Regular Article

TRANSPLANTATION

Haploidentical hematopoietic transplantation from KIR ligand–mismatched donors with activating KIRs reduces nonrelapse mortality

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Key Points

- Haploidentical transplantation from KIR ligand–mismatched donors with activating KIRs reduces nonrelapse mortality and improves survival.
- Activating KIR genetics should be considered when selecting donors for T cell–depleted haploidentical hematopoietic transplantation.

Because activating killer cell immunoglobulinlike receptors (KIRs) are heterogeneously expressed in the population, we investigated the role of donor activating KIRs in haploidentical hematopoietic transplants for acute leukemia. Transplants were grouped according to presence vs absence of KIR-ligand mismatches in the graft-vs-host direction (ie, of donor-vs-recipient natural killer [NK]-cell alloreactivity). In the absence of donor-vs-recipient NK-cell alloreactivity, donor activating KIRs had no effects on outcomes. In the 69 transplant pairs with donor-vs-recipient NK-cell alloreactivity, transplantation from donors with *KIR2DS1* and/or *KIR3DS1* was associated with reduced risk of nonrelapse mortality, largely infection related (*KIR2DS1* present vs absent: hazard ratio [HR], 0.25; $P = .01$; *KIR3DS1* present vs absent: HR, 0.18; $P = .006$), and better event-free survival (*KIR2DS1* present vs absent: HR, 0.31; $P = .011$; *KIR3DS1* present vs absent: HR, 0.30; $P = .008$). Transplantation from donors with *KIR2DS1* and/or *KIR3DS1* was also associated with a 50% reduction in infection rate ($P = .003$). In vitro analyses showed that KIR2DS1 binding to its HLA-C2 ligand upregulated inflammatory cytokine production by alloreactive NK cells in response to infectious challenges. Because ~40% of donors able to exert donor-vs-recipient NK-cell alloreactivity carry *KIR2DS1* and/or *KIR3DS1*, searching for them may become a feasible, additional criterion in donor selection. (*Blood*. 2015;125(20):3173-3182)

Introduction

Hematopoietic transplantation from human leukocyte antigen (HLA) haplotype–mismatched (haploidentical) donors is an established treatment of patients with high-risk acute leukemia who do not have a matched donor.[1-12]

In haploidentical, T cell–depleted peripheral blood stem cell transplants without posttransplant graft-versus-host disease (GVHD) prophylaxis,[3-5] donor natural killer (NK) cells play a beneficial role in outcomes.[13-17] Human NK cells possess clonally distributed, inhibitory receptors termed "killer cell immunoglobulinlike receptors" (KIRs), which recognize epitopes shared by groups of HLA-class I alleles (KIR ligands).[18-20] KIR2DL1 recognizes HLA-C group 2 alleles, KIR2DL2 and KIR2DL3 recognize HLA-C group 1 alleles, and KIR3DL1 is the receptor for HLA-Bw4 alleles. NK cells become fully functional ("licensed/educated") upon recognition of self-HLA.[18] KIRs are randomly expressed in the NK-cell repertoire. NK cells that express, as their only inhibitory receptor for self, a KIR for an HLA-class I group that is absent on allogeneic targets, sense the missing expression

of the self-HLA-class I KIR ligand and mediate alloreactions ("missing self" recognition).[21-24] In clinical hematopoietic stem cell transplantation, KIR-ligand mismatches in the graft-vs-host (GVH) direction trigger donor-vs-recipient NK-cell alloreactivity.[13-17] This occurs when the donor possesses an HLA-class I KIR ligand (either HLA-C groups 1 or 2 and/or the HLA-Bw4 group) that is absent in the recipient. Under such mismatch conditions, engrafted stem cells give rise to an NK-cell repertoire that is licensed by HLA KIR ligands on donor hematopoietic cells and, thus, enabled to recognize and react to missing self on recipient targets.[17,25] Donor-vs-recipient NK-cell alloreactions control leukemia relapse and improve survival in adult and pediatric patients.[13-17,26,27]

Homologs of the inhibitory KIRs, with shorter cytoplasmic tails, activating KIRs transduce signals that activate NK cells.[28-31] Activating *KIR* genes are variably present in the population. Approximately 25% of whites are homozygous for group *A KIR* haplotypes that contain the main inhibitory *KIR* genes and the activating *KIR2DS4* gene (however,

a *KIR2DS4* deletion variant allele encodes for a nonfunctional receptor in as much as ~80% of individuals).[32] The others are either heterozygous or homozygous for group *B KIR* haplotypes that carry not only inhibitory *KIR* genes but also various combinations of activating *KIR* genes (*KIR2DS1, 2, 3, 5,* and *3DS1*). Little is known about their ligands except that KIR2DS1 binds HLA-C group 2 molecules.[33,34] In vitro studies showed KIR2DS1 plays a role in NK cell killing of allogeneic targets that express HLA-C2.[27,34-37] Moreover, in clinical studies, activating *KIR*s were found to be associated with less leukemia relapse in matched unrelated and haploidentical transplants[38-43] and with protection against AIDS progression and hepatitis C virus infection.[44,45]

In the present study, we investigated the role of donor-activating KIRs in haploidentical transplants for acute leukemia grouped according to presence or absence of donor-vs-recipient NK-cell alloreactivity. We found that transplantation from donors who exerted donor-vs-recipient NK-cell alloreactivity and carried *KIR2DS1* and/or *KIR3DS1* genes was associated with markedly reduced nonrelapse mortality (NRM), which was largely infection related, and with significantly better event-free survival (EFS). Interestingly, our in vitro analyses showed that KIR2DS1 binding to HLA-C2 upregulated inflammatory cytokine production by alloreactive NK cells in response to infectious challenges.

Patients and methods

Patients

The role of the donor *KIR* genotype was retrospectively analyzed in 161 haploidentical hematopoietic transplants, 121 for acute myeloid leukemia (AML) and 40 for acute lymphoblastic leukemia (ALL), performed between 1993 and 2012. One-hundred fifty-nine patients had been included in previous studies.[3-7,13,14,16] Patients received haploidentical, T cell–depleted granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood CD34^{+} cell grafts.[3-7] Forty-five patients also received an infusion of donor regulatory and conventional T cells (Treg/Tcon).[6,7] No posttransplant GVHD prophylaxis was given. Approval was obtained from the Institutional Ethics Committee, and participants gave written informed consent in accordance with the Declaration of Helsinki.

GVHD was assessed by consensus criteria.[46] NRM was defined as death preceding leukemia relapse, and EFS was defined as the interval from transplantation to leukemia relapse, to death from all causes, or to last follow-up.

Donor selection

Candidate transplant donors were assessed for HLA compatibility by serology (*HLA-A, HLA-B*) and by high-resolution molecular analysis (*HLA-C, HLA-DR, HLA-DP, HLA-DQ*). The donor selection algorithm was: donor/recipient cytomegalovirus (CMV) serostatus, donor-vs-recipient NK-cell alloreactivity,[13,14,16] and donor/recipient relationship (maternal donors),[47] in this order.

Donors were considered able to exert donor-vs-recipient NK-cell alloreactivity (termed "NK alloreactive donors" in the paper) when: (1) *HLA-C* and *HLA-B* typing showed KIR-ligand mismatches in the GVH direction—that is, the donor possessed an HLA-class I KIR ligand (either HLA-C groups 1 or 2 and/ or HLA-B group Bw4) that was absent in the recipient; (2) donors possessed the relevant inhibitory *KIR* gene(s) for missing self-recognition of recipient targets (*KIR2DL2* and/or *KIR2DL3* were present in everyone, *KIR2DL1* was absent in ~3% of individuals, and *KIR3DL1* was absent in ~10%)[31]; (3) donors possessed alloreactive NK-cell clones against recipient targets (see NK-cell isolation, cloning, and allocytotoxicity assay). Because our previous analyses in large numbers of individuals had shown they possessed alloreactive NK clones against HLA-C group–mismatched targets,[16] in the present study, NK-cell cloning and allocytotoxicity assays were not considered necessary and were therefore not routinely performed in HLA-C group–mismatched transplant donors (however, this information was available for 29/60 HLA-C group–mismatched transplant

donors included in the present study). Thus, HLA typing and donor *KIR* genotyping were considered sufficient to define HLA-C group–mismatched transplant donors as "NK alloreactive." Because previous analyses had shown that alloreactive NK clones against HLA-Bw4–mismatched targets are undetectable in approximately one-third of KIR3DL1[+] individuals,[16] NK-cell cloning and allocytotoxicity assays were considered necessary and were therefore routinely performed.

KIR genotyping

Genomic DNA was prepared from donor peripheral blood mononuclear cells (PBMCs) using the Nucleospin Tissue Kit (Macherey-Nagel, Düren, Germany). *KIR* genes were typed as described elsewhere[16] or with the Olerup SSP *KIR* Genotyping kit (Olerup SSP AB, Stockholm, Sweden). *KIR* genes of the centromeric and telomeric segments of *A* and *B* haplotypes[48-50] were analyzed as described elsewhere.[39] Figure 1 shows the genomic organization of the *KIR* locus. Donor *KIR* genotypes are indicated as *A/A* when they did not contain *B* haplotypes; the centromeric segment is termed "*Cen-A/A*" and the telomeric "*Tel-A/A*." Donor *KIR* genotypes are indicated as *B/x* when they contained at least 1 *B* haplotype; the centromeric segments are termed "*Cen-B/x*" and the telomeric "*Tel-B/x*."

