with the diagnosis of AML or ALL as indicated; P_{RR} , P_{RC} , and P_{CC} , P values when comparing ALL and AML patients who received a similar RR, RC, or CC donor graft.

malignancies, the difference in progression rate was still significant statistically ($P_{RR, RC/CC} = .01$; Fig 2B). There was no significant difference in the risk of disease progression between AML and ALL patients among all three donor KIR2DL1 allelic groups (all $P \geq .54$; Fig 2C). After adjustment for other risk factors, the PFS remained significantly higher in patients with an RR ($P = .0001$) or RC donor ($P = .0049$) than in those with a CC donor (Table 3).

GvHD

There was no significant correlation between grade II to IV acute GvHD in the transplantation recipients and the donor KIR2DL1 polymorphism ($P_{RR/CC} = .78$; $P_{RC/CC} = .8$; Table 2). Of the patients who received an RR graft, 25.1% had grade II to IV GvHD. The corresponding rates were 31.6% in the RC and 27.3% in the CC groups.

Effects of Receptor-Ligand Mismatch

We then examined the effect of HLA-C receptor-ligand mismatch in the context of our KIR2DL1 polymorphism analysis. Among the 231 patients with hematologic malignancies, those with a donor who was RR

or RC in KIR2DL1 and was HLA-C receptor-ligand mismatched had the best 5-year survival rates (48%) when compared with those with a donor who was RR or RC in KIR2DL1 but was not HLA-C receptor-ligand mismatched (5-year survival rate, 31%) or those with a CC donor with or without receptor-ligand mismatch (5-year survival rate, 0%; Fig 3A). The cumulative incidence of diseases progression was the lowest in the group with donors who were RR or RC in KIR2DL1 and were HLA-C receptor-ligand mismatched (Fig 3B).

DISCUSSION

We found that patients who received an HSCT graft from donors with KIR2DL1-R²⁴⁵ had better overall survival and progression-free survival than those who received HSCT from donors with KIR2DL1-C²⁴⁵ alleles only. The outcome of HSCT was also dependent on other known factors, including primary disease, disease status, conditioning, and T-cell depletion. After adjusting for these prognostic factors, our analysis showed that the probability of patient survival was still

Table 3. Multivariate Analysis of Mortality, PFS, and Acute GvHD

Variable	Mortality			PFS			Grade II-IV Acute GvHD		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Donor KIR2DL1									
CC	1			1			1		
RC	0.44	0.26 to 0.74	.0024	0.45	0.26 to 0.78	.0049	0.59	0.23 to 1.49	.26
RR	0.4	0.25 to 0.64	.0001	0.38	0.23 to 0.63	.0001	0.46	0.19 to 1.09	.08
Age, years									
< 10	1						1		
> 10	1.2	0.88 to 1.63	.24				1.54	0.98 to 2.42	.06
Race									
Black	1			1			1		
Other	0.66	0.38 to 1.15	.14	0.63	0.35 to 1.15	.13	0.92	0.39 to 2.2	.85
White	1.05	0.7 to 1.57	.81	1.07	0.69 to 1.66	.75	1.48	0.75 to 2.92	.26
Primary diseases									
Nonmalignant	1						1		
Hematologic malignancy	1.94	1.15 to 3.27	.013	1			1.54	0.8 to 2.96	.2
Solid tumors	3.55	1.83 to 6.89	.0001	1.49	0.87 to 2.54	.15	2.98	1.25 to 7.13	.01
Malignant diseases status									
CR1				1					
CR2				1.29	0.8 to 2.07	.3			
CR3/CR4				1.31	0.62 to 2.76	.48			
Refractory/progressive				1.34	0.88 to 2.02	.17			
Conditioning intensity									
Nonmyeloablative	1			1			1		
Myeloablative	0.7	0.49 to 1.0	.049	0.67	0.46 to 0.99	.046	0.44	0.27 to 0.72	.001
T cell depleted									
No	1			1					
Yes	1.45	1.05 to 1.99	.023	1.3	0.92 to 1.85	.14			

Abbreviations: CC, KIR2DL1-C²⁴⁵ homozygous; GvHD, graft-versus-host disease; HR, hazard ratio; PFS, progression-free survival; RC, KIR2DL1-R²⁴⁵/C²⁴⁵ heterozygous; RR, KIR2DL1-R²⁴⁵ homozygous.

much higher if the donor was KIR2DL1-R²⁴⁵-positive. The effect of donor KIR2DL1 alleles seemed to be comparable in various subset analyses in Figure 1 and Appendix Figure A1; however, the numbers of patients in some subsets were small and similar investigations in other patient cohorts will be necessary to assess the generalizability of our findings. Previous studies, for example, have shown that KIR effect on relapse was only observed in adult patients with AML but not with ALL.⁷ Several pediatric studies, however, did not observe such a distinction between AML and ALL.^{18,23,33-35} In our study, the effect of KIR2DL1 allelic polymorphism on the patterns of survival estimates was similar in patients with childhood AML or ALL among all allele groups.