NK-cell isolation, cloning, and allocytotoxicity assay

NK cells were isolated from PBMCs using the Human NK Isolation Kit (Miltenyi Biotech, Bergisch Gladbach, Germany). Limiting dilution cloning and cytotoxicity assays determined alloreactive NK-cell repertoires in 110 HLA-C1[+] healthy individuals (that included 10 HLA-C1–mismatched transplant donors) against allogeneic HLA-C1–missing cell targets. NK cells were plated under limiting-dilution conditions, activated with phytohemagglutinin (PHA) (Biochrom AG, Berlin, Germany), and cultured with interleukin-2 (Novartis Farma S.p.A., Origgio, Italy) and irradiated feeder cells. NK clones were screened for alloreactivity by [51]Cr (PerkinElmer Inc., Waltham, MA) release assay against PHA T-cell blasts or cryopreserved primary AML cells at an effector-to-target (E:T) ratio of 10:1. Clones exhibiting >30% lysis were scored as alloreactive.

Flow cytometry

FACSCanto flow cytometer with FACSDiva software (BD Biosciences, San José, CA) and FlowJo software (Tree Star Inc., Ashland, OR) were used for multicolor immunofluorescence analyses and intracellular staining.

NK clones that were alloreactive against HLA-C1–missing cell targets were phenotyped with the following monoclonal antibodies (mAbs): anti-CD56-APC (clone NKH-1, IgG1), anti-CD3-PE-Cy7 (clone UCHT1, IgG1) (Beckman Coulter Inc., Fullerton, CA), and anti-KIR2DL2/L3/S2-FITC (clone CH-L, IgG2b) (BD Biosciences) in combination with either anti-KIR2DL1/S1-PE (clone EB6B, IgG1), anti-KIR3DL1/S1-PE (clone Z27, IgG1), anti-NKG2A-PE (clone Z199, IgG2b) (Beckman Coulter Inc.), or purified anti-KIR2DL3 (clone ECM-41, IgM, courtesy of Daniela Pende, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy) conjugated with anti-mouse IgM-PE (Southern Biotech, Birmingham, AL). Anti-KIR2DL1/S1-PE (clone EB6B) was used in combination with anti-KIR2DL1-FITC (clone #143211, IgG1) (R&D Systems Inc., Minneapolis, MN) to distinguish between KIR2DL1 and KIR2DS1 expression. Anti-KIR3DL1/S1-PE (clone Z27) combined with anti-KIR3DL1-FITC (clone DX9, IgG1) (Miltenyi Biotech) discriminated between KIR3DL1 and KIR3DS1 expression. Alloreactive NK clones that coexpressed KIR3DS1 were not considered when investigating killing of HLA-C1–missing targets.

KIR gene expression

Gene expression confirmed KIR phenotypes in randomly selected NK clones. RNA was extracted from NK-cell clones using RNAqueous-4PCR kit (Life Technologies, Carlsbad, CA) and reverse transcribed with iScript Reverse Transcription Supermix (Bio-Rad Laboratories, Hercules, CA). cDNA was subjected to real-time polymerase chain reaction analysis using the specific primer set for the *KIR* genes published elsewhere[51] and SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories). Amplification and

KIR Haplotypes	Cen	Centromeric Segment									Telomeric Segment							Tel	KIR Haplotypes
		3DL3	2DS2	2DL2/3	2DL5B	2DS3/5	2DP1	2DL1	3DP1		2DL4	3DL1/S1	2DL5A	2DS3/5	2DS1	2DS4	3DL2		
A	A			2DL3			2DP1	2DL1				3DL1				2DS4		A	A
B	A			2DL3			2DP1	2DL1				3DS1	2DL5A	2DS3/5	2DS1			B	B
	B		2DS2	2DL2								3DL1				2DS4		A	
	B		2DS2	2DL2								3DS1	2DL5A	2DS3/5	2DS1			B	
	B		2DS2	2DL2	2DL5B	2DS3/5	2DP1	2DL1				3DL1				2DS4		A	
	B		2DS2	2DL2	2DL5B	2DS3/5	2DP1	2DL1				3DS1	2DL5A	2DS3/5	2DS1			B	

Figure 1. Genomic organization of the *KIR* locus. *KIR* genes segregate into groups *A* and *B* haplotypes. Framework genes located at the ends and in the central part of the locus (gray boxes) define 2 haplotype segments: the centromeric (Cen) and the telomeric (Tel). Common centromeric and telomeric segments are shown. In the *A* haplotype, *KIR2DL1* and *KIR2DL3* are found in the centromeric segment and *KIR3DL1* and *KIR2DS4* in the telomeric. Combinations of *KIR2DL1/L2/L5/S2/S3/S5* are found in *B*-haplotype centromeric segments and combinations of *KIR2DL5/S1/S3/S5* and *KIR3DS1* in *B*-haplotype telomeric segments.

data acquisition were carried out on a Chromo4 PTC-200 Thermal Cycler (Bio-Rad Laboratories). The $2^{-\Delta\Delta C_T}$ method was used for relative quantification to reference gene expression.

Dendritic cell generation and phenotyping

Monocytes were purified from healthy individual PBMCs by positive anti-CD14 immuno-magnetic selection (Miltenyi Biotech) and plated at a 1×10^6 cells/mL. To generate immature myeloid CD11c$^+$ CD14$^-$ CD1a$^+$ dendritic cells (DCs), monocytes were cultured for 6 days in the presence of 1000 UI/mL recombinant granulocyte-macrophage colony-stimulating factor and 500 UI/mL rIL-4 (Miltenyi Biotech). To generate CD1a$^+$ CD83$^+$ CD86$^+$ mature DCs, immature DCs were stimulated with 2 μg/mL lipopolysaccharide (LPS) (Sigma-Aldrich/Merck, St. Louis, MO) for 24 hours or were exposed to live conidia of *A fumigatus* at a DC/fungi ratio of 1:1 for 24 hours, as described elsewhere.[52]

Myeloid DCs were phenotyped with the following mAbs: anti-CD14-FITC (clone TUk4, IgG2a) (Miltenyi Biotech); and anti-CD11c-FITC (clone 3.9, IgG1), anti-CD1a-PE (clone HI149, IgG1), anti-CD83-APC (clone HB15e, IgG1), anti-CD86-PE (clone IT2.2, IgG2b) (eBiosciences, San Diego, CA).

NK cell cytokine production

Freshly isolated NK cells were incubated with DCs at an E:T ratio of 1:2 for 6 hours in the presence of Golgi Stop (BD Biosciences) according to kit instructions. Interferon (IFN)-γ production was analyzed by intracellular staining. Because available antibodies do not discriminate among KIR2DL2, KIR2DL3, and KIR2DS2, *KIR2DL3*-homozygous individuals were used as NK-cell donors. Individuals with known anti-KIR2DS1/DL1 mAb (clone EB6) crossreactivity with a *KIR2DL3* allele were excluded.[53] NK cells that were potentially alloreactive against HLA-C1–missing cell targets were identified with the following combination of mAbs: anti-CD56-PE-Cy7 (clone NCAM16.02, IgG1) (BD Biosciences), anti-KIR2DL2/L3/S2-FITC (clone CH-L, IgG2b), anti-KIR3DL1/S1-PE (clone Z27), anti-NKG2A-PE (clone Z199), anti-KIR2DL1-PE (clone #143211, IgG1), anti-KIR3DL1/L2-PE (clone 5.133, IgG1), and anti-KIR2DS4-PE (clone JJC11.6, IgG1) (Miltenyi Biotech). Subsequently, anti-KIR2DL1/S1-APC mAb (clone EB6B, IgG1) (Beckman Coulter Inc.) was added to discriminate between KIR2DL1 and KIR2DS1, as described elsewhere.[34] Cells were fixed and permeabilized with BD Cytofix/Cytoperm Plus Fixation/Permeabilization kit (BD Biosciences) and stained with anti-IFN-γ-PerCp-Cy5.5 (clone B27, IgG1) (BD Biosciences). Fluorescence compensation for spectral overlap was optimized with BD anti-mouse Ig, κ compensation beads set (BD Biosciences).

For IFN-γ production–blocking experiments, an average of 3 alloreactive NK clones per individual were pooled and incubated with DCs at an E:T ratio of 1:1 for 4 hours, in the presence or absence of anti-KIR2DL1/S1 (clone EB6B) F(ab')$_2$ (courtesy of Francois Romagné, Innate-Pharma, Marseille, France).[54] Human IFN-γ concentrations were measured in cell-culture supernatants by an enzyme-linked immunosorbent assay kit (Life Technologies).

Statistical analyses

Demographics and prognostic variables were compared using the Fisher exact test, the χ^2 test for categorical variables, and the Student *t* test or Mann-Whitney *U* test for continuous variables. The Kaplan-Meier method evaluated EFS. A log-rank test assessed the impact of donor *KIR* genotype on EFS.

Cumulative incidence estimates were used for relapse, NRM, because they are competing risks. The cumulative incidences of acute GVHD and infectious mortality were calculated using death from any cause as a competing risk. The Gray test compared univariate competing risk outcomes.[55]

Multivariate analyses assessed the impact of donor-vs-recipient NK-cell alloreactivity and donor *KIR* genotype. They included the following variables: disease, Treg/Tcon infusions, disease status at transplant (remission vs relapse), patient age, patient and donor gender, conditioning regimens, CD34$^+$ and CD3$^+$ cell-graft content, donor/recipient CMV serostatus, and donor/recipient relationship (maternal donor vs others). Variables were included in a Cox regression model in a conditional forward stepwise fashion to identify factors with a significant impact on outcomes. Donor-vs-recipient NK-cell alloreactivity and disease status at transplant were included in the final model for relapse. Donor-vs-recipient NK-cell alloreactivity, disease status at transplant, and patient age per increasing year were included in the final model for EFS. When evaluating outcomes of transplants from NK-alloreactive donors, donor *KIR* genotype and donor/recipient relationship were included in the final model for NRM and infectious mortality; donor *KIR* genotype, disease status at transplant, donor/recipient relationship, and donor/recipient CMV status were included in the final model for EFS.

All *P* values are 2-sided and considered significant at *P* < .05.

Results

Overall clinical outcomes

Transplant characteristics are shown in supplemental Table 1, available on the *Blood* Web site. Primary engraftment was obtained in 155 of 161 patients. Rejection in 6 patients was reversed with a second transplant from the same or another haploidentical donor. Incidence of relapse was 23%. Incidence of NRM was 38.5%, owing to the fact that 62 patients died in remission, 51 of infections. The incidence of NRM was stable over time: 39% in transplants performed from 1993 to 1999, 36% from 2000 to 2006, and 43% from 2007 to 2012. Grade II-IV acute GVHD developed in 19 patients (12%), with 6 progressing to chronic GVHD. De novo chronic GVHD developed in 2 patients. At a mean follow-up of 4.07 years (range, 0.03-21.32), the probability of EFS was 38.5%.

Sixty-nine patients received transplants from NK alloreactive donors and 92 patients from non–NK-alloreactive donors. In agreement with previous studies,[13,14,16] multivariate analyses showed that donor-vs-recipient NK-cell alloreactivity was associated with reduced relapse (transplantation from NK-alloreactive donors vs non–NK-alloreactive donors—HR, 0.42; 95% confidence interval [CI], 0.18-0.96; *P* = .04) and improved EFS in patients with AML (but not ALL) (transplantation from NK-alloreactive donors vs non–NK-alloreactive donors—HR, 0.60; 95% CI, 0.37-0.96; *P* = .034).

Transplantation from NK-alloreactive donors with activating *KIR* genes reduces NRM and improves EFS

We first evaluated the role of donor *B* vs *A KIR* haplotype centromeric segments (termed *Cen-B/x* and *Cen-A/A*, respectively) and telomeric

segments (termed *Tel-B/x* and *Tel-A/A*, respectively) on transplantation outcomes in the whole series of 161 transplants and found no effects on EFS. We therefore analyzed transplants grouped according to the presence or absence of donor-vs-recipient NK-cell alloreactivity. We found no effects on EFS in the 92 transplants from non–NK-alloreactive donors (*Cen-B/x* vs *Cen-A/A*: 24.6% vs 41.9%, *P* = NS; *Tel-B/x* vs *Tel-A/A*: 32.5% vs 28.8%, *P* = NS).

In contrast, donor activating *KIR* genotypes favorably affected outcomes of the 69 transplants from NK-alloreactive donors. Characteristics of patients transplanted from NK-alloreactive donors are shown in Table 1. Compared with transplantation from NK-alloreactive donors carrying *Tel-A/A* genes, transplantation from NK-alloreactive donors with *Tel-B/x* genes improved EFS (at 5 years after transplant, *Tel-B/x* vs *Tel-A/A*, 65.4% vs 39.6%; *P* = .013) (Figure 2A). Cox models incorporating interaction terms demonstrated that the effect of *Tel-B/x* genes depended on donor-vs-recipient NK-cell alloreactivity (*P* = .03). Transplantation from NK-alloreactive donors with *Tel-B/x* genes did not affect leukemia relapse (*Tel-B/x* vs *Tel-A/A*, 19.2% vs 11.6%; *P* = NS) (Figure 2B) or GVHD (*Tel-B/x* vs *Tel-A/A*, 11.5% vs 9.5%, *P* = NS). In fact, it reduced NRM (at 5 years after transplant, *Tel-B/x* vs *Tel-A/A*, 15.4% vs 48.8%; *P* = .005) (Figure 2C). *B* vs *A* haplotype centromeric *KIR* genes did not affect EFS (*Cen-B/x* vs *Cen-A/A*, 48.8% vs 50%; *P* = NS). Multivariate analyses showed that transplantation from NK-alloreactive donors with *Tel-B/x* genes was an independent factor predicting lower NRM (*Tel-B/x* vs *Tel-A/A*: HR, 0.23; 95% CI, 0.08-0.66; *P* = .007) and better EFS (*Tel-B/x* vs *Tel-A/A*: HR, 0.33; 95% CI, 0.14-0.76; *P* = .009). The other independent variables that improved EFS in NK-alloreactive transplants were maternal donors (HR, 0.25; 95% CI, 0.07-0.86; *P* = .028) and disease remission at transplant (HR, 0.45; 95% CI, 0.21-0.95; *P* = .037), with maternal donors affecting NRM (HR, 0.13; 95% CI, 0.02-0.99; *P* = .05) and disease remission affecting relapse (HR, 0.17; 95% CI, 0.04-0.75; *P* = .019). Multivariate analyses also showed that transplantation from donors with *KIR2DS1* and/or *KIR3DS1* (present only in *B*-haplotype telomeric segments) was associated with less NRM (*KIR2DS1* present vs absent: HR, 0.25; 95% CI, 0.08-0.72; *P* = .01; and *KIR3DS1* present vs absent: HR, 0.18; 95% CI, 0.05-0.62, *P* = .006) and better EFS (*KIR2DS1* present vs absent: HR, 0.31; 95% CI, 0.13-0.76; *P* = .011; and *KIR3DS1* present vs absent: HR, 0.30; 95% CI, 0.12-0.73; *P* = .008).

Infectious mortality accounted for 80% of NRM. In fact, transplantation from NK-alloreactive donors with *Tel-B/x* genes decreased infectious mortality (*Tel-B/x* vs *Tel-A/A*: 7.7% vs 41.9%, *P* = .0027) (Figure 3A). In multivariate analysis, it was an independent factor predicting lower infectious mortality (*Tel-B/x* vs *Tel-A/A*: HR, 0.13; 95% CI, 0.03-0.58; *P* = .007). Moreover, transplantation from NK-alloreactive donors with *Tel-B/x* genes was associated with a 50% reduction in the infection rate per month (*Tel-B/x* vs *Tel-A/A*: 0.91 ± 0.81 vs 0.45 ± 0.38; *P* = .003) (Figure 3B). The protective effect was mainly exerted on viral infection (*Tel-B/x* vs *Tel-A/A*: 0.45 ± 0.51 vs 0.20 ± 0.22; *P* = .0014) and, to a lesser extent, on fungal infections (*Tel-B/x* vs *Tel-A/A*: 0.22 ± 0.42 vs 0.07 ± 0.11; *P* = .09). Analysis of the ratio between the infection rate in patients transplanted from *Tel-B/x* donors and patients transplanted from *Tel-A/A* donors showed the protective effect was evident from the second to the fifth month after transplant (Figure 3C). Donor *B* vs *A* centromeric genotypes did not affect the infection rate (0.72 vs 0.73).

KIR2DS1 binding to HLA-C2 triggers cytokine production in alloreactive NK cells

Because KIR2DS1 is the only activating KIR for which binding to an HLA ligand (HLA-C2) is well documented,[33,34] cytotoxicity and cytokine production were evaluated in alloreactive NK cells from HLA-C1[+] healthy individuals upon triggering by HLA-C1–missing, HLA-C2/C2 targets.