The better survival rates and cancer control in patients receiving grafts from donors with KIR2DL1-R²⁴⁵ alleles may be related to the strength of KIR signaling. We have recently shown that KIR2DL1-R²⁴⁵ alleles are functionally stronger than KIR2DL1-C²⁴⁵ alleles.²⁸ It has been documented in several studies that NK cells lacking inhibitory receptor-mediated NK cell licensing or arming are hyporesponsive against target cells.^{16,36} In keeping with these observations, we previously found that NK cells expressing KIR2DL1-C²⁴⁵ have significantly less degranulation and interferon gamma (IFN- γ) secretion than those with R²⁴⁵-containing alleles after coculture with identical target cells.^{28,29} Yawata et al³⁷ demonstrated that enhancement of IFN- γ response to missing-self mediated by HLA-C2 in normal donors was less in KIR2DL1*004 (KIR2DL1-C²⁴⁵) NK cells than in those with other KIR alleles. Taken together, these studies suggested that the

functionally stronger KIR2DL1-R²⁴⁵ alleles are apparently more potent than the weaker KIR2DL1-C²⁴⁵ alleles in licensing NK cells, resulting in better cancer control and patient survival after HSCT.

KIR2DL1 is present in the centromeric motif of typical group A KIR haplotypes but in only approximately half of the group B haplotypes.^{38,39} An alternative or complementary explanation of our findings is the association of KIR2DL1-R²⁴⁵ with the Cen-A-containing haplotypes and KIR2DL1-C²⁴⁵ with the Cen-B haplotypes. For instance, the common alleles KIR2DL1*003 and KIR2DL1*002 in Cen-A haplotypes are KIR2DL1-R²⁴⁵.³⁸⁻⁴⁰ Our findings of uniformly poor outcomes with KIR2DL1-C²⁴⁵ homozygous donors, however, cannot be explained entirely by the association with the Cen-B KIR haplotypes, because some Cen-B-associated KIR2DL1 alleles are KIR2DL1-C²⁴⁵ (eg, KIR2DL1*004 and *007), whereas others are KIR2DL1-R²⁴⁵ (eg, KIR2DL1*010).⁴¹ The frequency of these two groups of Cen-B-associated KIR2DL1 alleles was similar (approximately 12%) in our previous study.²⁸ Furthermore, some KIR2DL1-C²⁴⁵ alleles (eg, KIR2DL1*006) are not associated with particular Cen-A or Cen-B regions.⁴¹ In some previous studies, donor Cen-B was a favorable factor in HSCTs,^{1,42,43} whereas others observed a worse outcome.⁴⁴⁻⁴⁶ In our study, Cen-A/B haplotypes were not associated statistically with overall survival or PFS (all $P \geq .15$).

The level of transcripts and the proportion of NK cells expressing KIR2DL1 are dependent on the gene copy number.^{27,47} Thus, we hypothesized that recipients with an RR donor might have better outcome than those with an RC donor. Although transplantations

Fig 2. Cumulative incidence of disease progression after transplantations stratified by donor KIR2DL1 functional allelic groups. Shown are the cumulative incidence (CI) of disease progression or relapse in (A) 256 patients with malignancy, (B) 231 patients with hematologic malignancy, and (C) patients with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) displayed separately after receiving a KIR2DL1-R²⁴⁵ homozygous (RR), KIR2DL1-R²⁴⁵/C²⁴⁵ heterozygous (RC), or KIR2DL1-C²⁴⁵ homozygous (CC) graft. Deaths unrelated to disease were considered competing events. ALL:RR, patients with ALL who received an RR donor graft; ALL:RC, patients with ALL who received an RC donor graft; ALL:CC, patients with ALL who received a CC donor graft; AML:RR, patients with AML who received an RR donor graft; AML:RC, patients with AML who received an RC donor graft; AML:CC, patients with AML who received a CC donor graft; $P_{RR, RC/CC}$, P value when comparing RR and RC groups with CC group; $P_{RR/RC}$, P value between the RR and RC groups; P_{RR} , P_{RC} , and P_{CC} indicate the P values when comparing ALL and AML patients who received a similar RR, RC, or CC donor graft.