Our present data show that frequencies of alloreactive NK-cell clones against HLA-C1–missing, HLA-C2/C2 PHA T-cell blasts in 45

Table 1. Characteristics of transplants with donor-vs-recipient NK-cell alloreactivity

	Donor *KIR* genotype*			
	Cen-A/A, n = 28	*Cen-B/x,* n = 41	*Tel-A/A,* n = 43	*Tel-B/x,* n = 26
Patient sex, n (%)				
Male	12 (43)	20 (49)	19 (44)	13 (50)
Female	16 (57)	21 (51)	24 (56)	13 (50)
Median age (range), years	42 (14-58)	35 (14-62)	39 (16-62)	30.5 (14-59)
Disease, n (%)				
AML	23 (82)	37 (90)	36 (84)	24 (92)
ALL	5 (18)	4 (10)	7 (16)	2 (8)
Disease status at transplant, n (%)				
High-risk† complete remission	21 (75)	28 (68)	28 (65)	21 (81)
Chemoresistant relapse‡	7 (25)	13 (32)	15 (35)	5 (19)
CMV serostatus (donor/recipient), n (%)				
Positive/positive	26 (93)	36 (88)	39 (91)	23 (88)
Positive/negative	1 (3.5)	2 (5)	2 (5)	1 (4)
Negative/positive	0 (0)	2 (5)	1 (2)	1 (4)
Negative/negative	1 (3.5)	1 (2)	1 (2)	1 (4)
Donor/recipient relationship, n (%)				
Maternal donor	6 (21)	7 (17)	7 (16)	6 (23)
Other donor	22 (79)	34 (83)	36 (84)	20 (77)
Transplant protocol, n (%)				
T cell–depleted§	20 (71)	33 (80)	33 (77)	20 (77)
Treg/Tcon¶	8 (29)	8 (20)	10 (23)	6 (23)
Conditioning regimen#, n (%)				
T cell–depleted protocol				
TBI, thiotepa, fludarabine, antithymocyte globulin**	20 (71)	33 (80.5)	33 (77)	20 (77)
Treg/Tcon protocol				
TBI, thiotepa, fludarabine, cyclophosphamide	5 (18)	7 (17)	7 (16)	5 (19)
TBI, thiotepa, fludarabine, alemtuzumab, or thymoglobulin	3 (11)	1 (2.5)	3 (7)	1 (4)
Graft composition (mean ± SD)				
CD34^{+} × 10[6]/kg	10.2 ± 3.3	11.9 ± 4.4	11.1 ± 4.0	11.4 ± 4.2
CD3^{+} × 10[4]/kg	2.3 ± 2.2	3.2 ± 3.9	2.5 ± 2.8	3.3 ± 4.0

TBI, total body irradiation.

*Donor *KIR* genotypes are indicated as *A/A* when they did not contain *B* haplotypes; the centromeric segment is termed "*Cen-A/A*" and the telomeric "*Tel-A/A*." Donor *KIR* genotypes are indicated as *B/x* when they contained at least 1 *B* haplotype; the centromeric segments are termed "*Cen-B/x*" and the telomeric "*Tel-B/x*." Distribution of variables did not differ significantly in transplants from *Cen-A/A* vs *Cen-B/x* donors and *Tel-A/A* vs *Tel-B/x* donors (*P* > .05).

†Myelodysplasia or unfavorable cytogenetics or first-line induction therapy failure in first to third complete remission.

‡Morphologic evidence of leukemic cells in the bone marrow and/or blood after treatment.

§T cell–depleted granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood CD34^{+} hematopoietic progenitor cell graft.[3-5]

¶T cell–depleted G-CSF–mobilized peripheral blood CD34^{+} hematopoietic progenitor cell graft and Treg/Tcon infusion.[6,7]

#See supplemental Table 1 for details on conditioning regimens.

**Four patients received cyclophosphamide instead of fludarabine.[3]

Figure 2. Transplantation from NK-alloreactive donors with *B*-haplotype telomeric *KIR* genes reduced NRM and improved EFS. (A) Probability of EFS; (B) cumulative incidence of leukemia relapse; and (C) cumulative incidence of NRM. *Tel-B/x*, 26 patients transplanted from NK-alloreactive donors with *B*-haplotype telomeric *KIR* genes; *Tel-A/A*, 43 patients transplanted from donors with *A*-haplotype telomeric *KIR* genes.
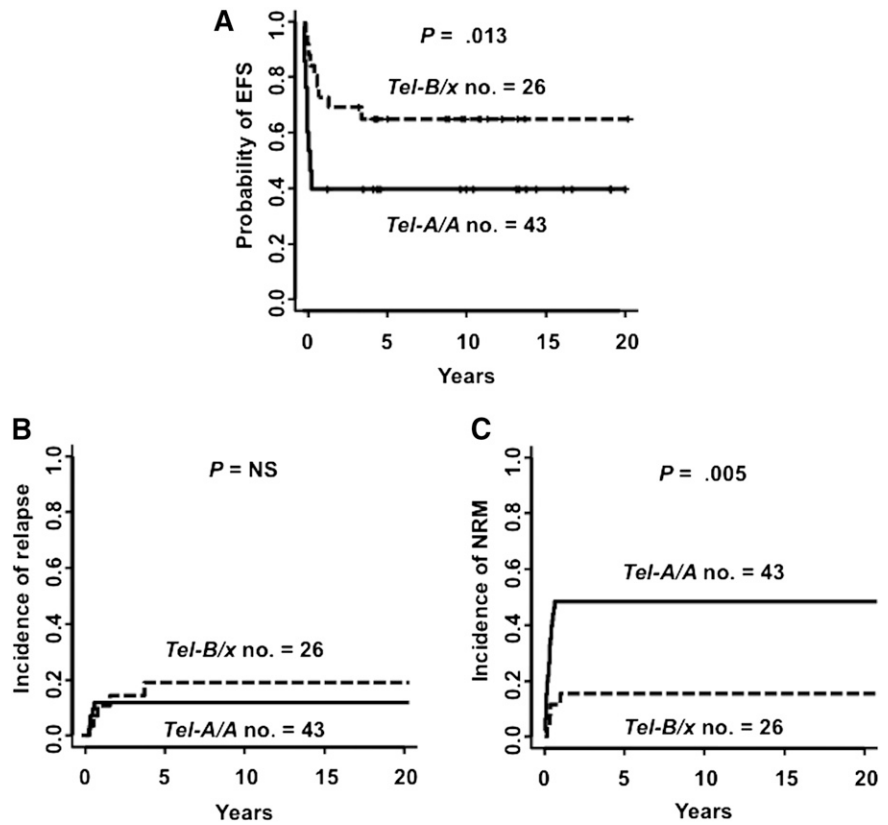

individuals with the *KIR2DS1* gene did not differ significantly from those in 65 individuals without the *KIR2DS1* gene (Figure 4A). Next, such analysis was performed separately in individuals possessing the *KIR2DL2* and/or *KIR2DL3* inhibitory receptor genes. In fact, in HLA-C1$^+$ individuals, NK cells that are alloreactive against HLA-C1–missing targets express KIR2DL2 and/or KIR2DL3 as their only
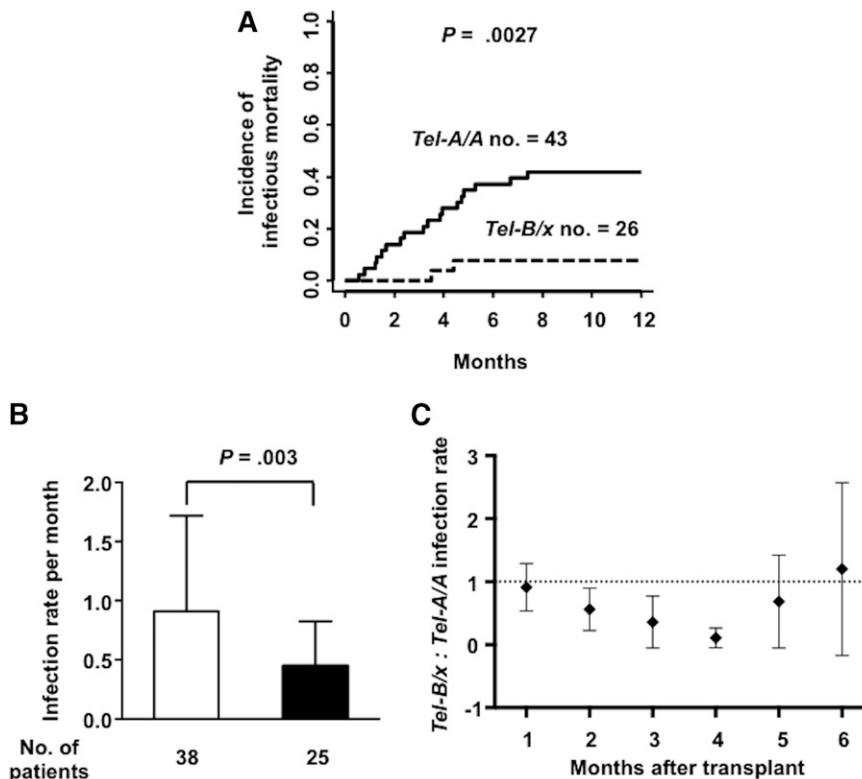

Figure 3. Transplantation from NK-alloreactive donors with *B*-haplotype telomeric *KIR* genes reduced infectious mortality and morbidity. (A) Cumulative incidence of infectious mortality in the 26 patients transplanted from NK-alloreactive donors with *Tel-B/x* genes and in the 43 patients transplanted from NK-alloreactive donors with *Tel-A/A* genes. Eighteen of 43 patients transplanted from donors with *Tel-A/A* genes died of infection (1 bacterial, 1 *Toxoplasma gondii*, 4 invasive fungal, 4 CMV, 2 human herpesvirus 6, 1 Epstein-Barr virus, 1 parainfluenza virus, 4 pneumonia and acute respiratory distress syndrome of unknown etiology). Only 2 of 26 patients transplanted from donors with *Tel-B/x* genes died of infection (1 CMV and 1 concomitant viral and fungal infection). (B) Data on infectious episodes were available for 63 of 69 patients transplanted from NK-alloreactive donors. Number of infectious episodes per month (mean ± standard deviation [SD]) in the first 6 months after transplant in patients transplanted from NK-alloreactive donors with *Tel-A/A* (open bar) and *Tel-B/x* genes (closed bar) are shown. Difference between the groups was tested by unpaired Student *t* test. (C) Ratios (mean with 95% CI) between the infection rates of patients transplanted from NK-alloreactive donors with *Tel-B/x* (diamonds) and infection rates of patients transplanted from NK-alloreactive donors *Tel-A/A* (dotted line) as evaluated monthly during the first 6 months after transplant.
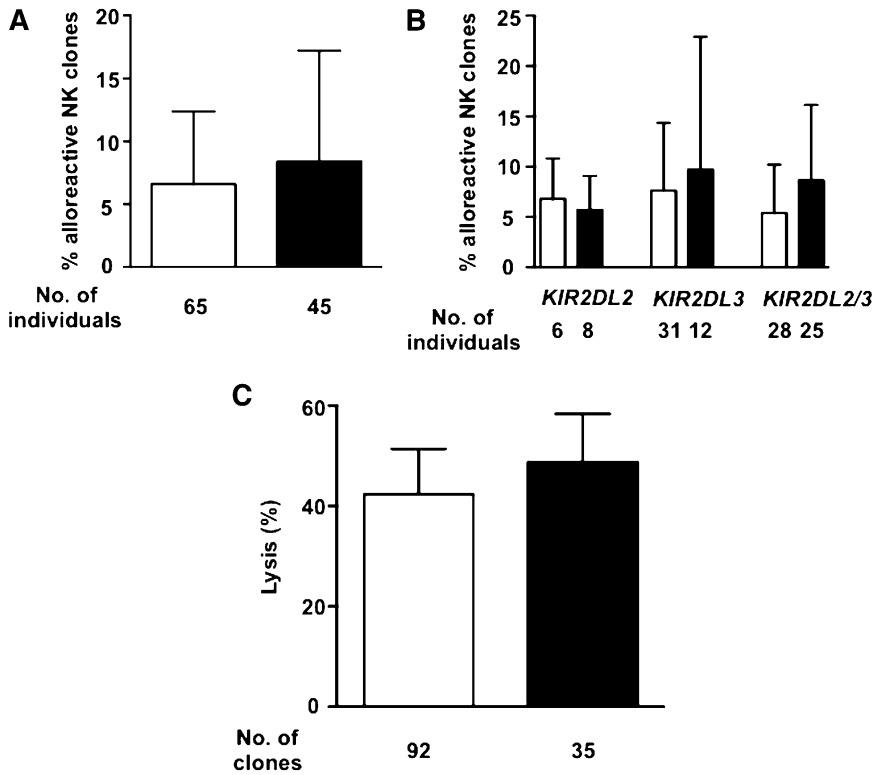
Figure 4. KIR2DS1 does not affect alloreactive NK-cell repertoires. (A) A total of 11 999 NK-cell clones were generated from 110 HLA-C1+ individuals (mean 109 clones/individual [30-302]) and were screened for cytotoxicity against HLA-C1–missing, HLA-C2/C2 PHA T-cell blasts. Frequencies (mean ± SD) of alloreactive NK-cell clones in the 65 individuals without the *KIR2DS1* gene (open bar) and in the 45 individuals with the *KIR2DS1* gene (closed bar) are shown (*P* = NS by unpaired Student *t* test). (B) The *KIR2DS1−* and *KIR2DS1+* individuals in (A) were grouped according to their *KIR2DL2* or *KIR2DL3* homozygous or *KIR2DL2/3* heterozygous inhibitory genotypes. Frequencies of alloreactive NK-cell clones in *KIR2DS1−* (open bar) and *KIR2DS1+* (closed bar) individuals are shown (*P* = NS by unpaired Student *t* test). (C) A total of 2281 clones obtained from 20 HLA-C1+ individuals (randomly selected from the 110 in [A]) were screened for allo-cytotoxicity against HLA-C1–missing, HLA-C2C2 PHA T-cell blasts. The contribution of KIR2DS1 to lysis was evaluated in 127 allocytotoxic NK-cell clones that expressed KIR2DL2/L3 (as evaluated by phenotypic analyses). Lysis (mean ± SD) by the 92 clones that did not express KIR2DS1 (open bar) and the 35 clones that expressed KIR2DS1 (closed bar) is shown.
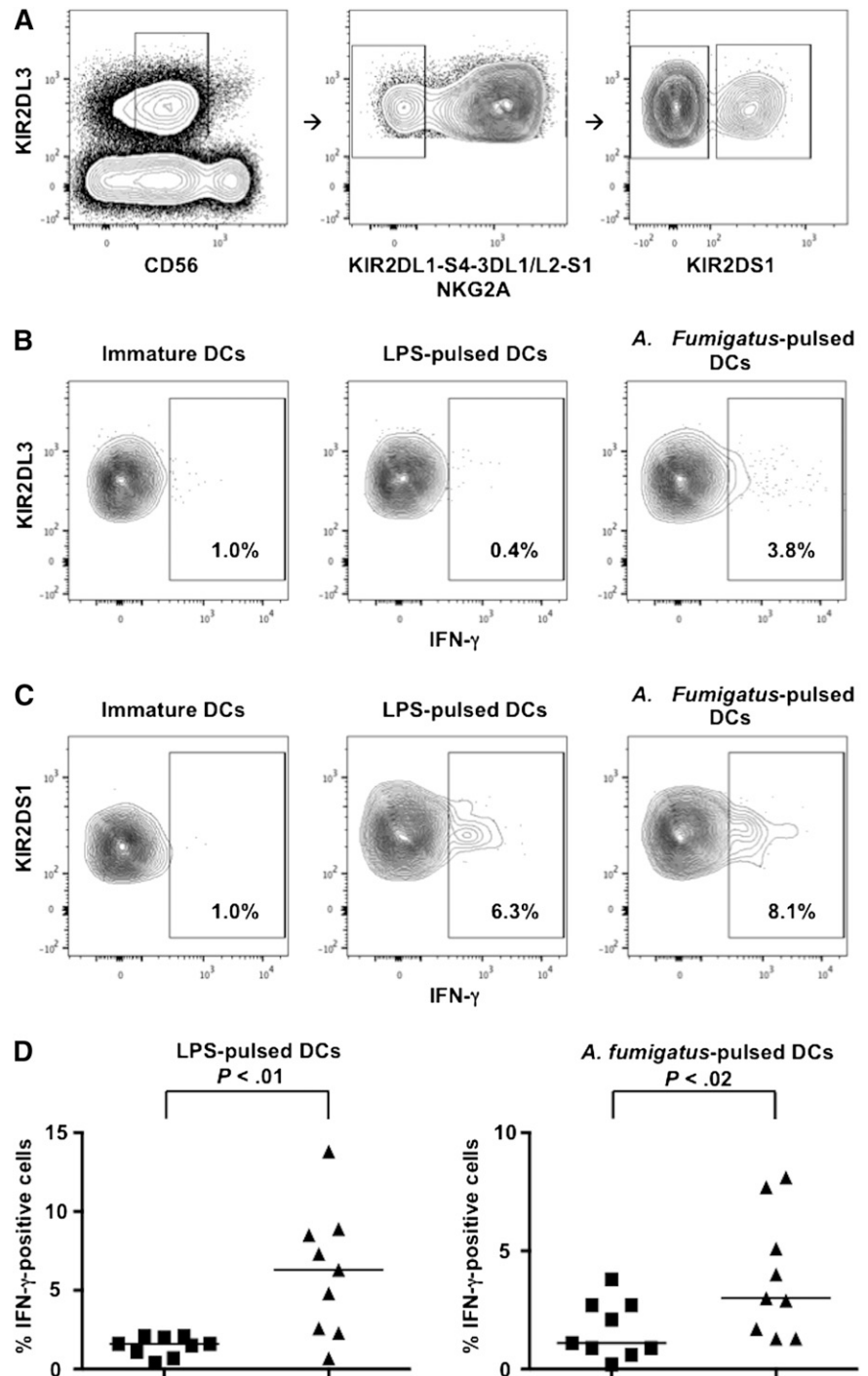
inhibitory receptor(s) for self–HLA-C1. KIR2DL2, and to a lesser extent KIR2DL3, crossreact with HLA-C2 molecules, thereby possibly hampering allorecognition of HLA-C1–missing, HLA-C2C2 targets.[27,56] Figure 4B shows that *KIR2DS1* did not significantly affect the frequencies of alloreactive NK-cell clones in *KIR2DL2* or *KIR2DL3*