from an RC donor was associated with a 15% higher risk in PFS analyses compared with those from an RR donor (HR, 1.15; 95% CI, 0.79 to 1.64; $P = .48$), the difference was not statistically significant, in part because the effect size was relatively small (compared with a

Fig 3. Survival and cumulative incidence (CI) of disease progression stratified by donor KIR2DL1 allelic groups and the presence of HLA-C receptor-ligand mismatch. Shown are the (A) overall survival probability and (B) CI of disease progression or relapse among different groups of patients with hematologic malignancy. Group 1, patients with a KIR2DL1-R²⁴⁵ homozygous (RR) or KIR2DL1-R²⁴⁵/C²⁴⁵ heterozygous (RC) donor and HLA-C receptor-ligand mismatch; group 2, patients with a KIR2DL1 RR or RC donor but not HLA-C receptor-ligand mismatched; group 3, patients who received a KIR2DL1-C²⁴⁵ homozygous (CC) graft with or without HLA-C receptor-ligand mismatch. $P_{group 1/group 3}$, P value between group 1 and group 3; $P_{group 2/group 3}$, P value between group 2 and group 3.

2.5-fold difference in HR between RR and CC), and the number of HSCTs in this study is limited. Nevertheless, analyses of larger cohorts of patients are warranted, as NK cells from RC donors have an intermediate cytolytic phenotype in vitro when compared with RR or CC homozygous NK cells.²⁹

Leukemic relapse and patient survival after HSCT can also be predicted by the mismatches between donor inhibitory KIRs and recipient ligands.⁷⁻⁹ Based on the Memphis receptor-ligand model,^{9,18} patients who do not have a ligand for one or more inhibitory KIRs of the donor are predicted to have a low relapse rate and favorable survival probability. Our new findings support this model and show that receptor-ligand mismatch is also prognostic in the context of KIR2DL1 functional allele polymorphism. Patients with HLA-C receptor-ligand mismatch had better survival rates and leukemic control than those without a mismatch after receiving a donor graft with the strong KIR2DL1-R²⁴⁵ functional allele. In contrast, patients who received a donor graft with the weak KIR2DL1-C²⁴⁵ allele had poor survival, regardless of the presence or absence of receptor-ligand mismatch, in agreement with our prior laboratory finding that KIR2DL1-C²⁴⁵ NK cells are hyporeactive even in the setting of missing ligands.^{28,29}

In the field of donor HLA typing, we have witnessed the progression from low-resolution serology typing, to intermediate-resolution antigen typing, and finally to high-resolution allele typing. The advance in donor KIR typing is reminiscent of that in HLA typing. KIR-mediated alloreactivity was first predicted based on the donor HLA ligand repertoire.^{5,13} KIR genotyping was then used to directly measure the donor gene content.^{18,48} A KIR expression assay was subsequently implemented to select the donor with the largest number of receptor-ligand mismatched NK cells.⁴⁹ Now, based on the findings of our study, we propose to include functional allele typing in the selection of HSCT donors. This extension should not slow down donor work-up, because HLA and KIR typing can be performed using the same blood sample collected from potential donors during routine HLA matching. Functional KIR allele typing and KIR ligand typing can be performed simultaneously and can be completed in one day using the SNP assays, which are much cheaper and faster than existing high-resolution typing.²⁹ Importantly, this high-throughput SNP assay provides readouts that are informative in predicting NK cell activ-

ity and patient outcome in HSCT. Because the amount of DNA required is small (100 ng), KIR allele and ligand typing should also be feasible before or after cord blood units are banked.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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Appendix

Fig A1. Overall survival stratified by donor KIR2DL1 functional allelic groups. (A) Survival of patients who received a graft from a haploidentical (H), sibling (S), or unrelated (U) donor. (B) Survival of patients who received a T cell depleted (yes) or replete (no) graft. (C) Survival of patients who received a myeloablative (yes) or nonmyeloablative (no) conditioning. CC, KIR2DL1-C²⁴⁵ homozygous graft; P_{RR} , P_{RC} , and P_{CC} , P values when comparing patients who received a similar RR, RC, or CC donor graft, respectively; $P_{RR,RC/CC}^X$, P value when comparing the RR and RC groups with the CC group in the corresponding subset; RC, KIR2DL1-R²⁴⁵/C²⁴⁵ heterozygous graft; KIR2DL1-R²⁴⁵ homozygous graft.