homozygous or *KIR2DL2/3* heterozygous individuals. Clones that expressed KIR2DS1 killed targets with equal efficacy than clones that did not express KIR2DS1 (Figure 4C). Finally, clones that expressed KIR2DS1 killed primary AML cells as efficiently as those that did not express KIR2DS1 (Figure 5).
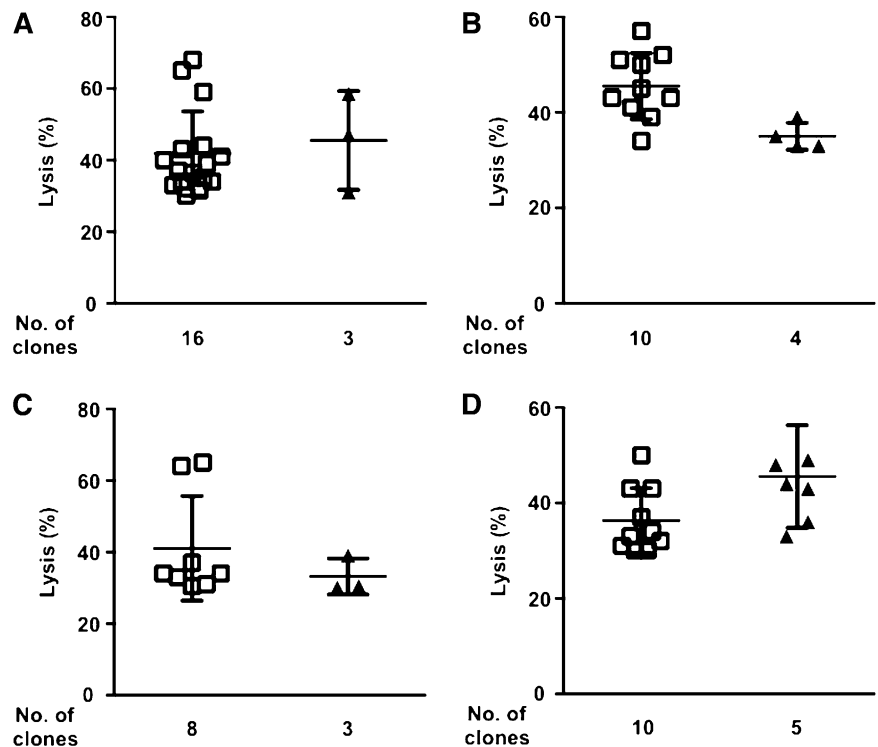
Figure 5. KIR2DS1 does not affect NK-cell alloreactivity against AML cells. 1265 NK-cell clones from 10 HLA-C1+ individuals were screened for cytotoxicity against a panel of 4 HLA-C1–missing, HLA-C2/C2 AML (A-D). The contribution of KIR2DS1 to lysis was evaluated in 59 allocytotoxic NK-cell clones that expressed KIR2DL2/L3 (as evaluated by phenotypic analyses). Lysis (mean ± SD) exerted by KIR2DS1− (squares) and KIR2DS1+ alloreactive NK-cell clones (triangles) is shown. (A) Lysis by 19 alloreactive NK-cell clones of 270 clones obtained from 2 individuals. (B) Lysis by 14 alloreactive NK-cell clones of 224 clones obtained from 2 individuals. (C) Lysis by 11 alloreactive NK-cell clones of 258 clones obtained from 3 individuals. (D) Lysis by 15 alloreactive NK-cell clones of 479 clones obtained from 3 individuals.
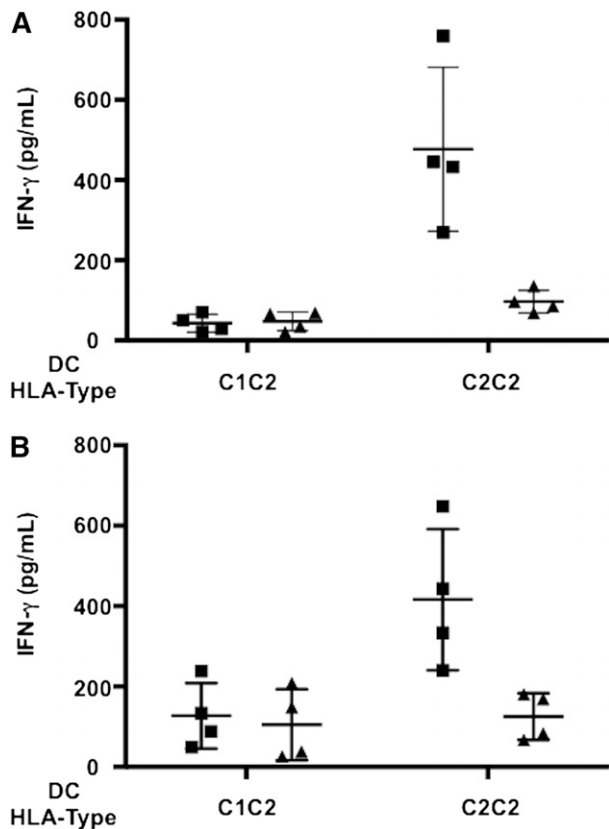
Figure 6. KIR2DS1⁺ alloreactive NK cells produce more IFN-γ than KIR2DS1⁻ alloreactive NK cells. Resting NK cells were incubated with immature or LPS- or *A fumigatus*–pulsed HLA-C1–missing, HLA-C2/C2 DCs. Cells were stained with the following mAb combination: anti-CD56-PE-Cy7, anti-KIR2DL2/L3/S2-FITC, anti-KIR2DL1-PE, anti-KIR3DL1/S1-PE, anti-KIR3DL1/L2-PE, anti-KIR2DS4-PE, anti-NKG2A-PE, anti-KIR2DL1/S1-APC, and anti-IFN-γ-PerCp-Cy5.5. (A) Gating strategy that identified potentially alloreactive NK cells against HLA-C1–missing, HLA-C2/C2 cell targets. Left panel: identification of KIR2DL3⁺ NK cells within CD56^dim lymphocytes. Middle panel: identification of potentially alloreactive NK cells, which expressed KIR2DL3 and did not express KIR2DL1, KIR3DL1/S1, KIR3DL2, KIR2DS4, and NKG2A receptors. Right panel: identification of KIR2DL3⁺ potentially alloreactive NK cells that expressed KIR2DS1 (right quadrant) or did not express KIR2DS1 (left quadrant) (a minimum of 300 events were analyzed). (B) IFN-γ production by KIR2DS1⁻ alloreactive NK cells. (C) IFN-γ production by KIR2DS1⁺ alloreactive NK cells. (D) Frequencies of IFN-γ⁺ cells in KIR2DS1⁻ (squares) and KIR2DS1⁺ (triangles) alloreactive NK cells in 9 HLA-C1⁺ individuals. Each symbol represents NK cells from 1 individual. Bars represent median frequency of IFN-γ⁺ cells. Differences between the groups were tested by Wilcoxon matched-pairs test.

IFN-γ production analyses were performed in resting NK cells that expressed KIR2DL3 as their only inhibitory receptor for self and were therefore potentially capable of allorecognition of HLA-C1–missing targets (Figure 6A). Both KIR2DS1⁻ and KIR2DS1⁺ alloreactive NK cells produced negligible IFN-γ levels upon interaction with immature DCs. KIR2DS1⁺ alloreactive NK cells produced significantly more IFN-γ than KIR2DS1⁻ alloreactive NK cells upon activation with LPS- or *A fumigatus*–pulsed DCs (Figure 6B-D). Furthermore, IFN-γ release by alloreactive KIR2DL2/3⁺ NK clones that coexpressed KIR2DS1 was markedly reduced upon antibody blocking of KIR2DS1–HLA-C2 interaction (Figure 7).

Discussion

In T cell–depleted haploidentical hematopoietic transplantation, donor-vs-recipient NK-cell alloreactivity controls high-risk leukemia relapse.[13-17,26,27] The present study identifies a further advantage of donor-vs-recipient NK-cell alloreactivity. It demonstrates that transplantation from NK-alloreactive donors who possessed *KIR2DS1* and/or *KIR3DS1* activating genes was associated with a markedly reduced infection rate and infectious mortality, and significantly better EFS.

Figure 7. KIR2DS1 binding to HLA-C2 triggers IFN-γ production in alloreactive NK cells. NK cell clones from 4 HLA-C1[+] individuals were screened for alloreactivity against HLA-C1–missing, HLA-C2/C2 PHA T-cell blasts. Alloreactive KIR2DL2/3[+] clones that coexpressed KIR2DS1 obtained from each individual were pooled and incubated with NK-resistant HLA-C1/C2 DCs or NK-susceptible HLA-C1–missing, HLA-C2/C2 DCs, pulsed with LPS (A) or *A fumigatus* (B). IFN-γ release (mean ± SD) in the absence (squares) or presence (triangles) of an anti-KIR2DS1 F(ab')₂ is shown. Each symbol represents IFN-γ release by NK cells from 1 individual.

The study included patients transplanted under 2 protocols: our standard T cell–depleted CD34^{+} hematopoietic progenitor cell graft protocol[3-5] and the more recent protocol with Treg/Tcon addbacks.[6,7] It might be questionable to include in the analysis patients who received Treg/Tcon cell addbacks because T cells in the graft were shown to antagonize NK-cell recovery and prevent clinically relevant NK-cell effects in unrelated and unmanipulated haploidentical transplants.[9,57-60] However, in patients receiving Treg/Tcon addbacks, we previously demonstrated that reconstitution of donor-vs-recipient alloreactive NK cells was as efficient as in patients under the T cell–depleted transplant protocol.[6] In fact, the survival advantage of transplantation from NK-alloreactive donors with activating *KIR* genes was more significant in the entire cohort (HR, 0.33; 95% CI, 0.14-0.76; *P* = .009) than in the T cell–depleted cohort (HR, 0.37; 95% CI, 0.15-0.88; *P* = .025).

Apparently, KIR-ligand mismatches and the consequent NK-cell release from recipient HLA blockade allowed activating KIRs to enhance NK-cell functions upon binding to their ligands on recipient cells. KIR2DS1 is the only activating KIR for which binding to an HLA ligand (HLA-C2) is well documented.[33,34] Because *KIR2DS1* is in strong linkage disequilibrium with *KIR3DS1*[48-50] and the present transplant series is relatively small, it was not possible to analyze the effect of donor *KIR2DS1* interaction with recipient HLA-C2 independently of *KIR3DS1*. Likewise, it was not possible to analyze the effect of the *KIR2DS4* gene (located in the *A*-haplotype telomeric segment) because of a deletion variant allele that encodes for a nonfunctional receptor in as much as ~80% of individuals.[32]

In an attempt to define the underlying mechanism in vitro, we found that KIR2DS1 triggered NK cells to release IFN-γ upon interaction with KIR-ligand–mismatched, HLA-C2/C2 LPS–, or *A fumigatus*–pulsed DCs and that IFN-γ release was inhibited when KIR2DS1–HLA-C2 interaction was blocked. One may speculate that enhanced IFN-γ production played a role in improving responsiveness to pathogens. In fact, IFN-γ has antiviral effects, activates macrophage microbicidal functions, enhances antigen-presenting cell functions, and promotes Th1 polarization.[61-66] Protective immunity against *A fumigatus* depends on inflammatory cytokines and Th1 responses.[67] The KIR2DS1-triggered IFN-γ release in response to *A fumigatus*–pulsed DCs may have clinical implications because invasive Aspergillosis is a major cause of mortality in immune-compromised hosts.

Previous studies showed that KIR2DS1 boosted allocytotoxicity against HLA-C1–missing, HLA-C2/C2 targets.[27,34-37] KIR2DL2, and to a lesser extent KIR2DL3, crossreact with HLA-C2 molecules, thereby possibly hampering allorecognition of HLA-C1–missing, HLA-C2C2 targets.[27,56] KIR2DS1 binding to HLA-C2 was reported to overcome such inhibition, and analyses at the bulk NK-cell level suggested NK cells from HLA-C1[+] individuals with the *KIR2DS1* gene were more alloreactive.[27,34,35] Our analyses at the clonal level documented even low-frequency alloreactive NK cells in the repertoire and were therefore particularly suited to identify donors able to mount donor-vs-recipient NK-cell alloreactivity.[16] Such analyses, to date performed with >10 000 NK clones from >100 HLA-C1[+] individuals, showed that alloreactive NK cells against HLA-C1–missing targets were present in *KIR2DS1*[−] individuals in frequencies that were only slightly lower than those in *KIR2DS1*[+] individuals. Moreover, *KIR2DS1* did not significantly shape the alloreactive repertoires of HLA-C1[+] individuals regardless of whether they were *KIR2DL2* or *KIR2DL3* homozygous or *KIR2DL2/L3* heterozygous. In agreement with these findings, NK-alloreactive donors exerted a remarkable graft-vs-leukemia effect regardless of their activating *KIR* genetics.

Studies in matched unrelated and haploidentical transplants showed that, even in the absence of KIR-ligand mismatches, donor activating KIRs were associated with less leukemia relapse[38-43] or NRM.[68] Under these circumstances, one may speculate that activating KIRs overrode NK-cell blockade by recipient HLA, or that T cells coexpressing activating KIRs were responsible because they expand after transplant[69] and their effector functions are costimulated by activating KIRs.[70-72]

A previous study in adult and pediatric acute leukemia patients demonstrated that transplantation from maternal donors favorably affected survival.[47] Interestingly, the present analysis in adult leukemia patients shows that such beneficial effect is evident only in transplants from NK-alloreactive donors. Studies in larger series of transplants are underway to confirm this finding.

The present investigation shows that transplantation from NK-alloreactive donors who possess *KIR2DS1* and/or *KIR3DS1* activating genes is associated with a remarkable reduction in infectious morbidity and mortality, and it consequently improves EFS. Because ~40% of NK-alloreactive donors carry *KIR2DS1* and/or *KIR3DS1*, searching for them may become a feasible, additional criterion in donor selection.

Acknowledgments

Authorship

Contribution: A.M. designed the study, performed experiments, analyzed data, and wrote the paper; L. Ruggeri designed the study and analyzed data; E.U., A. Tosti, and F.T. contributed to NK cell cloning and cytotoxicity assays; A.P., M.S.M., A. Terenzi, A.C., and F.F. took care of patients and provided clinical data; S.B. provided *A fumigatus*–pulsed DCs; R.T. performed serologic and high-resolution molecular HLA typing; M.S. contributed to statistical analyses; L. Romani, F.A., and M.F.M. contributed to the design and interpretation of the study; and A.V. designed the study, analyzed data, and wrote the paper.

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References

1. Velardi A. Haplo-BMT: which approach? [commentary] *Blood.* 2013;121(5):719-720.

2. Reisner Y, Hagin D, Martelli MF. Haploidentical hematopoietic transplantation: current status and future perspectives. *Blood.* 2011;118(23): 6006-6017.

3. Aversa F, Tabilio A, Terenzi A, et al. Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. *Blood.* 1994;84(11):3948-3955.

4. Aversa F, Tabilio A, Velardi A, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *N Engl J Med.* 1998; 339(17):1186-1193.

5. Aversa F, Terenzi A, Tabilio A, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *J Clin Oncol.* 2005;23(15):3447-3454.

6. Di Ianni M, Falzetti F, Carotti A, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood.* 2011;117(14):3921-3928.

7. Martelli MF, Di Ianni M, Ruggeri L, et al. HLA-haploidentical transplantation with regulatory and conventional T-cell adoptive immunotherapy prevents acute leukemia relapse. *Blood.* 2014; 124(4):638-644.

8. Ciceri F, Bregni M, Peccatori J. Innovative platforms for haploidentical stem cell transplantation: the role of unmanipulated donor graft. *J Cancer.* 2011;2:339-340.

9. Fuchs EJ. Human leukocyte antigen-haploidentical stem cell transplantation using T-cell-replete bone marrow grafts. *Curr Opin Hematol.* 2012;19(6):440-447.

10. Wang Y, Liu DH, Liu KY, et al. Long-term follow-up of haploidentical hematopoietic stem cell transplantation without in vitro T cell depletion for the treatment of leukemia: nine years of experience at a single center. *Cancer.* 2013; 119(5):978-985.

11. Di Bartolomeo P, Santarone S, De Angelis G, et al. Haploidentical, unmanipulated, G-CSF-primed bone marrow transplantation for patients with high-risk hematologic malignancies. *Blood.* 2013;121(5):849-857.

12. Raiola AM, Dominietto A, Ghiso A, et al. Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning. *Biol Blood Marrow Transplant.* 2013;19(1):117-122.

13. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood.* 1999;94(1):333-339.

14. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science.* 2002;295(5562):2097-2100.

15. Kärre K. Immunology. A perfect mismatch. [commentary] *Science.* 2002;295(5562): 2029-2031.

16. Ruggeri L, Mancusi A, Capanni M, et al. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. *Blood.* 2007;110(1):433-440.

17. Velardi A, Ruggeri L, Mancusi A, Aversa F, Christiansen FT. Natural killer cell allorecognition of missing self in allogeneic hematopoietic transplantation: a tool for immunotherapy of leukemia. *Curr Opin Immunol.* 2009;21(5): 525-530.

18. Vivier E, Raulet DH, Moretta A, et al. Innate or adaptive immunity? The example of natural killer cells. *Science.* 2011;331(6013):44-49.

19. Caligiuri MA. Human natural killer cells. *Blood.* 2008;112(3):461-469.

20. Moretta L, Moretta A. Unravelling natural killer cell function: triggering and inhibitory human NK receptors. *EMBO J.* 2004;23(2):255-259.

21. Kärre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature.* 1986;319(6055):675-678.

22. Ljunggren HG, Kärre K. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today.* 1990;11(7):237-244.

23. Ciccone E, Pende D, Viale O, et al. Evidence of a natural killer (NK) cell repertoire for (allo) antigen recognition: definition of five distinct NK-determined allospecificities in humans. *J Exp Med.* 1992;175(3):709-718.

24. Colonna M, Brooks EG, Falco M, Ferrara GB, Strominger JL. Generation of allospecific natural killer cells by stimulation across a polymorphism of HLA-C. *Science.* 1993;260(5111):1121-1124.

25. Haas P, Loiseau P, Tamouza R, et al. NK-cell education is shaped by donor HLA genotype after unrelated allogeneic hematopoietic stem cell transplantation. *Blood.* 2011;117(3):1021-1029.

26. Leung W, Iyengar R, Turner V, et al. Determinants of antileukemia effects of allogeneic NK cells. *J Immunol.* 2004;172(1):644-650.

27. Pende D, Marcenaro S, Falco M, et al. Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity. *Blood.* 2009;113(13):3119-3129.

28. Moretta A, Sivori S, Vitale M, et al. Existence of both inhibitory (p58) and activatory (p50) receptors for HLA-C molecules in human natural killer cells. *J Exp Med.* 1995;182(3):875-884.

29. Biassoni R, Cantoni C, Falco M, et al. The human leukocyte antigen (HLA)-C-specific "activatory" or "inhibitory" natural killer cell receptors display highly homologous extracellular domains but differ in their transmembrane and intracytoplasmic portions. *J Exp Med.* 1996;183(2):645-650.

30. Uhrberg M, Valiante NM, Shum BP, et al. Human diversity in killer cell inhibitory receptor genes. *Immunity.* 1997;7(6):753-763.

31. Parham P. MHC class I molecules and KIRs in human history, health and survival. *Nat Rev Immunol.* 2005;5(3):201-214.

32. Maxwell LD, Wallace A, Middleton D, Curran MD. A common KIR2DS4 deletion variant in the human that predicts a soluble KIR molecule analogous to the KIR1D molecule observed in the rhesus monkey. *Tissue Antigens.* 2002;60(3): 254-258.

33. Stewart CA, Laugier-Anfossi F, Vély F, et al. Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors. *Proc Natl Acad Sci USA.* 2005;102(37): 13224-13229.

34. Fauriat C, Ivarsson MA, Ljunggren HG, Malmberg KJ, Michaëlsson J. Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. *Blood.* 2010;115(6):1166-1174.

35. Chewning JH, Gudme CN, Hsu KC, Selvakumar A, Dupont B. KIR2DS1-positive NK cells mediate alloresponse against the C2 HLA-KIR ligand group in vitro. *J Immunol.* 2007;179(2):854-868.

36. Foley B, De Santis D, Lathbury L, Christiansen F, Witt C. KIR2DS1-mediated activation overrides NKG2A-mediated inhibition in HLA-C C2-negative individuals. *Int Immunol.* 2008;20(4):555-563.

37. Sivori S, Carlomagno S, Falco M, Romeo E, Moretta L, Moretta A. Natural killer cells expressing the KIR2DS1-activating receptor efficiently kill T-cell blasts and dendritic cells: implications in haploidentical HSCT. *Blood.* 2011; 117(16):4284-4292.

38. Cooley S, Trachtenberg E, Bergemann TL, et al. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. *Blood.* 2009;113(3): 726-732.

39. Cooley S, Weisdorf DJ, Guethlein LA, et al. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. *Blood.* 2010;116(14):2411-2419.

40. Venstrom JM, Pittari G, Gooley TA, et al. HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. *N Engl J Med.* 2012; 367(9):805-816.

41. Cooley S, Weisdorf DJ, Guethlein LA, et al. Donor killer cell Ig-like receptor B haplotypes, recipient HLA-C1, and HLA-C mismatch enhance the clinical benefit of unrelated transplantation for acute myelogenous leukemia. *J Immunol.* 2014; 192(10):4592-4600.

42. Michaelis SU, Mezger M, Bornhäuser M, et al. KIR haplotype B donors but not KIR-ligand mismatch result in a reduced incidence of relapse after haploidentical transplantation using reduced intensity conditioning and CD3/CD19-depleted grafts. *Ann Hematol.* 2014;93(9):1579-1586.

43. Oevermann L, Michaelis SU, Mezger M, et al. KIR B haplotype donors confer a reduced risk of relapse after haploidentical transplantation in children with ALL. *Blood.* 2014;124(17): 2744-2747.

44. Martin MP, Gao X, Lee JH, et al. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat Genet.* 2002;31(4): 429-434.

45. Khakoo SI, Thio CL, Martin MP, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science.* 2004;305(5685): 872-874.

46. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant.* 2005;11(12):945-956.

47. Stern M, Ruggeri L, Mancusi A, et al. Survival after T cell-depleted haploidentical stem cell transplantation is improved using the mother as donor. *Blood.* 2008;112(7):2990-2995.

48. Wilson MJ, Torkar M, Haude A, et al. Plasticity in the organization and sequences of human KIR/ILT gene families. *Proc Natl Acad Sci USA.* 2000; 97(9):4778-4783.

49. Pyo CW, Guethlein LA, Vu Q, et al. Different patterns of evolution in the centromeric and telomeric regions of group A and B haplotypes of the human killer cell Ig-like receptor locus. *PLoS ONE.* 2010;5(12):e15115.

50. Vierra-Green C, Roe D, Hou L, et al. Allele-level haplotype frequencies and pairwise linkage disequilibrium for 14 KIR loci in 506 European-American individuals. *PLoS ONE.* 2012;7(11): e47491.

51. Cooley S, Xiao F, Pitt M, et al. A subpopulation of human peripheral blood NK cells that lacks inhibitory receptors for self-MHC is developmentally immature. *Blood.* 2007;110(2): 578-586.

52. Bozza S, Perruccio K, Montagnoli C, et al. A dendritic cell vaccine against invasive aspergillosis in allogeneic hematopoietic transplantation. *Blood.* 2003;102(10):3807-3814.

53. Falco M, Romeo E, Marcenaro S, et al. Combined genotypic and phenotypic killer cell Ig-like receptor analyses reveal KIR2DL3 alleles displaying unexpected monoclonal antibody reactivity: identification of the amino acid residues critical for staining. *J Immunol.* 2010;185(1): 433-441.

54. Romagné F, André P, Spee P, et al. Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. *Blood.* 2009;114(13):2667-2677.

55. Gray R. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat.* 1988;16:1141-1154.

56. Moesta AK, Norman PJ, Yawata M, Yawata N, Gleimer M, Parham P. Synergistic polymorphism at two positions distal to the ligand-binding site makes KIR2DL2 a stronger receptor for HLA-C than KIR2DL3. *J Immunol.* 2008;180(6): 3969-3979.

57. Davies SM, Ruggieri L, DeFor T, et al. Evaluation of KIR ligand incompatibility in mismatched unrelated donor hematopoietic transplants. Killer immunoglobulin-like receptor. *Blood.* 2002; 100(10):3825-3827.

58. Farag SS, Bacigalupo A, Eapen M, et al; KIR Study Group, Center for International Blood and Marrow Transplantation Research. The effect of KIR ligand incompatibility on the outcome of unrelated donor transplantation: a report from the center for international blood and marrow transplant research, the European blood and marrow transplant registry, and the Dutch registry. *Biol Blood Marrow Transplant.* 2006;12(8): 876-884.

59. Shilling HG, McQueen KL, Cheng NW, Shizuru JA, Negrin RS, Parham P. Reconstitution of NK cell receptor repertoire following HLA-matched hematopoietic cell transplantation. *Blood.* 2003; 101(9):3730-3740.

60. Cooley S, McCullar V, Wangen R, et al. KIR reconstitution is altered by T cells in the graft and correlates with clinical outcomes after unrelated donor transplantation. *Blood.* 2005;106(13): 4370-4376.

61. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol.* 2004; 75(2):163-189.

62. Cooper MA, Fehniger TA, Fuchs A, Colonna M, Caligiuri MA. NK cell and DC interactions. *Trends Immunol.* 2004;25(1):47-52.

63. Walzer T, Dalod M, Robbins SH, Zitvogel L, Vivier E. Natural-killer cells and dendritic cells: "l'union fait la force." *Blood.* 2005;106(7):2252-2258.

64. Marcenaro E, Dondero A, Moretta A. Multi-directional cross-regulation of NK cell function during innate immune responses. *Transpl Immunol.* 2006;17(1):16-19.

65. Zanoni I, Granucci F, Foti M, Ricciardi-Castagnoli P. Self-tolerance, dendritic cell (DC)-mediated activation and tissue distribution of natural killer (NK) cells. *Immunol Lett.* 2007;110(1):6-17.

66. Martín-Fontecha A, Thomsen LL, Brett S, et al. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. *Nat Immunol.* 2004;5(12):1260-1265.

67. Lass-Flörl C, Roilides E, Löffler J, Wilflingseder D, Romani L. Minireview: host defence in invasive aspergillosis. *Mycoses.* 2013;56(4):403-413.

68. Symons HJ, Leffell MS, Rossiter ND, Zahurak M, Jones RJ, Fuchs EJ. Improved survival with inhibitory killer immunoglobulin receptor (KIR) gene mismatches and KIR haplotype B donors after nonmyeloablative, HLA-haploidentical bone marrow transplantation. *Biol Blood Marrow Transplant.* 2010;16(4):533-542.

69. Albi N, Ruggeri L, Aversa F, et al. Natural killer (NK)-cell function and antileukemic activity of a large population of CD3+/CD8+ T cells expressing NK receptors for major histocompatibility complex class I after "three-loci" HLA-incompatible bone marrow transplantation. *Blood.* 1996;87(9):3993-4000.

70. Mandelboim O, Davis DM, Reyburn HT, et al. Enhancement of class II-restricted T cell responses by costimulatory NK receptors for class I MHC proteins. *Science.* 1996;274(5295): 2097-2100.

71. Mandelboim O, Kent S, Davis DM, et al. Natural killer activating receptors trigger interferon gamma secretion from T cells and natural killer cells. *Proc Natl Acad Sci USA.* 1998;95(7): 3798-3803.

72. Snyder MR, Nakajima T, Leibson PJ, Weyand CM, Goronzy JJ. Stimulatory killer Ig-like receptors modulate T cell activation through DAP12-dependent and DAP12-independent mechanisms. *J Immunol.* 2004;173(6):3725-3731.

Haploidentical hematopoietic transplantation from KIR ligand–mismatched donors with activating KIRs reduces nonrelapse mortality

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